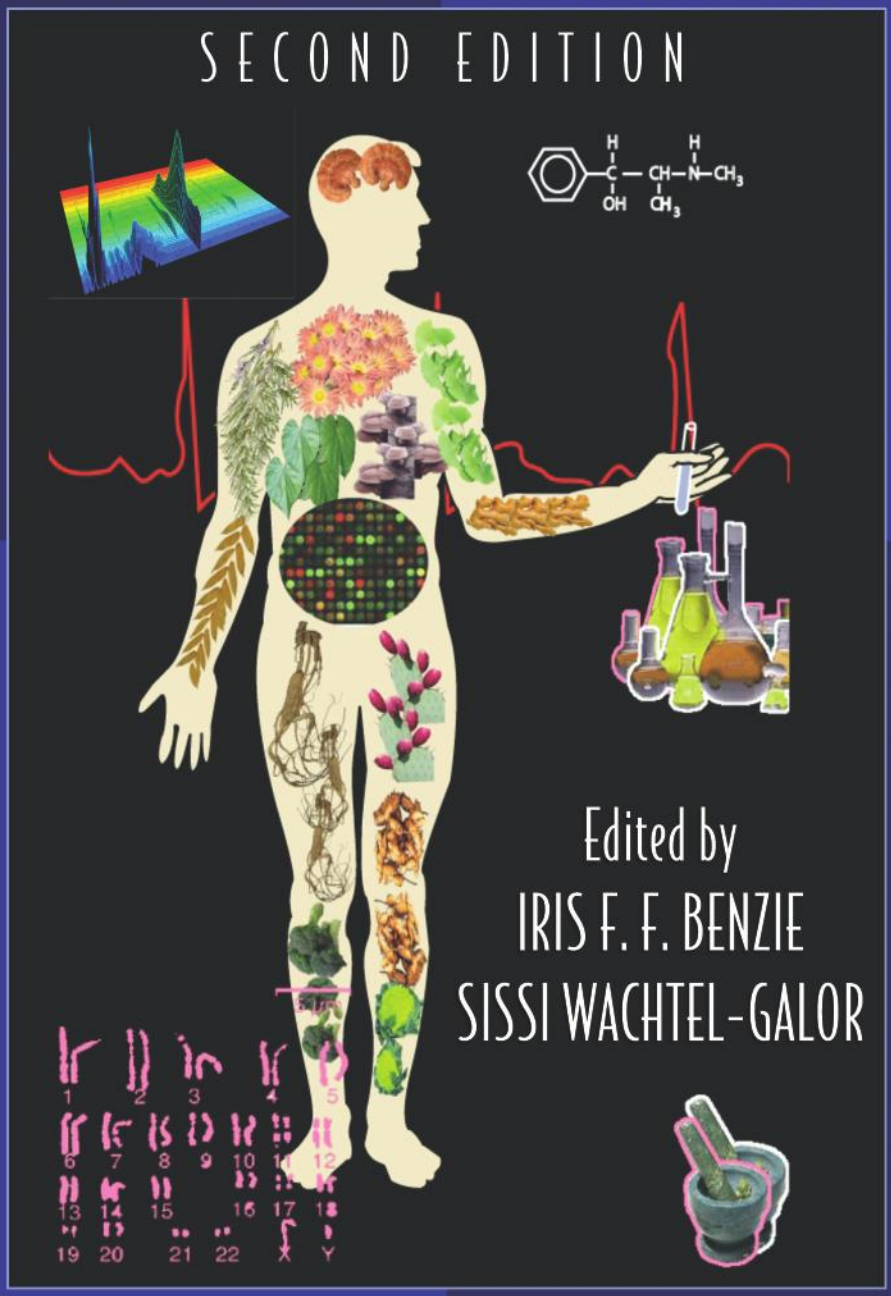
HERBAL MEDICINE

BIOMOLECULAR AND CLINICAL ASPECTS

SECOND EDITION



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HERBAL MEDICINE
BIOMOLECULAR AND CLINICAL ASPECTS

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OXIDATIVE STRESS AND DISEASE

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Enrique Cadenas, M.D., Ph.D.

University of Southern California School of Pharmacy
Los Angeles, California

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Edited by
IRIS F. F. BENZIE
SISSI WACHTEL-GALOR

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Contents

Series Preface ix
Foreword xi
Preface xiii
Editors xv
Contributors xvii

Chapter 1 Herbal Medicine: An Introduction to Its History, Usage, Regulation, Current Trends, and Research Needs 1
Sissi Wachtel-Galor and Iris F. F. Benzie

Chapter 2 Antioxidants in Herbs and Spices: Roles in Oxidative Stress and Redox Signaling 11
Ingvild Paur, Monica H. Carlsen, Bente Lise Halvorsen, and Rune Blomhoff

Chapter 3 Evaluation of the Nutritional and Metabolic Effects of *Aloe vera* 37
Meika Foster, Duncan Hunter, and Samir Samman

Chapter 4 Bilberry (*Vaccinium myrtillus* L.) 55
Wing-kwan Chu, Sabrina C. M. Cheung, Roxanna A. W. Lau, and Iris F. F. Benzie

Chapter 5 *Cordyceps* as an Herbal Drug 73
Bao-qin Lin and Shao-ping Li

Chapter 6 Cranberry 107
Catherine C. Neto and Joe A. Vinson

Chapter 7 The Amazing and Mighty Ginger 131
Ann M. Bode and Zigang Dong

Chapter 8 Biological Activities of Ginseng and Its Application to Human Health 157
Jae Joon Wee, Kyeong Mee Park, and An-Sik Chung

Chapter 9 *Ganoderma lucidum* (Lingzhi or Reishi): A Medicinal Mushroom 175
Sissi Wachtel-Galor, John Yuen, John A. Buswell, and Iris F. F. Benzie

Chapter 10 Pomegranate Ellagitannins 201
David Heber

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Chapter 11 Medical Attributes of St. John's Wort (*Hypericum perforatum*)..... 211

Kenneth M. Klemow, Andrew Bartlow, Justin Crawford, Neil Kocher, Jay Shah, and Michael Ritsick

Chapter 12 Health Benefits of Tea 239

Mauro Serafini, Daniele Del Rio, Yao Denis N'Dri, Saverio Bettuzzi, and Ilaria Peluso

Chapter 13 Turmeric, the Golden Spice: From Traditional Medicine to Modern Medicine 263

Sahdeo Prasad and Bharat B. Aggarwal

Chapter 14 Biomolecular and Clinical Aspects of Chinese Wolfberry 289

Peter Bucheli, Qiutao Gao, Robert Redgwell, Karine Vidal, Junkuan Wang, and Weiguo Zhang

Chapter 15 Botanical Phenolics and Neurodegeneration 315

Albert Y. Sun, Qun Wang, Agnes Simonyi, and Grace Y. Sun

Chapter 16 Cardiovascular Disease 333

Richard Walden and Brian Tomlinson

Chapter 17 Herbs and Spices in Cancer Prevention and Treatment 361

Christine M. Kaefer and John A. Milner

Chapter 18 Herbal Treatment for Dermatologic Disorders..... 383

Philip D. Shenefelt

Chapter 19 Diabetes and Herbal (Botanical) Medicine..... 405

William T. Cefalu, Jaqueline M. Stephens, and David M. Ribnicky

Chapter 20 Bioactive Components in Herbal Medicine: Experimental Approaches 419

Foo-tim Chau, Kwok-pui Fung, Chi-man Koon, Kit-man Lau, Shui-yin Wei, and Ping-chung Leung

Chapter 21 Ethics of Using Herbal Medicine as Primary or Adjunct Treatment and Issues of Drug–Herb Interaction 439

Lauren Girard and Sunita Vohra

Chapter 22 Integration of Herbal Medicine into Evidence-Based Clinical Practice: Current Status and Issues 453

Anthony Lin Zhang, Charlie Changli Xue, and Harry H. S. Fong

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Series Preface

During evolution, oxygen—itself a free radical—was chosen as the terminal electron acceptor for respiration; hence, the formation of oxygen-derived free radicals is a consequence of aerobic metabolism. These oxygen-derived radicals are involved in oxidative damage to cell components inherent in several pathophysiological situations. Conversely, cells convene antioxidant mechanisms to counteract the effects of oxidants in either a highly specific manner (e.g., by superoxide dismutases) or a less-specific manner (e.g., through small molecules such as glutathione, vitamin E, and vitamin C). Oxidative stress, as defined classically, entails an imbalance between oxidants and antioxidants. However, the same free radicals that are generated during oxidative stress are produced during normal metabolism and, as a corollary, are involved in both human health and disease by virtue of their involvement in the regulation of signal transduction and gene expression, activation of receptors and nuclear transcription factors, antimicrobial and cytotoxic actions of immune system cells, and aging and age-related degenerative diseases.

In recent years, research disciplines focusing on oxidative stress have increased our knowledge of the importance of the cell redox status and the recognition of oxidative stress as a process with implications for many pathophysiological states. From this multi- and interdisciplinary interest in oxidative stress emerges a concept that attests the vast consequences of the complex and dynamic interplay of oxidants and antioxidants in cellular and tissue settings. Consequently, our view of oxidative stress is both growing in scope and following new directions. Likewise, the term "reactive oxygen species," adopted at some stage to highlight nonradical/radical oxidants, now fails to reflect the rich variety of other species in free-radical biology and medicine, encompassing nitrogen-, sulfur-, oxygen-, and carbon-centered radicals. These reactive species are involved in the redox regulation of cell functions and, as a corollary, oxidative stress is increasingly viewed as a major upstream component in cell-signaling cascades involved in inflammatory responses, stimulation of cell adhesion molecules, and chemoattractant production and as an early component of age-related neurodegenerative disorders such as Alzheimer's, Parkinson's, and Huntington's diseases, and amyotrophic lateral sclerosis. Hydrogen peroxide is probably the most important redox-signaling molecule that, among others, can activate nuclear factor κB (NF-κB), NF-E2 related factor 2 (Nrf2), and other universal transcription factors, and that is involved in the redox regulation of insulin and mitogen-activated protein kinase (MAPK) signaling. These pleiotropic effects of hydrogen peroxide are largely accounted for by changes in the thiol/disulfide status of a cell, an important determinant of the cell's redox status with clear involvement in adaptation, proliferation, differentiation, apoptosis, and necrosis.

The identification of oxidants in the regulation of redox cell signaling and gene expression is a significant breakthrough in the field of oxidative stress. The classical definition of oxidative stress as an imbalance between the production of oxidants and the occurrence of antioxidant defenses now seems to provide a limited depiction of oxidative stress, although it emphasizes the significance of cell redox status. Because individual signaling and control events occur through discrete redox pathways rather than through global balances, a new definition of oxidative stress was advanced by Dean P. Jones as a disruption of redox signaling and control that recognizes the occurrence of compartmentalized cellular redox circuits. These concepts are anticipated to serve as platforms for the development of tissue-specific therapeutics tailored to discrete, compartmentalized redox circuits. This, in essence, dictates the principles of drug development–guided knowledge of the mechanisms of oxidative stress. Hence, successful interventions will take advantage of new knowledge of compartmentalized redox control and free-radical scavenging.

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Virtually all diseases examined thus far involve free radicals. Although in most cases free radicals are secondary to the disease process, in some instances causality is established for free radicals. Thus, there is a delicate balance between oxidants and antioxidants in health, and disease clearly associates with and in at least some cases is caused by loss of such balance. Their proper balance is essential for ensuring healthy aging. Compelling support for the involvement of free radicals in disease development originates from epidemiological studies showing that enhanced antioxidant status is associated with reduced risk of several diseases. Of great significance is the role played by micronutrients in modulation of cell signaling. This establishes a strong linking of diet, health, and disease centered on the abilities of micronutrients to regulate redox cell signaling and modify gene expression.

Oxidative stress is an underlying factor in health and disease. In this series of books, the importance of oxidative stress and diseases associated with organ systems is highlighted by exploring the scientific evidence and clinical applications of this knowledge. This series is intended for researchers in basic biomedical sciences and for clinicians. The potential of such knowledge in facilitating healthy aging and disease prevention warrants further knowledge about how oxidants and antioxidants modulate cell and tissue functions.

Lester Packer
Enrique Cadenas

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Foreword

This book, *Herbal Medicine: Biomolecular and Clinical Aspects, Second Edition,* edited by Iris F. F. Benzie and Sissi Wachtel-Galor, reports updated information on some of the most widely investigated traditional and herbal medicines, and establishes continuity with a previous book in this series, *Herbal and Traditional Medicine*: *Molecular Aspects of Health*, edited by Lester Packer, Choon Nam Ong, and Barry Halliwell. This new edition is timely because there is unprecedented interest in understanding the molecular basis of the biological activity of traditional remedies—many of which are derived from plants and herbs (phytomedicines) or from products that are available as herbal supplements—and because such herbal supplements enjoy much popularity throughout the world. This popularity is the result of the recognition of alternative and complementary forms of medicine by governmental and nongovernmental changes; for example, the U.S. Public Health Service and the National Institutes of Health established the Office of Dietary Supplement and the National Center for Complementary and Alternative Medicine (NCCAM), which seek to verify health claims. Western and traditional medicines hold great promise once research and medical practice are appropriately coordinated.

The number of herbal remedies recognized to date is staggering, and an extensive literature has documented their existence and reported on their beneficial effects toward health and well-being, disease prevention, and disease treatment. *Herbal Medicine: Biomolecular and Clinical Aspects, Second Edition* selects some of the best-case scenarios and widely used and known herbal remedies to report on their effects on health in light of the current knowledge concerning their basic biological mechanisms of action. Coeditors Iris F. F. Benzie and Sissi Wachtel-Galor must be congratulated for their excellent effort.

Lester Packer
Enrique Cadenas

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Preface

Herbal medicine has been used throughout history and within every culture to prevent and treat diseases. In any individual culture, the materials used were those that were available within the geographical location and addressed local health concerns. With immigration and trade, cultural traditions were exposed and often overwhelmed by modern scientific concepts and medical practices. However, the mix and movement of cultures that began only fairly recently, along with modern transportation, storage, and communication tools, brought an enormous increase in the general availability of herbs from different cultures and geographical areas. In a different culture, an herb would often be used for its appearance, coloring, or taste rather than for any perceived health benefits. Indeed, some of the herbs discussed in this book, such as curcumin, garlic, and cumin, are often referred to as "spices," or regarded as simple, if somewhat exotic, ingredients of foods from faraway lands. Nonetheless, the long history and powerful reputation of many types of herbs, spices, and fungi are impressive. In this time of increasing need for effective, affordable health promotion and treatment strategies for our aging populations and growing problems posed by new and antibiotic-resistant microbes, the history and reputation of herbal medicines must be examined in a rigorous and scientific way so that their biomolecular effects, if confirmed, can be translated into clinical benefit. Because of the strong associations among oxidative stress, aging, and disease, there is increasing interest in the biomolecular effects of herbs, which may be related to antioxidant action.

By biomolecular effects, we mean the measurable or observable changes (biomarkers) that occur in cells, animals, and human subjects, healthy or otherwise, under controlled conditions of treatment with an herb. Biomarkers reflect organ, cell, and organelle function or damage, and homeostatic control mechanisms. These are not limited to the genomic level, although this is a level on which interest and insight are growing, but include a wide range and variety of biochemical and "metabolomic" biomarkers, such as hemoglobin A1c (HbA1c) for glycemic control, plasma high-sensitivity C-reactive protein (hsCRP) for inflammation, the number and function of immune cells, plasma cholesterol and triglycerides for lipid balance, and biomarkers of liver and renal function. In addition, herbs contain many compounds with powerful antioxidant properties, and herb-induced changes in biomarkers that assess antioxidant status and oxidative stress, such as plasma ascorbic acid and lipid peroxides, antioxidant enzyme activity/induction, and oxidation-induced damage to DNA, are of interest in relation to the mechanisms of herbal protection. In cell-culture studies, direct cytotoxicity and protection, gene expression, protein synthesis, and transport mechanisms can be measured, and the morphology and growth of cells can be assessed. In animal studies, tumor occurrence and size can be examined. By clinical effects, we mean the outcome of the biomolecular effects in terms of human health preservation and restoration.

This book focuses on presenting the current scientific evidence of biomolecular effects of selected herbs in relation to clinical outcomes and therapy for promotion of human health. Although the terms "herb" and "herbal medicine" in traditional medicine are sometimes used in relation to animal or insect parts, our use of the term is limited to plants and fungi. Also, whereas many herbal medicines are made by mixing different herbs, the focus in this book is on single herbs. The herbs selected cover a wide range and include flowering herbs, leaves, and leaf exudate (St. John's wort, tea, *aloe vera*), fruits and berries (pomegranate, cranberry, wolfberry, bilberry), roots and rhizomes (ginseng, ginger, and turmeric), and fungi (lingzhi and cordyceps). There is a chapter that focuses on the antioxidant properties and effects of herbs (Chapter 2), and other chapters shift the focus into the clinical arena and the use of herbs in relation to cardiovascular disease, cancer, diabetes, skin disorders, and neurodegenerative disease. The ethics of using herbal medicine and its integration into modern, evidence-based medicine are also discussed. Finally, the use of new technologies

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of mass spectrometry and chemometric fingerprinting in the authentication of herbs is presented. These technologies will bring a previously unknown level of quality control to the production of herbal extracts. Currently, many commercially available herbal products are uncharacterized and of questionable quality or content. The composition of natural products such as herbs can vary greatly with season, growing conditions, preparation, and storage. However, there is also adulteration, contamination, and misidentification of herbs and herbal products. Improved quality control techniques and processes for the identification of herbs and the establishment of characteristic chemical "fingerprints" for herbs and herbal medicines are badly needed.

For well-designed clinical trials to be performed and for them to generate valid findings, herbs that are safe and of consistent quality and composition are needed. For findings to be valid in terms of human health and disease, a wide and diverse range of biomarkers needs to be investigated in controlled human trials. To translate the positive findings of science-based studies and clinical trials into action for health promotion, consumers need to be supplied with the same herbal products as the ones shown to have these effects. Quality, consistency, and control are needed across all aspects of production, testing, and promotion of herbal medicines; further, at the heart of ethically acceptable and responsible use of herbal medicines in modern health care, there must be science-based evidence of biomolecular and clinical effects.

In closing, we would like to express our sincere thanks to the authors who contributed their expertise and time to the production of this volume. Individually the authors are leaders in their field. Collectively they embody a truly international collection of wisdom and experience in the biomolecular and clinical aspects of herbal medicine. We are honored to be in their company.

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Editors



Dr. Iris F. F. Benzie is a chair professor of biomedical science in the Department of Health Technology and Informatics at the Hong Kong Polytechnic University, Hong Kong SAR, People's Republic of China (http://www.polyu.edu.hk/hti). She has a doctorate in biomedical science from the University of Ulster, United Kingdom, and a master's degree in clinical and pathological science from the Chinese University of Hong Kong. She is a fellow of the Institute of Biomedical Science in the United Kingdom, a fellow of the Hong Kong Society of Clinical Chemistry, an accredited clinical biochemist, a chartered biologist, and a chartered scientist. Originally from Scotland, she has lived and worked in Hong Kong for many years. Her research interest is in the area of dietary antioxidants, herbs, and functional foods, and the role of antioxidants and oxidative stress in aging and chronic degenerative disease using a biomarker approach. She has published over 100 scientific papers and book chapters, and she developed the patented ferric reducing ability of plasma (FRAP) assay, a widely used test for measuring the total antioxidant/reducing power of foods, herbs, drugs, and biological fluids.



Dr. Sissi Wachtel-Galor is a research fellow in the Department of Health Technology and Informatics at the Hong Kong Polytechnic University, Hong Kong SAR, People's Republic of China. She has a bachelor's degree in industrial engineering and a master's degree in biotechnology from the Technion–Israel Institutes of Technology, Haifa, Israel. Dr. Wachtel-Galor received her PhD in biomedical sciences from the Hong Kong Polytechnic University, Hong Kong, where she was awarded the first distinguished thesis prize for her outstanding achievements. Dr. Wachtel-Galor was born in Italy, grew up in Israel, and has traveled and lived in the United States and in Asia. During her travels, she was introduced to different types of traditional and herbal medicine, which sparked her interest in her present research areas. Her research interests include herbal medicine with a focus on Chinese medicine, vegetarian diets, endogenous antioxidants, and antioxidants from dietary sources and their effects on inflammatory mechanisms. Her approach is to use biomarkers in the assessment of antioxidant status and oxidative stress, and she has extensive experience in both animal and human supplementation studies.

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Contributors

Bharat B. Aggarwal
Cytokine Research Laboratory
Department of Experimental Therapeutics
The University of Texas

and

Anderson Cancer Center
Houston, Texas

Andrew Bartlow
Department of Biology
Wilkes University
Wilkes-Barre, Pennsylvania

Iris F. F. Benzie
Department of Health Technology and Informatics
The Hong Kong Polytechnic University
Kowloon, Hong Kong, People's Republic of China

Saverio Bettuzzi
Division of Biochemistry
Department of Experimental Medicine
University of Parma
Parma, Italy

Rune Blomhoff
Department of Nutrition
Institute of Basic Medical Sciences
Faculty of Medicine
University of Oslo
Oslo, Norway

Ann M. Bode
The Hormel Institute
University of Minnesota
Austin, Minnesota

Peter Bucheli
Manufacturing Support Department
Nestlé Product Technology Centre
Konolfingen, Switzerland

John A. Buswell
Institute of Edible Fungi
Shanghai Academy of Agricultural Sciences
Shanghai, People's Republic of China

Monica H. Carlsen
Department of Nutrition
Institute of Basic Medical Sciences
Faculty of Medicine
University of Oslo
Oslo, Norway

William T. Cefalu
The Botanical Research Center
Pennington Biomedical Research Center
Louisiana State University System
Baton Rouge, Louisiana

and

Biotech Center
Rutgers University
New Brunswick, New Jersey

Foo-tim Chau
Department of Applied Biology and Chemical Technology
The Hong Kong Polytechnic University
Kowloon, Hong Kong, People's Republic of China

Sabrina C. M. Cheung
Department of Health Technology and Informatics
The Hong Kong Polytechnic University
Kowloon, Hong Kong, People's Republic of China

Wing-kwan Chu
Department of Health Technology and Informatics
The Hong Kong Polytechnic University
Kowloon, Hong Kong, People's Republic of China

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An-Sik Chung
Department of Biological Sciences
Korea Advanced Institute of Science and Technology
Daejeon, Korea

Justin Crawford
Department of Biology
Wilkes University
Wilkes-Barre, Pennsylvania

Zigang Dong
The Hormel Institute
University of Minnesota
Austin, Minnesota

Harry H. S. Fong
University of Illinois at Chicago
Chicago, Illinois

and

The Royal Melbourne Institute of Technology (RMIT)
Melbourne, Australia

Meika Foster
Discipline of Nutrition and Metabolism
School of Molecular Bioscience
University of Sydney
Sydney, Australia

Kwok-pui Fung
Institute of Chinese Medicine
The Chinese University of Hong Kong
Shatin, Hong Kong, People's Republic of China

Qiutao Gao
Nestlé Research Centre
Nestlé R&D Centre Beijing Ltd.
Beijing, People's Republic of China

Lauren Girard
CARE Program
Department of Pediatrics
Faculty of Medicine and Dentistry
University of Alberta
Edmonton, Alberta, Canada

Bente Lise Halvorsen
Department of Nutrition
Institute of Basic Medical Sciences
Faculty of Medicine
University of Oslo
Oslo, Norway

David Heber
Department of Medicine
UCLA Center for Human Nutrition
David Geffen School of Medicine at University of California
Los Angeles, California

Duncan Hunter
Discipline of Nutrition and Metabolism
School of Molecular Bioscience
University of Sydney
Sydney, Australia

Christine M. Kaefer
Epidemiology and Genetics Research Program
Division of Cancer Control and Populations Sciences
National Cancer Institute
Rockville, Maryland

Kenneth M. Klemow
Department of Biology
Wilkes University
Wilkes-Barre, Pennsylvania

Neil Kocher
Department of Biology
Wilkes University
Wilkes-Barre, Pennsylvania

Chi-man Koon
Institute of Chinese Medicine
The Chinese University of Hong Kong
Shatin, Hong Kong, People's Republic of China

Kit-man Lau
Institute of Chinese Medicine
The Chinese University of Hong Kong
Shatin, Hong Kong, People's Republic of China

Roxanna A. W. Lau
Department of Health Technology and Informatics
The Hong Kong Polytechnic University
Kowloon, Hong Kong, People's Republic of China

Ping-chung Leung
Institute of Chinese Medicine
The Chinese University of Hong Kong
Shatin, Hong Kong, People's Republic of China

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Shao-ping Li
Institute of Chinese Medical Sciences
University of Macau
Macao, People's Republic of China

Bao-qin Lin
Institute of Chinese Medical Sciences
University of Macau
Macao, People's Republic of China

John A. Milner
Nutrional Science Research Group
Division of Cancer Prevention
National Cancer Institute
Rockville, Maryland

Yao Denis N'Dri
Human Nutrition Unit
Department of Public Health
University of Parma
Parma, Italy

Catherine C. Neto
Department of Chemistry and Biochemistry
University of Massachusetts–Dartmouth
North Dartmouth, Massachusetts

Kyeong Mee Park
Department of Oriental Medicine
Daejon University
Daejeon, South Korea

Ingvild Paur
Department of Nutrition
Institute of Basic Medical Sciences
Faculty of Medicine
University of Oslo
Oslo, Norway

Ilaria Peluso
Area of Nutrition Sciences
Antioxidant Research Laboratory
Rome, Italy

Sahdeo Prasad
Cytokine Research Laboratory
Department of Experimental Therapeutics
The University of Texas
Austin, Texas

and

Anderson Cancer Center
Houston, Texas

Robert Redgwell
Food Science and Technology Department
Nestlé Research Centre
Vers-chez-les-Blanc, Lausanne, Switzerland

David M. Ribnicky
The Botanical Research Center
Pennington Biomedical Research Center
Louisiana State University System
Baton Rouge, Louisiana

and

Biotechnology Center
Rutgers University
New Brunswick, New Jersey

Daniele Del Rio
Human Nutrition Unit
Department of Public Health
University of Parma
Parma, Italy

Michael Ritsick
Department of Biology
Wilkes University
Wilkes-Barre, Pennsylvania

Samir Samman
Discipline of Nutrition and Metabolism
School of Molecular Bioscience
University of Sydney
Sydney, Australia

Mauro Serafini
Area of Nutrition Sciences
Antioxidant Research Laboratory
Rome, Italy

Jay Shah
Department of Biology
Wilkes University
Wilkes-Barre, Pennsylvania

Philip D. Shenefelt
Department of Dermatology and
Cutaneous Surgery
College of Medicine
University of South Florida
Tampa, Florida

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Agnes Simonyi
Department of Biochemistry
University of Missouri
Columbia, Missouri

Jaqueline M. Stephens
The Botanical Research Center
Pennington Biomedical Research Center
Louisiana State University System
Baton Rouge, Louisiana

and

Biotechnology Center
Rutgers University
New Brunswick, New Jersey

Albert Y. Sun
Department of Medical Pharmacology and Physiology and Department of Pathology and Anatomical Sciences
University of Missouri
Columbia, Missouri

Grace Y. Sun
Department of Pathology and Anatomical Sciences and Department of Biochemistry
University of Missouri
Columbia, Missouri

Brian Tomlinson
Department of Medicine and Therapeutics
The Chinese University of Hong Kong
Shatin, Hong Kong, People's Republic of China

Karine Vidal
Food Immunology Department
Nestlé Research Centre
Vers-chez-les-Blanc
Lausanne, Switzerland

Joe A. Vinson
Department of Chemistry
University of Scranton
Scranton, Pennsylvania

Sunita Vohra
CARE Program
Department of Pediatrics
Faculty of Medicine and Dentistry
University of Alberta
Edmonton, Alberta, Canada

Sissi Wachtel-Galor
Department of Health Technology and Informatics
The Hong Kong Polytechnic University
Kowloon, Hong Kong, People's Republic of China

Richard Walden
Centre for Clinical Pharmacology and Therapeutics
Division of Medicine
University College London

Junkuan Wang
Nestlé Research Centre
Nestlé R&D Centre Beijing Ltd.
Beijing, People's Republic of China

Qun Wang
Department of Medical Pharmacology and Physiology
University of Missouri
Columbia, Missouri

Jae Joon Wee
KT&G Central Research Institute
Daejeon, South Korea

Shui-yin Wei
Department of Applied Biology and Chemical Technology
The Hong Kong Polytechnic University
Kowloon, Hong Kong, People's Republic of China

Charlie Changli Xue
Discipline of Chinese Medicine
School of Health Sciences
RMIT University
Melbourne, Australia

John Yuen
Department of Health Technology and Informatics
The Hong Kong Polytechnic University
Kowloon, Hong Kong, People's Republic of China

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Anthony Lin Zhang
Discipline of Chinese Medicine
School of Health Sciences
RMIT University
Melbourne, Australia

Weiguo Zhang
Nestlé Research Centre
Nestlé R&D Centre Beijing Ltd.
Beijing, People's Republic of China

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1 Herbal Medicine
An Introduction to Its History, Usage, Regulation, Current Trends, and Research Needs

Sissi Wachtel-Galor and Iris F. F. Benzie

CONTENTS

1.1 Herbal Medicine: A Growing Field with a Long Tradition 1
1.2 Herbal Medicine and the Aging Population 4
1.3 Herbal Medicines: Challenges and Regulations 4
1.3.1 International Diversity and National Policies 5
1.3.2 Quality, Safety, and Scientific Evidence 6
1.4 Research Needs 7
1.5 Conclusions 8
Acknowledgments 9
References 9

1.1 HERBAL MEDICINE: A GROWING FIELD WITH A LONG TRADITION

Traditional medicine is "the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness" (World Health Organization, http://www.who.int/topics/traditional_medicine/en/). There are many different systems of traditional medicine, and the philosophy and practices of each are influenced by the prevailing conditions, environment, and geographic area within which it first evolved (WHO 2005), however, a common philosophy is a holistic approach to life, equilibrium of the mind, body, and the environment, and an emphasis on health rather than on disease. Generally, the focus is on the overall condition of the individual, rather than on the particular ailment or disease from which the patient is suffering, and the use of herbs is a core part of all systems of traditional medicine (Engebretson 2002; Conboy et al. 2007; Rishton 2008; Schmidt et al. 2008).

Traditional Chinese medicine (TCM) is an important example of how ancient and accumulated knowledge is applied in a holistic approach in present day health care. TCM has a history of more than 3000 years (Xutian, Zhang, and Louise 2009). The book *The Devine Farmer's Classic of Herbalism* was compiled about 2000 years ago in China and is the oldest known herbal text in the world, though the accumulated and methodically collected information on herbs has been developed into various herbal pharmacopoeias and many monographs on individual herbs exist.

Diagnosis and treatment are based on a holistic view of the patient and the patient's symptoms, expressed in terms of the balance of yin and yang. Yin represents the earth, cold, and femininity, whereas yang represents the sky, heat, and masculinity. The actions of yin and yang influence the interactions of the five elements composing the universe: metal, wood, water, fire, and earth.

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TCM practitioners seek to control the yin and yang levels through 12 meridians, which bring and channel energy (*Q*i) through the body. TCM is a growing practice around the world and is used for promoting health as well as for preventing and curing diseases. TCM encompasses a range of practices, but herbal medicine is a core part (Engebretson 2002; Nestler 2002; Schmidt et al. 2008; Xutian, Zhang, and Louise 2009). Three of the top-selling botanical products, namely *Ginkgo biloba, Allium sativum* (garlic), and *Panax ginseng*, can be traced back to origins in TCM and are today used to treat various diseases (Li, Jiang, and Chen 2008; Xutian, Zhang, and Louise 2009).

Over the past 100 years, the development and mass production of chemically synthesized drugs have revolutionized health care in most parts of the word. However, large sections of the population in developing countries still rely on traditional practitioners and herbal medicines for their primary care. In Africa up to 90% and in India 70% of the population depend on traditional medicine to help meet their health care needs. In China, traditional medicine accounts for around 40% of all health care delivered and more than 90% of general hospitals in China have units for traditional medicine (WHO 2005). However, use of traditional medicine is not limited to developing countries, and during the past two decades public interest in natural therapies has increased greatly in industrialized countries, with expanding use of ethnobotanicals. In the United States, in 2007, about 38% of adults and 12% of children were using some form of traditional medicine (Ernst, Schmidt, and Wider 2005; Barnes, Bloom, and Nahin 2008). According to a survey by the National Center for Complementary and Alternative Medicine (Barnes, Bloom, and Nahin 2008), herbal therapy or the usage of natural products other than vitamins and minerals was the most commonly used alternative medicine (18.9%) when all use of prayer was excluded. A survey conducted in Hong Kong in 2003 reported that 40% of the subjects surveyed showed marked faith in TCM compared with Western medicine (Chan et al. 2003). In a survey of 21,923 adults in the United States, 12.8% took at least one herbal supplement (Harrison et al. 2004) and in another survey (Qato et al. 2008), 42% of respondents used dietary or nutritional supplements, with multivitamins and minerals most commonly used, followed by saw palmetto, flax, garlic, and Ginkgo, at the time of the interview.

The most common reasons for using traditional medicine are that it is more affordable, more closely corresponds to the patient's ideology, allays concerns about the adverse effects of chemical (synthetic) medicines, satisfies a desire for more personalized health care, and allows greater public access to health information. The major use of herbal medicines is for health promotion and therapy for chronic, as opposed to life-threatening, conditions. However, usage of traditional remedies increases when conventional medicine is ineffective in the treatment of disease, such as in advanced cancer and in the face of new infectious diseases. Furthermore, traditional medicines are widely perceived as natural and safe, that is, not toxic. This is not necessarily true, especially when herbs are taken with prescription drugs, over-the-counter medications, or other herbs, as is very common (Canter and Ernst 2004; Qato et al. 2008; Loya, Gonzalez-Stuart, and Rivera 2009; Cohen and Ernst 2010).

Regardless of why an individual uses it, traditional medicine provides an important health care service whether people have physical or financial access to allopathic medicine, and it is a flourishing global commercial enterprise (Engebretson 2002; Conboy et al. 2007; Evans et al. 2007). In 1990, expenditure associated with "alternative" therapy in the United States was estimated to be US$13.7 billion. This had doubled by the year 1997, with herbal medicines growing faster than any other alternative therapy (Eisenberg et al. 1998). In Australia, Canada, and the United Kingdom, annual expenditure on traditional medicine is estimated to be US$80 million, US$1 billion, and US$2.3 billion, respectively. These figures reflect the incorporation of herbal and other forms of traditional medicine into many health care systems and its inclusion in the medical training of doctors in many parts of the developed world.

The total commercial value of the ethnobotanicals market cannot be ignored. For example, in 1995, the total turnover of nonprescription-bound herbal medicines in pharmacies was equal to almost 30% of the total turnover of nonprescription-bound medicines in Germany, and in the United States, the annual retail sales of herbal products was estimated to be US$5.1 billion. In India,

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herbal medicine is a common practice, and about 960 plant species are used by the Indian herbal industry, of which 178 are of a high volume, exceeding 100 metric tons per year (Sahoo 2010). In China, the total value of herbal medicine manufactured in 1995 reached 17.6 billion Chinese yuan (approximately US$2.5 billion; Eisenberg et al. 1998; WHO 2001). This trend has continued, and annual revenues in Western Europe reached US$5 billion in 2003–2004 (De Smet 2005). In China, sales of herbal products totaled US$14 billion in 2005, and revenue from herbal medicines in Brazil was US$160 million in 2007 (World Health Organization; http://www.who.int/topics/traditional_medicine/en/). It is estimated that the annual worldwide market for these products approached US$60 billion (Tilburt and Kaptchuk 2008).

Currently, herbs are applied to the treatment of chronic and acute conditions and various ailments and problems such as cardiovascular disease, prostate problems, depression, inflammation, and to boost the immune system, to name but a few. In China, in 2003, traditional herbal medicines played a prominent role in the strategy to contain and treat severe acute respiratory syndrome (SARS), and in Africa, a traditional herbal medicine, the Africa flower, has been used for decades to treat wasting symptoms associated with HIV (De Smet 2005; Tilburt and Kaptchuk 2008). Herbal medicines are also very common in Europe, with Germany and France leading in over-the-counter sales among European countries, and in most developed countries, one can find essential oils, herbal extracts, or herbal teas being sold in pharmacies with conventional drugs.

Herbs and plants can be processed and can be taken in different ways and forms, and they include the whole herb, teas, syrup, essential oils, ointments, salves, rubs, capsules, and tablets that contain a ground or powdered form of a raw herb or its dried extract. Plants and herbs extract vary in the solvent used for extraction, temperature, and extraction time, and include alcoholic extracts (tinctures), vinegars (acetic acid extracts), hot water extract (tisanes), long-term boiled extract, usually roots or bark (decoctions), and cold infusion of plants (macerates). There is no standardization, and components of an herbal extract or a product are likely to vary significantly between batches and producers.

Plants are rich in a variety of compounds. Many are secondary metabolites and include aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins (Hartmann 2007; Jenke-Kodama, Müller, and Dittmann 2008). Many of these compounds have antioxidant properties (see Chapter 2 on antioxidants in herbs and spices). Ethnobotanicals are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds (Li and Vederas 2009). About 200 years ago, the first pharmacologically active pure compound, morphine, was produced from opium extracted from seeds pods of the poppy *Papaver somniferum*. This discovery showed that drugs from plants can be purified and administered in precise dosages regardless of the source or age of the material (Rousseaux and Schachter 2003; Hartmann 2007). This approach was enhanced by the discovery of penicillin (Li and Vederas 2009). With this continued trend, products from plants and natural sources (such as fungi and marine microorganisms) or analogs inspired by them have contributed greatly to the commercial drug preparations today. Examples include antibiotics (e.g., penicillin, erythromycin); the cardiac stimulant digoxin from foxglove (*Digitalis purpurea*); salicylic acid, a precursor of aspirin, derived from willow bark (*Salix spp.*); reserpine, an antipsychotic and antihypertensive drug from *Rauwolfia spp.*; and antimalarials such as quinine from *Cinchona* bark and lipid-lowering agents (e.g., lovastatin) from a fungus (Rishton 2008; Schmidt et al. 2008; Li and Vederas 2009). Also, more than 60% of cancer therapeutics on the market or in testing are based on natural products. Of 177 drugs approved worldwide for treatment of cancer, more than 70% are based on natural products or mimetics, many of which are improved with combinatorial chemistry. Cancer therapeutics from plants include paclitaxel, isolated from the Pacific yew tree; camptothecin, derived from the Chinese "happy tree" *Camptotheca acuminata* and used to prepare irinotecan and topotecan; and combretastatin, derived from the South African bush willow (Brower 2008). It is also estimated that about 25% of the drugs prescribed worldwide are derived from plants, and 121 such active compounds are in use (Sahoo et al. 2010). Between 2005 and 2007, 13 drugs derived

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from natural products were approved in the United States. More than 100 natural product–based drugs are in clinical studies (Li and Vederas 2009), and of the total 252 drugs in the World Health Organization's (WHO) essential medicine list, 11% are exclusively of plant origin (Sahoo et al. 2010).

1.2 HERBAL MEDICINE AND THE AGING POPULATION

Average life expectancy at birth has increased from around 41 years in the early 1950s to approaching 80 years in many developed countries. Consequently, the percentage of elderly people (65 years and above) in our populations is increasing. The graying of our populations brings an increasing burden of chronic age-related disease and dependency. Aging is associated with a progressive decline in physiological function and an increased risk of pathological changes leading to cancer, cardiovascular disease, dementia, diabetes, osteoporosis, and so on. Lifestyle factors such as nutrition or exercise play an important role in determining the quality and duration of healthy life and in the treatment of chronic diseases (Bozzetti 2003; Benzie and Wachtel-Galor 2009, 2010). It is most likely that there is no one cause of aging, and different theories of aging have been suggested over the years. Genetic factors are undoubtedly important, but among all the metabolic theories of aging, the oxidative stress theory is the most generally supported theory (Harman 1992; Beckman and Ames 1998). This theory postulates that aging is caused by accumulation of irreversible, oxidation-induced damage (oxidative stress) resulting from the interaction of reactive oxygen species with the DNA, lipid, and protein components of cells. However, even if the aging process itself is found to be unrelated to oxidative stress, highly prevalent chronic age-related diseases all have increased oxidative stress (Holmes, Bernstein, and Bernstein 1992; Beckman and Ames 1998; Finkel and Holbrook 2000; Rajah et al. 2009). Antioxidants in herbs may contribute at least part of their reputed therapeutic effects (Balsano and Alisi 2009; Tang and Halliwell 2010).

With the growing popularity of herbal medicine, the "traditional" ways of identification and preparation of herbs need to be replaced with more accurate and reproducible methods (see Chapter 20) so as to ensure the quality, safety, and consistency of the product. Given the market value, potential toxicity and increasing consumer demand, particularly in the sick and elderly members of our populations, regulation of production and marketing of herbal supplements and medicines require attention.

1.3 HERBAL MEDICINES: CHALLENGES AND REGULATIONS

WHO has recognized the important contribution of traditional medicine to provide essential care (World Health Organization, http://www.who.int/topics/traditional_medicine/en/). In 1989, the U.S. Congress established the Office of Alternative Medicine within the National Institutes of Health to encourage scientific research in the field of traditional medicine (http://nccam.nih.gov, last access: November 5, 2010), and the European Scientific Cooperative on Phytotherapy (ESCOP) was founded in 1989 with the aim of advancing the scientific status and harmonization of phytomedicines at the European level (www.escop.com, last access: November 5, 2010). This led to an increase in investment in the evaluation of herbal medicines. In the United States, the National Center for Complementary and Alternative Medicine at the National Institutes of Health spent approximately US$33 million on herbal medicines in the fiscal year 2005; in 2004, the National Canadian Institute committed nearly US$89 million for studying a range of traditional therapies. While this scale of investment is low compared to the total research and development expenses of the pharmaceutical industry, it nevertheless reflects genuine public, industry, and governmental interest in this area (Li and Vederas 2009).

With tremendous expansion in the interest in and use of traditional medicines worldwide, two main areas of concern arise that bring major challenges. These are international diversity and national policies regarding the regulation of the production and use of herbs (and other complemen-

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tary medicines) and their quality, safety, and scientific evidence in relation to health claims (WHO 2005; Sahoo et al. 2008).

1.3.1 International Diversity and National Policies

The diversity among countries with the long history and holistic approach of herbal medicines makes evaluating and regulating them very challenging. In addition, there are a great number of different herbs used. Legislative criteria to establish traditionally used herbal medicines as part of approved health care therapies faces several difficulties. In a survey conducted across 129 countries, WHO reported the following issues regarding herbal medicines: lack of research data, appropriate mechanisms for control of herbal medicines, education and training, expertise within the national health authorities and control agency, information sharing, safety monitoring, and methods to evaluate their safety and efficacy. The support needed from different countries includes information sharing on regulatory issues, workshops on herbal medicines safety monitoring, general guidelines on research and evaluation of herbal medicines, provision of databases, herbal medicine regulation workshops, and international meetings.

National policies are the basis for defining the role of traditional medicines in national health care programs, ensuring that the necessary regulatory and legal mechanisms are established for promoting and maintaining good practice, assuring the authenticity, safety, and efficacy of traditional medicines and therapies, and providing equitable access to health care resources and their resource information (WHO 2005). Another fundamental requirement is harmonization of the market for herbal medicines for industry, health professionals, and consumers (Mahady 2001). Herbal medicines are generally sold as food supplements, but a common regulatory framework does not exist in different countries. As a result, information on clinical indications for their use, efficacy, and safety are influenced by the traditional experience available in each place. A brief outline of the legislation in United States, Canada, and Europe is given in this section, and could be used to guide the legal aspects of the herbal medicine industry in other countries.

In the United States, under the Dietary Supplement Health and Education Act (DSHEA) of 1994, any herb, botanical and natural concentrate, metabolite and constituent of extract, is classified as a dietary supplement. Dietary supplements do not need approval from the Food and Drug Administration (FDA) before they are marketed (FDA 2010). Under DSHEA, herbal medicines, which are classified as dietary supplements, are presumed safe, and the FDA does not have the authority to require them to be approved for safety and efficacy before they enter the market, which is the case for drugs. This means that the manufacturer of the herbal medicine is responsible for determining that the dietary supplements manufactured or distributed are indeed safe and that any representations or claims made about them are sustained by adequate evidence to show that they are not false or misleading. However, a dietary supplement manufacturer or distributor of a supplement with a "new dietary ingredient," that is, an ingredient that was not marketed in the United States before October 1994, may be required to go through premarket review for safety data and other information. Also, all domestic and foreign companies that manufacture package labels or hold dietary supplements must follow the FDA's current good manufacturing practice (GMP) regulations, which outline procedures for ensuring the quality of supplements intended for sale (FDA 2010; Gao 2010). Regarding contamination, the FDA has not issued any regulations addressing safe or unsafe levels of contaminants in dietary supplements but has set certain advisory levels in other foods (FDA 2010; Gao 2010). A product being sold as an herbal supplement (dietary supplement) in the United States cannot suggest on its label or in any of its packaging that it can diagnose, treat, prevent, or cure a specific disease or condition without specific approval from the FDA. A claim also cannot suggest an effect on an abnormal condition associated with a natural state or process, such as aging (FDA 2010; Gao 2010).

In Canada, herbal remedies must comply with the Natural Health Products Regulations (Health Canada 2003). According to these regulations, all natural products require a product license before

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they can be sold in Canada. In order to be granted a license, detailed information on the medicinal ingredients, source, potency, nonmedicinal ingredients, and recommended use needs to be furnished. Once a product has been granted a license, it will bear the license number and follow standard labeling requirements to ensure that consumers can make informed choices. A site license is also needed for those who manufacture, pack, label, and import herbal medicines. In addition, GMPs must be employed to ensure product safety and quality. This requires that appropriate standards and practices regarding the manufacture, storage, handling, and distribution of natural health products be met. The GMPs are designed to be outcome based, ensuring safe and high-quality products, while giving the flexibility to implement quality control systems appropriate to the product line and business. Product license holders are required to monitor all adverse reactions associated with their product and report serious adverse reactions to the Canadian Department of Health.

In Europe, the European Directive 2004/24/EC released in 2004 by the European Parliament and by the Council of Europe provides the guidelines for the use of herbal medicines (Calapai 2008). The directive establishes that herbal medicines released on the market need authorization by the national regulatory authorities of each European country and that these products must have a recognized level of safety and efficacy (Calapai 2008). The registration of herbal medicinal products needs sufficient evidence for the medicinal use of the product throughout a period of at least 30 years in the European Union (EU), at least 15 years within the EU, and 15 years elsewhere for products from outside the EU. With regard to the manufacturing of these products and their quality, products must fulfill the same requirements as applications for a marketing authorization. Information is based on the availability of modern science–based public monographs in the *European Pharmacopeia* and their equivalents developed by the pharmaceutical industry. The standards put forward allow not only to define the quality of products but also to eliminate harmful compounds, adulteration, and contamination. Within the EU, a number of committees were set up to attempt and standardize the information and guidelines related to herbal medicines. A variety of materials has been produced, such as monographs on herbs and preparations, guidelines on good agricultural and collection practice for starting materials of herbal origin, and guidelines on the standardization of applications and setting up pragmatic approaches for identification and quantitative determination of herbal preparations and their complex compositions (Routledge 2008; Vlietinck, Pieters, and Apers 2009).

1.3.2 Quality, Safety, and Scientific Evidence

Herbal medicine has been commonly used over the years for treatment and prevention of diseases and health promotion as well as for enhancement of the span and quality of life. However, there is a lack of a systematic approach to assess their safety and effectiveness. The holistic approach to health care makes herbal medicine very attractive to many people, but it also makes scientific evaluation very challenging because so many factors must be taken into account. Herbal medicines are in widespread use and although many believe herbal medicines are safe, they are often used in combination and are drawn from plant sources with their own variability in species, growing conditions, and biologically active constituents. Herbal extracts may be contaminated, adulterated, and may contain toxic compounds. The quality control of herbal medicines has a direct impact on their safety and efficacy (Ernst, Schmidt, and Wider 2005; Ribnicky et al. 2008). But, there is little data on the composition and quality of most herbal medicines not only due to lack of adequate policies or government requirements but also due to a lack of adequate or accepted research methodology for evaluating traditional medicines (WHO 2001; Kantor 2009). In addition, there is very little research on whole herbal mixtures because the drug approval process does not accommodate undifferentiated mixtures of natural chemicals. To isolate each active ingredient from each herb would be immensely time-consuming at a high cost, making it not cost-effective for manufacturers (Richter 2003).

Another problem is that despite the popularity of botanical dietary and herbal supplements, some herbal products on the market are likely to be of low quality and suspect efficacy, even if the herb

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has been shown to have an effect in controlled studies using high-quality product. There is a belief that herbs, as natural products, are inherently safe without side effects and that efficacy can be obtained over a wide range of doses. Although herbs may well have undesirable side effects, there are no set "doses," and herb–drug or herb–herb interactions are possible.

A major hypothetical advantage of botanicals over conventional single-component drugs is the presence of multiple active compounds that together can provide a potentiating effect that may not be achievable by any single compound. This advantage presents a unique challenge for the separation and identification of active constituents. Compounds that are identified by activity-guided fractionation must be tested in appropriate animal models to confirm in vivo activity. Ideally, the composition of the total botanical extract must be standardized and free of any potential hazards, and plants should be grown specifically for the production of botanical extracts under controlled conditions and originate from a characterized and uniform genetic source with a taxonomic record of the genus, species, and cultivar or other additional identifiers. Records should be maintained for the source of the seed, locations and conditions of cultivation, and exposure to possible chemical treatments such as pesticides. Because the environment can significantly affect phytochemical profiles and the efficacy of the botanical end product, botanical extracts can vary from year to year and may be significantly affected by temperature, drought, or flood as well as by geographic location. Therefore, biochemical profiling must be used to ensure that a consistent material is used to produce a botanical. The concentration step can also be challenging, and the process to concentrate active compounds to a sufficient level can negatively affect their solubility and bioavailability. Therefore, improving efficacy by increasing concentration can be counterproductive, and the use of solubilizers and bioenhancers needs to be considered just as for drugs (Ribnicky et al. 2008). However, there are major challenges to achieving this.

Although in theory botanicals should be well characterized and herbal supplements should be produced to the same quality standards as drugs, the situation in practice is very different from that of a pure drug. Herbs contain multiple compounds, many of which may not be identified and often there is no identifier component, and chemical fingerprinting is in its early stages and is lacking for virtually all herbs (see Chapter 20). This makes standardization of botanicals difficult, although some can be produced to contain a standardized amount of a key component or class of components, such as ginsenosides for ginseng products or anthocyanins for bilberry products (see chapter 4 on bilberry and chapter 8 on ginseng in this volume). However, even when such key compounds have been identified and a standard content is agreed or suggested, there is no guarantee that individual commercial products will contain this.

Another interesting point to consider is that herbal materials for commercial products are collected from wild plant populations and cultivated medicinal plants. The expanding herbal product market could drive overharvesting of plants and threaten biodiversity. Poorly managed collection and cultivation practices could lead to the extinction of endangered plant species and the destruction of natural resources. It has been suggested that 15,000 of 50,000–70,000 medicinal plant species are threatened with extinction (Brower 2008). The efforts of the Botanic Gardens Conservation International are central to the preservation of both plant populations and knowledge on how to prepare and use herbs for medicinal purposes (Brower 2008; Li and Vederas 2009).

1.4 RESEARCH NEEDS

Research needs in the field of herbal medicines are huge, but are balanced by the potential health benefits and the enormous size of the market. Research into the quality, safety, molecular effects, and clinical efficacy of the numerous herbs in common usage is needed. Newly emerging scientific techniques and approaches, many of which are mentioned in this book, provide the required testing platform for this. Genomic testing and chemical fingerprinting techniques using hyphenated testing platforms are now available for definitive authentication and quality control of herbal products. They should be regulated to be used to safeguard consumers, but questions of efficacy will

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remain unless and until adequate amounts of scientific evidence accumulate from experimental and controlled human trials (Giordano, Engebretson, and Garcia 2005; Evans 2008; Tilburt and Kaptchuk 2008). Evidence for the potential protective effects of selected herbs is generally based on experiments demonstrating a biological activity in a relevant in vitro bioassay or experiments using animal models. In some cases, this is supported by both epidemiological studies and a limited number of intervention experiments in humans (WHO 2001). In general, international research on traditional herbal medicines should be subject to the same ethical requirements as all research related to human subjects, with the information shared between different countries. This should include collaborative partnership, social value, scientific validity, fair subject selection, favorable risk-benefit ratio, independent review, informed consent, and respect for the subjects (Giordano, Engebretson, and Garcia 2005; Tiburt and Kaptchuk 2008). However, the logistics, time, and cost of performing large, controlled human studies on the clinical effectiveness of an herb are prohibitive, especially if the focus is on health promotion. Therefore, there is an urgent need to develop new biomarkers that more clearly relate to health (and disease) outcomes. Predictor biomarkers and subtle but detectable signs of early cellular change that are mapped to the onset of specific diseases are needed.

Research is needed also to meet the challenges of identifying the active compounds in the plants, and there should be research-based evidence on whether whole herbs or extracted compounds are better. The issue of herb–herb and herb–drug interactions is also an important one that requires increased awareness and study, as polypharmacy and polyherbacy are common (Canter and Ernst 2004; Qato et al. 2008; Loya, Gonzalez-Stuart, and Rivera 2009; Cohen and Ernst 2010). The use of new technologies, such as nanotechnology and novel emulsification methods, in the formulation of herbal products, will likely affect bioavailability and the efficacy of herbal components, and this also needs study. Smart screening methods and metabolic engineering offer exciting technologies for new natural product drug discovery. Advances in rapid genetic sequencing, coupled with manipulation of biosynthetic pathways, may provide a vast resource for the future discovery of pharmaceutical agents (Li and Vederas 2009). This can lead to reinvestigation of some agents that failed earlier trials and can be restudied and redesigned using new technologies to determine whether they can be modified for better efficacy and fewer side effects. For example, maytansine isolated in the early 1970s from the Ethiopian plant *Maytenus serrata*, looked promising in preclinical testing but was dropped in the early 1980s from further study when it did not translate into efficacy in clinical trials; later, scientists isolated related compounds, ansamitocins, from a microbial source. A derivative of maytansine, DM1, has been conjugated with a monoclonal antibody and is now in trials for prostate cancer (Brower 2008).

1.5 CONCLUSIONS

Plants, herbs, and ethnobotanicals have been used since the early days of humankind and are still used throughout the world for health promotion and treatment of disease. Plants and natural sources form the basis of today's modern medicine and contribute largely to the commercial drug preparations manufactured today. About 25% of drugs prescribed worldwide are derived from plants. Still, herbs, rather than drugs, are often used in health care. For some, herbal medicine is their preferred method of treatment. For others, herbs are used as adjunct therapy to conventional pharmaceuticals. However, in many developing societies, traditional medicine of which herbal medicine is a core part is the only system of health care available or affordable. Regardless of the reason, those using herbal medicines should be assured that the products they are buying are safe and contain what they are supposed to, whether this is a particular herb or a particular amount of a specific herbal component. Consumers should also be given science-based information on dosage, contraindications, and efficacy. To achieve this, global harmonization of legislation is needed to guide the responsible production and marketing of herbal medicines. If sufficient scientific evidence of benefit is available

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for an herb, then such legislation should allow for this to be used appropriately to promote the use of that herb so that these benefits can be realized for the promotion of public health and the treatment of disease.

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2 Antioxidants in Herbs and Spices
Roles in Oxidative Stress and Redox Signaling

Ingvild Paur, Monica H. Carlsen, Bente Lise Halvorsen, and Rune Blomhoff

CONTENTS

2.1 Introduction 11
2.2 Reactive Oxygen Species: Complex Roles in Normal Physiology 12
2.2.1 Role of Reactive Oxygen Species in Cell Signaling 12
2.2.2 Production of Reactive Oxygen Species 13
2.2.3 How Are Reactive Oxygen Species Perceived? 13
2.3 Examples of the Dual Roles of Reactive Oxygen Species in Pathologies 14
2.3.1 Reactive Oxygen Species in Rheumatoid Arthritis 14
2.3.2 Exploitation of Reactive Oxygen Species Signaling by Cancer Cells to Survive and Grow 14
2.3.3 Positive Role of Reactive Oxygen Species in Exercise 14
2.4 Is There a Role of Dietary Antioxidants in Oxidative Stress? 15
2.5 Total Antioxidant Content of Foods and Drinks: Largest Density of Antioxidants Contained in Spices and Herbs 15
2.6 Total Amounts of Antioxidants in Herbs and Spices 17
2.7 Research Needs: Potential Health Effects of Dietary Antioxidants 19
2.8 Conclusions 33
References 33

2.1 INTRODUCTION

Herbs and spices are traditionally defined as any part of a plant that is used in the diet for their aromatic properties with no or low nutritional value (Davidson 1999; Hacskaylo 1996; Smith and Winder 1996). However, more recently, herbs and spices have been identified as sources of various phytochemicals, many of which possess powerful antioxidant activity (Larson 1988; Velioglu et al. 1998; Kähkönen et al. 1999; Dragland et al. 2003). Thus, herbs and spices may have a role in antioxidant defense and redox signaling.

In the scientific and public literature, antioxidants and oxidative stress are very often presented in a far too simple manner. First, reactive oxygen species (ROS) are lumped together as one functional entity. However, there are many different ROS that have separate and essential roles in normal physiology and are required for a variety of normal processes. These physiological functions are not overlapping, and the different ROS that exist cannot, in general, replace each other. Different ROS are also strongly implicated in the etiology of diseases such as cancers, atherosclerosis,

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neurodegenerative diseases, infections, chronic inflammatory diseases, diabetes, and autoimmune diseases (Gutteridge and Halliwell 2000; McCord 2000). Second, the various antioxidants that exist are often viewed as a single functional entity. However, the different endogenous antioxidants that are produced by the body (e.g., glutathione, thioredoxins, glutaredoxin, and different antioxidant enzymes) cannot, in general, replace each other. They have specific chemical and physiological characteristics that ensure all parts of the cells and the organs or tissues are protected against oxidative damage. Dietary antioxidants also exist in various forms, with polyphenols and carotenoids being the largest groups of compounds. These have different functions and are produced by plants to protect plant cells against oxidative damage (Halliwell 1996; Lindsay and Astley 2002).

Based on the complex nature of antioxidants and ROS, it would thus be extremely unlikely that a magic bullet with a high dose of one or a few particular antioxidants such as vitamin C, vitamin E, or β-carotene would protect all parts of the cells, organs, and tissues against oxidative damage and oxidative stress, at the same time without destroying any of the numerous normal and beneficial functions of ROS. Indeed, supplementation with antioxidants has often resulted in no effect or even adverse disease outcomes. Recently, several reviews and meta-analyses have concluded that there is now a strong body of evidence indicating that there is no beneficial effect for supplemental vitamin C, vitamin E, or β-carotene (Vivekananthan et al. 2003; Eidelman et al. 2004; Bjelakovic et al. 2007; Bjelakovic et al. 2008). An alternative and much more likely antioxidant strategy to test protection against oxidative stress and related diseases would be to test the potential beneficial effects of antioxidant-rich foods, since such foods typically contain a large combination of different antioxidants that are selected, through plant evolution, to protect every part of the plant cells against oxidative damage. This is especially relevant for herbs and spices. The aim of this chapter is to discuss the potential role of antioxidants in herbs and spices in normal physiology, oxidative stress, and related diseases. We begin with a brief introduction of ROS and their role in normal physiology and oxidative stress, and then present data that demonstrate herbs and spices are the most antioxidant-dense dietary source of antioxidants that has been described. We end the chapter with a discussion on the potential role of herb and spice antioxidants in oxidative stress.

2.2 REACTIVE OXYGEN SPECIES: COMPLEX ROLES IN NORMAL PHYSIOLOGY

ROS molecules are simply oxygen-containing molecules that are reactive. They can be divided into free-radical ROS and nonradical ROS. Free-radical ROS have unpaired electrons in their outer orbits; examples of such molecules are superoxide and hydroxyl radical. Nonradical ROS do not have unpaired electrons; however, these are chemically reactive and can be converted into free-radical ROS. One example of a nonradical ROS is hydrogen peroxide.

2.2.1 Role of Reactive Oxygen Species in Cell Signaling

To survive, cells must sense their immediate surroundings and change their activity according to their microenvironment. This is accomplished through cell signaling. A basic signaling pathway relays a signal through the cell by modulating the activities of proteins along the pathway. A "mediator" or "second messenger" is a molecule that promotes (or inhibits) a step in a signaling pathway. Functions of ROS have been described at different locations of signaling pathways. The ROS molecules have been described as the very first stimulus that starts the cascade of a signaling pathway, the "initiator," and also as the last step of a signaling pathway, the so-called effector. Furthermore, ROS can also be involved somewhere between the start and the end of the signaling pathway, either as the molecule that relays the signal itself or by promoting a step in the signaling pathway. In both cases, ROS can be seen as the mediator in the particular pathway (for review, see the work by Hancock [2009]). However, for ROS to function as signaling mediators, they should be produced where and when they are needed, sensed by some mechanism, and should be rapidly removed to stop the signal from being sustained.

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2.2.2 Production of Reactive Oxygen Species

ROS molecules are created during the reduction of oxygen to water. The addition of one electron to oxygen creates superoxide, whereas further reduction gives hydrogen peroxide. Production of ROS can also be a consequence of endogenous or exogenous stimuli, including ultraviolet (UV) radiation, chemotherapy, environmental toxins, and exercise (Blomhoff 2005). Deliberate production of ROS occurs in different cellular compartments from enzymes such as nicotinamide adenine dinucleotide phosphate (NADPH), oxidases (NOX and dual oxidase [DUOX]), nitric oxide (NO) synthase (NOS), xanthine oxidase, and from the electron transport chain of the mitochondria.

There are seven NADPH oxidases (i.e., NOX1 to NOX5 and DUOX1 and DUOX2). These are transmembrane proteins that produce superoxide or hydrogen peroxide. The oxidases NOX1 through 5 produce superoxide by the transfer of an electron through the membrane from NADPH to oxygen. The enzymes DUOX1–2 are calcium-dependent enzymes and produce hydrogen peroxide directly by virtue of a peroxide-like subunit located on the outer side of the membrane in addition to the transfer of an electron from NADPH. The enzymes further differ in their cellular compartmentalization, their upstream activators, and the associated subunits. Known inducers of NOX are growth factors, cytokines, and vitamin D (Brown and Griendling 2009; Chen et al. 2009; Leto et al. 2009).

Mitochondria have traditionally been thought to produce ROS only as an unwanted by-product of energy production in the electron transfer chain. However, deliberate ROS production also occurs from the mitochondria. This occurs at least partially by the inhibition of cytochrome *c* oxidase by NO leading to increased superoxide production without affecting energy production. Mitochondrial superoxide dismutase converts superoxide to hydrogen peroxide, which can cross the membrane and take part in cytosolic signaling (Brookes et al. 2002).

2.2.3 How Are Reactive Oxygen Species Perceived?

ROS can alter the production, stability, or function of proteins. The redox status may alter the activity of transcription factors in the nucleus. In general, the reduced transcription factor binds to deoxyribonucleic acid (DNA) and promotes transcription, whereas an oxidized transcription factor will not be able to bind to DNA and thus will not promote transcription. Furthermore, the stability of proteins can be affected by the oxidation of proteasomes. Oxidation of proteasomes may render them inactive and unable to degrade proteins, thus maintaining or increasing the level of proteins. Finally, the function of proteins and molecules can be modified through oxidation by the following three different strategies: (1) Proteins, such as thioredoxin, can be oxidized, resulting in alteration of the activity of the protein directly. (2) The oxidation targets a chaperone protein that usually inhibits protein activity; on oxidation, the protein can dissociate from its inhibitor and thus become active. (3) Phosphatases and kinases can be targets for oxidation, and subsequently alter the activity of proteins through posttranslational modifications. Protein tyrosine phosphatases are often inactivated by oxidation, whereas the different kinases are generally activated. The most common targets of oxidation are cysteine residues, but other amino acids like tyrosine and methionine can also be targets. Further oxidation of target molecules may lead to irreversible oxidative damage. Oxidized cysteine residues can be protected from further oxidation by the formation of thiol bridges.

In phagocytosis, ROS is an effector that is produced by NOX2 inside the phagosome to kill phagocytozed microbes. Targets of ROS in signaling pathways include transcription factors, redox sensors, and phosphatases/kinases. Transcription factors include Nrf2, NF-kB, p53, AP-1, cyclic adenosine monophosphate response element binding (CREB), HomeoboxB5, and nuclear receptors such as the estrogen receptor. Redox sensors include thioredoxin, glutharedoxins, peroxiredoxins, glutathione, and redox effector factor-1 (Ref-1), whereas phosphatases/kinases include PTP, Akt, JNK, ERK, Src, and CDK (Brown and Gutteridge 2007; Halliwell and Gutteridge 2007; Kamata et al. 2005; Kiley and Storz 2004; Trachootham et al. 2008). To counteract the possible toxic effects of ROS and enable ROS to act in signaling pathways, intricate systems of antioxidants have evolved.

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This system is highly specialized in terms of both removal of specific ROS and compartmentalization of the different antioxidants. For a discussion of various antioxidant systems, please see the excellent book by Halliwell and Gutteridge (2007).

2.3 EXAMPLES OF THE DUAL ROLES OF REACTIVE OXYGEN SPECIES IN PATHOLOGIES

Increased levels of ROS have been implicated in numerous chronic degenerative diseases such as cardiovascular diseases, cancers, type 2 diabetes, neurodegenerative diseases, obesity, and hypertension. However, ROS may have dual roles in many pathologies.

2.3.1 Reactive Oxygen Species in Rheumatoid Arthritis

Dual roles of ROS have been found in many types of autoimmune diseases. Most often, the focus was on lowering the levels of ROS as a treatment in diseases such as rheumatoid arthritis (Hultqvist et al. 2009). As NOX2 has been found to produce ROS in rheumatoid arthritis, it would, therefore, be a natural target for therapy. In a murine model of rheumatoid arthritis, mice with dysfunctional NOX2 were found to have decreased ROS production; however, these mice had increased rather than decreased symptoms of rheumatoid arthritis. These mice had more active T cells, and that this increased T-cell activity was due to the dysfunction of NOX2 in macrophages, which rendered the macrophages unable to downregulate T-cell activity. By restoring ROS signaling in the macrophages, the altered T-cell activation was reversed and the increased rheumatoid arthritis symptoms were decreased (Hultqvist et al. 2004; Gelderman et al. 2007).

2.3.2 Exploitation of Reactive Oxygen Species Signaling by Cancer Cells to Survive and Grow

Normal cells have a low level of ROS. Increased ROS, for example, due to inflammation or environmental factors, are generally thought to increase mutations in DNA and thereby risk of cancer. However, the increased level of ROS in cancer cells is balanced by an increased defense against ROS so that the cell does not exceed the ROS threshold for cell death. The increase in ROS leads to activation of signaling pathways that favor cell growth, migration, and proliferation. Furthermore, many cancer therapies (e.g., radiation, chemotherapy) induce massive amounts of ROS that exceed the ROS threshold and induce cancer cell death (reviewed by Trachootham, Alexandre, and Huang 2009). Thus, although antioxidants may theoretically prevent transformation of normal cells to cancerous cells, they may theoretically also lower the efficacy of cancer treatment.

2.3.3 Positive Role of Reactive Oxygen Species in Exercise

During exercise, several adaptive responses occur that are related to the increased level of ROS production via mitochondria. These adaptations include increased antioxidant defense, increased insulin sensitivity in muscle, and biogenesis of mitochondria. Thus, physical activity and exercise decreases the risk of several diseases, although exercise is known to induce the production of ROS. A study by Ristow and collaborators (2009) shed new light on the effect of exercise on ROS production. In their clinical trial, subjects were divided into previously trained or untrained individuals, and these two groups were randomized to consume either high doses of vitamin C and E supplements or placebo during an exercise regimen. Exercise was found to increase ROS, induce ROS defense, and insulin sensitivity. However, these changes were not found in those subjects who had consumed vitamin C and E supplements. Furthermore, these differences were most evident in the previously untrained subjects (Ristow et al. 2009.) Thus, these data suggest that adaptive responses to ROS are an important mechanism that mediates the beneficial effects of exercise.

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2.4 IS THERE A ROLE OF DIETARY ANTIOXIDANTSIN OXIDATIVE STRESS?

Based on the dual role of ROS described in Section 2.3 and the large variety of ROS and mechanisms involved, it is clear that a beneficial effect of a large intake of one single antioxidant (such as high-dose vitamin C, vitamin E, or β-carotene supplement) would not be expected. An alternative and much more likely strategy would be to test the potential beneficial effects of antioxidant-rich foods, since such foods typically contain a large combination of different antioxidants, which are selected through plant evolution to protect every part of the plant cells against oxidative damage. Moreover, this "package" of antioxidants with different functions is also present in much lower doses than those that are typically used in antioxidant supplements. Thus, we suggest that dietary antioxidants taken in their usual form of food may decrease risk of chronic diseases without compromising the normal functions of ROS (Blomhoff 2005).

There are numerous antioxidants in dietary plants. Carotenoids are ubiquitous in the plant kingdom, and as many as 1000 naturally occurring variants have been identified. At least 60 carotenoids occur in the fruits and vegetables commonly consumed by humans (Lindsay and Astley 2002). Besides the pro-vitamin A carotenoids, α- and β-carotene, and β-cryptoxanthin, lycopene and the hydroxy carotenoids (xanthophylls) lutein and zeaxanthin are the main carotenoids present in the diet. Their major role in plants is related to light harvesting as auxiliary components and quenching of excited molecules, such as singlet oxygen, that might be formed during photosynthesis. Phenolic compounds are also ubiquitous in dietary plants (Lindsay and Astley 2002). They are synthesized in large varieties, and belong to several molecular families, such as benzoic acid derivatives, flavonoids, proanthocyanidins, stilbenes, coumarins, lignans, and lignins. Over 8000 plant phenols have been isolated. Plant phenols are antioxidants by virtue of the hydrogen-donating properties of the phenolic hydroxyl groups.

We hypothesize that antioxidant-rich foods may be beneficial and provide a balanced combination of a variety of antioxidants in appropriate doses that would protect against excessive oxidative stress and oxidative damage without disturbing the normal role of ROS. In order to test this hypothesis, we first need to identify antioxidant-rich foods, that is, foods that contain relatively large amounts of total antioxidants. Therefore, we perform a systematic screening of the total antioxidant content (Benzie and Strain 1996) of more than 3500 foods (Halvorsen et al. 2002; Halvorsen et al. 2006; Carlsen et al. 2010). This novel and unique antioxidant food table enables us to calculate the total antioxidant content of complex diets, identify and rank potentially good sources of antioxidants, and provide the research community with data on the relative antioxidant capacity of a wide range of foods.

There is not necessarily a direct relationship between the antioxidant content of a food sample consumed and the subsequent antioxidant activity in the target cell. Factors influencing the bioavailability of phytochemical antioxidants include the food matrix and food preparation methods, as well as absorption, metabolism, and catabolism. With the present study, food samples with high antioxidant content are identified, but further investigation into each individual food is needed to identify those samples that may have biological relevance and the mechanisms involved in antioxidant activity. Such studies, including mechanistic cell-culture and experimental animal research, preclinical studies on bioavailability and bioefficacy, as well as clinical trials, are in progress.

2.5 TOTAL ANTIOXIDANT CONTENT OF FOODS AND DRINKS: LARGEST DENSITY OF ANTIOXIDANTS CONTAINED IN SPICES AND HERBS

The results of our study show large variations both between different food categories and within each category; all the food categories contain products almost devoid of antioxidants (Table 2.1). Please refer to the antioxidant food table published as an electronic supplement to the paper by Carlsen et al. (2010) for the antioxidant results on all products analyzed. The updated database is available online at http://www.blomhoff.no (link to "Scientific Online Material").

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TABLE 2.1
Characteristics of the Antioxidant Food Table

	Antioxidant Content (mmol/100 g)							
	n	Mean	Median	Min	Max	25th Percentile	75th Percentile	90th Percentile
Plant-based foods*	1943	11.57	0.88	0.00	2897.11	0.27	4.11	24.30
Animal-based foods†	211	0.18	0.10	0.00	1.00	0.05	0.21	0.46
Mixed foods‡	854	0.91	0.31	0.00	18.52	0.14	0.68	1.50
Categories								
1. Berry products	119	9.86	3.34	0.06	261.53	1.90	6.31	37.08
2. Beverages	283	8.30	0.60	0.00	1347.83	0.15	2.37	3.64
3. Breakfast cereals	90	1.09	0.89	0.16	4.84	0.53	1.24	1.95
4. Chocolates/sweets	80	4.93	2.33	0.05	14.98	0.82	8.98	13.23
5. Dairy products	86	0.14	0.06	0.00	0.78	0.04	0.14	0.44
6. Desserts and cakes	134	0.45	0.20	0.00	4.10	0.09	0.52	1.04
7. Egg	12	0.04	0.04	0.00	0.16	0.01	0.06	0.14
8. Fats and oils	38	0.51	0.39	0.19	1.66	0.30	0.50	1.40
9. Fish and seafood	32	0.11	0.08	0.03	0.65	0.07	0.12	0.21
10. Fruit and fruit juices	278	1.25	0.69	0.03	55.52	0.31	1.21	2.36
11. Grain products	227	0.34	0.18	0.00	3.31	0.06	0.38	0.73
12. Herbal medicine	59	91.72	14.18	0.28	2897.11	5.66	39.67	120.18
13. Infant foods	52	0.77	0.12	0.02	18.52	0.06	0.43	1.17
14. Legumes	69	0.48	0.27	0.00	1.97	0.12	0.78	1.18
15. Meat products	31	0.31	0.32	0.00	0.85	0.11	0.46	0.57
16. Miscellaneous	44	0.77	0.15	0.00	15.54	0.03	0.41	1.70
17. Mixed food entrees	189	0.19	0.16	0.03	0.73	0.11	0.23	0.38
18. Nuts and seeds	90	4.57	0.76	0.03	33.29	0.44	5.08	15.83
19. Poultry products	50	0.23	0.15	0.05	1.00	0.12	0.23	0.59
20. Snacks, biscuits	66	0.58	0.61	0.00	1.17	0.36	0.77	0.97
21. Soups, sauces, etc.	251	0.63	0.41	0.00	4.67	0.25	0.68	1.27
22. Spices and herbs	425	29.02	11.30	0.08	465.32	4.16	35.25	74.97
23. Vegetable products	303	0.80	0.31	0.00	48.07	0.17	0.68	1.50
24. Supplements	131	98.58	3.27	0.00	1052.44	0.62	62.16	316.93

* Categories 1, 2, 3, 10, 11, 12, 14, 18, 22, 23.

† Categories 5, 7, 9, 15, 19.

‡ Categories 4, 6, 8, 13, 16, 17, 20, 21.

Source: The values are taken from Carlsen, M. H. et al. 2010. *Nutr J* 22 (epub, January 22 2010. doi: 10.1186/1475-2891-9-3)

Interestingly, the categories "spices and herbs" and "herbal/traditional plant medicine" include the most antioxidant-rich products analyzed in the study. The categories "berries and berry products," "fruit and fruit juices," "nuts and seeds," "breakfast cereals," "chocolate and sweets," "beverages," and "vegetables and vegetable products" include most of the common foods and beverages, which have medium to high antioxidant values. We find that plant-based foods are generally higher in antioxidant content than animal-based and mixed food products, with median antioxidant values of 0.88, 0.10, and 0.31 mmol/100 g, respectively. Furthermore, the 75th percentile of antioxidant-content threshold for plant-based foods is 4.11 mmol/100 g, compared to that of 0.21

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and 0.68 mmol/100 g for animal-based and mixed foods, respectively. The high mean value of plant-based foods is due to a minority of products with very high antioxidant values, found among plant medicines, spices, and herbs. Table 2.1 summarize results from the 24 food categories tested.

2.6 TOTAL AMOUNTS OF ANTIOXIDANTS IN HERBS AND SPICES

Herbal/traditional plant medicine is the most antioxidant-rich category in the present study and also the category with the largest variation between products (Table 2.2). Half of the products have antioxidant values above the 90th percentile of the complete food table and the mean and median values are 91.7 and 14.2 mmol/100 g, respectively. The 59 products included originate from India, Japan, Mexico, and Peru. Sangre de grado ("dragon's blood") from Peru has the highest antioxidant content of all the products in the database (2897.1 mmol/100 g). Other antioxidant-rich products are triphala, amalaki, and arjuna from India and goshuyu-tou, a traditional kampo medicine from

TABLE 2.2
Antioxidants in Herbal/Traditional Plant Medicine

Product	Manufacturer/Product Label/ Country of Origin	Procured in	Antioxidant Content (mmol/100 g)
Amalaki (amla), powder in capsule	The Himalaya Herbal Health Care	India	301.14
Angelicae radix	Tsumura Pharmaceutical Company, Japan	Japan	2.96
Arjuna, powder in capsule	The Himalaya Herbal Health Care	India	146.95
Arnica (*Arnica montana*), flower and seeds, dried	Mexico	Mexico	36.28
Arnica (*Arnica montana*), leaves	Mexico	Mexico	3.72
Astragali radix	Tsumura Pharmaceutical Company, Japan	Japan	4.87
Atractylodis lanceae rhizoma	Tsumura Pharmaceutical Company, Japan	Japan	7.37
Aurantii nobilis pericarpium	Tsumura Pharmaceutical Company, Japan	Japan	17.48
AyurSlim, powder in capsule	The Himalaya Herbal Health Care	India	4.94
Blood Purifier, powder in capsule	The Himalaya Herbal Health Care	India	25.42
Bordelobo	Mexico	Mexico	17.98
Brahmi, powder in capsule	The Himalaya Herbal Health Care	India	10.40
Bupleuri radix	Tsumura Pharmaceutical Company, Japan	Japan	5.66
Cancerina	Mexico	Mexico	19.14
Cascara sagrada	Mexico	Mexico	47.15
Chyavanprash, Dabur	Dabur India Limited	India	35.70
Chyavanprash, Zandu in asli ghee	The Zandu Pharmaceutical Works	India	18.32
Cimicifugae rhizoma	Tsumura Pharmaceutical Company, Japan	Japan	64.31
Cinnamoni cortex	Tsumura Pharmaceutical Company, Japan	Japan	120.18
Cnidii Rhizoma	Tsumura Pharmaceutical Company, Japan	Japan	6.68
Digestiv, powder in capsule	The Himalaya Herbal Health Care	India	7.68
Domiana de San Luis	Mexico	Mexico	10.69

(*Continued*)

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TABLE 2.2 (*Continued*)
Antioxidants in Herbal/Traditional Plant Medicine

Product	Manufacturer/Product Label/ Country of Origin	Procured in	Antioxidant Content (mmol/100 g)
Eucalipto	Mexico	Mexico	47.30
Ginseng radix	Tsumura Pharmaceutical Company, Japan	Japan	1.45
Glycyrrhizae Radix	Tsumura Pharmaceutical Company, Japan	Japan	11.58
Goshuyutou, kampo, traditional medicine from Japan, powder	Japan	Japan	132.58
Hochuekkito	Tsumura Pharmaceutical Company, Japan	Japan	9.67
Holelen	Tsumura Pharmaceutical Company, Japan	Japan	2.82
Huacharable	Mexico	Mexico	39.18
Juzentaihoto	Tsumura Pharmaceutical Company, Japan	Japan	14.18
Kampo, traditional medicine from Japan, powder	Japan	Japan	4.02
Karela (bitter gourd), powder in capsule	The Himalaya Herbal Health Care	India	7.57
Lasuna (garlic), powder in capsule	The Himalaya Herbal Health Care	India	0.80
Neem Guard, powder in capsule	—	India	89.23
Nimba (neem tree), powder in capsule	The Himalaya Herbal Health Care	India	19.99
Paeoniae radix	Tsumura Pharmaceutical Company, Japan	Japan	55.13
Pinelliae tuber	Tsumura Pharmaceutical Company, Japan	Japan	0.28
Pinguica	Mexico	Mexico	2.31
Rhemanniae radix	Tsumura Pharmaceutical Company, Japan	Japan	3.94
Saikokeishito	Tsumura Pharmaceutical Company, Japan	Japan	21.35
Sangre de grado (*Croton lechleri*), liquid solution	Iquitos, Peru	Peru	2897.11
Sano Sano	Peru	Peru	0.47
Scutellariae radix	Tsumura Pharmaceutical Company, Japan	Japan	111.33
Shallaki, powder in capsule	The Himalaya Herbal Health Care	India	2.58
Shuddha guggulu, powder in capsule	The Himalaya Herbal Health Care	India	13.77
Stress Guard, antistress, powder in capsule	—	India	6.39
Tagara, valerian, powder in capsule	The Himalaya Herbal Health Care	India	6.44
Tepezcohuite	Mexico	Mexico	64.58
Tetzar	Mexico	Mexico	5.88
Tila	Mexico	Mexico	19.49
Triphala, powder in capsule	The Himalaya Herbal Health Care	India	706.25
Hangebyakujutsutemmato	Tsumura Pharmaceutical Company, Japan	Japan	5.15
Tulasi, (holy basil), powder in capsule	The Himalaya Herbal Health Care	India	39.67
Un Compuesto, herbal condiment against insomnia	Mexico	Mexico	40.89

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TABLE 2.2 (*Continued*)
Antioxidants in Herbal/Traditional Plant Medicine

Product	Manufacturer/Product Label/ Country of Origin	Procured in	Antioxidant Content (mmol/100 g)
Uncaria tomentosa (Uña de gato)	Cusco, Peru	Peru	37.10
Zapote	Mexico	Mexico	38.78
Zarzaparrilla, root	Cusco, Peru	Peru	13.73
Zingiberis rhizoma	Tsumura Pharmaceutical Company, Japan	Japan	17.52
Zizyphi fructus	Tsumura Pharmaceutical Company, Japan	Japan	5.88
Soyatein (protein-rich soya)	Vital Soya Industry, Sri Goindwal Sahib, District Amritsar	India	1.32

Japan, with antioxidant values in the range 132.6–706.3 mmol/100 g. Only four products in this category have values less than 2.0 mmol/100 g.

A summary of the 425 spices and herbs analyzed in our study is presented in Table 2.3. The study includes spices and herbs from 59 different manufacturers or countries. Although 27 single products have a total antioxidant content in the range 100–465 mmol/100 g, the variation is from 0.08 mmol/100 g in raw garlic paste procured in Japan to 465 mmol/100 g in dried and ground clove purchased from Norway. When sorted by antioxidant content, clove has the highest mean antioxidant value, followed by peppermint, allspice, cinnamon, oregano, thyme, sage, rosemary, saffron, and estragon, all dried and ground, with mean values ranging from 44 to 277 mmol/100 g. When analyzed in fresh samples compared to the dried herbs, oregano, rosemary, and thyme have lower values, in the range 2.2–5.6 mmol/100 g. This is also true for basil, chives, dill, and parsley. In addition to common spices and culinary herbs, we also analyzed other herbs, such as birch leaves, wild marjoram, and wood cranesbill, among others. Most of the spices and herbs analyzed have very high antioxidant content. Although spices and herbs contribute little weight to the dinner plate, they may still be important contributors to antioxidant intake, especially in dietary cultures where spices and herbs are used regularly. We interpret the elevated concentration of antioxidants observed in several dried herbs compared to fresh samples as a normal consequence of the drying process leaving most of the antioxidants intact in the dried end product.

2.7 RESEARCH NEEDS: POTENTIAL HEALTH EFFECTS OF DIETARY ANTIOXIDANTS

Only a few spices have been relatively extensively studied in terms of possible health effects (those include turmeric and ginger, both of which are described in more detail elsewhere in this book). Clove, oregano, and thyme are all among the commercially available spices with the highest total antioxidant capacity (Table 2.3). Several phytochemicals found in these spices, such as rosmarinic acid (Lee et al. 2006) in thyme and oregano (Shan et al. 2005), eugenol in clove and allspice (Chainy et al. 2000) and gallic acid in clove, have all been identified as inhibitors of NF-κB, a transcription factor which is crucial in the orchestration of immune and inflammatory responses. Thyme and oregano essential oils in combination decreased the levels of IL-1β and IL-6, as well as inflammation related tissue damage in a model of colitis (Bukovska et al. 2007), both of which may also be related to NF- κB. We found an extract of clove, oregano, thyme, together with walnuts and coffee to inhibit NF-κB activation in a synergistic manner in vitro, and also in vivo in transgenic mice (Paur et al. 2010). Furthermore, thyme has been found to induce or maintain levels of endogenous

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TABLE 2.3
Antioxidants in Herbs and Spices

Product	Manufacturer/Product Label/ Country of Origin	Procured in	Antioxidant Content (mmol/100 g)
A condiment with red pepper and six other spices, dried ground	Japan	Japan	6.08
Ajwain fruit pods, whole	—	Iran	0.94
Ajwain fruit pods, dried	India	India	28.42
Allspice, dried ground	Hindu, Norway	Norway	99.28
Allspice, dried ground	Black Boy, Rieber og søn	Norway	101.52
Alpine lady's mantle, leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	130.36
Angelica, fresh	The Norwegian Crop Research Institute, Norway	Norway	0.66
Angelica, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	25.25
Angelica, seeds, dried	Norsk Øko-Urt AB, Norway	Norway	8.66
Anisisop, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	33.14
Ash, young leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	47.78
Barbeque spicemix	Santa Maria, Sweden	Norway	1.65
Barbeque spicemix	Engebretsen AS, Norway	Norway	2.13
Barberry, bark	The Norwegian Crop Research Institute, Norway	Norway	55.63
Basil, dried	Gökqehan ,Turkey	Norway	9.86
Basil, dried	Norsk Øko-Urt AB, Norway	Norway	28.10
Basil, dried	—	United States	12.31
Basil, dried	Black Boy, Rieber og søn	Norway	30.86
Basil, dried	Spice Cargo	Mexico	18.24
Basil, dried	Natures Treats Australia PTY LTD, Australia	New Zealand	0.49
Basil, fresh	Norway	Norway	1.12
Basil, fresh	—	Norway	0.67
Basil, fresh	—	United States	0.82
Bay leaves, dried	Santa Maria	Norway	31.29
Bay leaves, dried	Black Boy, Rieber og søn	Norway	24.29
Bay leaves, fresh	Natures Treats Australia PTY LTD, Australia	New Zealand	15.05
Bearberry (*Arctostaphylos uvaursi*), leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	182.10
Bee balm (*Monarda didyma*), flower, dried	Norsk Øko-Urt AB, Norway	Norway	46.56
Betonica officinalis, dried	The Norwegian Crop Research Institute, Norway	Norway	9.41
Betterave	Mali	Mali	2.34
Birch, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	30.44
Birch, leaves, fresh	Norway	Norway	26.23
Bird cherry, flower, dried	The Norwegian Crop Research Institute, Norway	Norway	23.08
Biting stonecrop, dried	The Norwegian Crop Research Institute, Norway	Norway	11.89
Blackberry, leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	23.31

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TABLE 2.3 (*Continued*)
Antioxidants in Herbs and Spices

Product	Manufacturer/Product Label/ Country of Origin	Procured in	Antioxidant Content (mmol/100 g)
Blackcurrant, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	97.83
Calamus root (*Acorus calamus*), rhizome	The Norwegian Crop Research Institute, Norway	Norway	6.65
Caper, flower	Turkey	Norway	1.00
Caper, fruits and stem	Cervera, Denmark	Norway	0.84
Caper, small	Turkey	Norway	0.94
Car magaz, whole kernels	Asian Bazaar	Mexico	0.77
Caraway seeds, dried	Norsk Øko-Urt AB, Norway	Norway	3.35
Caraway seeds, dried	Black Boy, Rieber og søn	Norway	4.48
Cardamom pod, green, whole	India	India	1.85
Cardamom seeds (from green pod)	Roopaks, Ajmal Khan, New Delhi	India	1.64
Cardamom seeds, dried	India	India	1.13
Cardamom seeds, dried	Black Boy, Rieber og søn	Norway	0.48
Cardamom, dried ground	Engebretsen AS, Norway	Norway	2.35
Cardamom, dried ground	Santa Maria, Sweden	Norway	1.65
Cardamom, whole fruit, dried	India	India	1.64
Cayenne pepper, dried ground	Spice Cargo	Mexico	5.38
Cayenne pepper, dried ground	Santa Maria	Norway	4.18
Cayenne pepper, dried ground	Black Boy, Rieber og søn	Norway	5.90
Celery seeds, whole	Spice Cargo	Mexico	8.17
Celery, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	16.91
Chervil, dried	The Norwegian Crop Research Institute, Norway	Norway	17.67
Chili, Chile ancho, dark, whole, dried	Mexico	Mexico	5.09
Chili, Chile de arcbol, small red, whole, dried	Mexico	Mexico	3.11
Chili, Chile don piquin, with seeds, crushed, dried	Mexico	Mexico	4.20
Chili, Chile guajillo, dark, whole, dried	Mexico	Mexico	2.25
Chili, Chile pasilla, dark, whole, dried	Verde Valle, Mexico	Mexico	7.54
Chili, Chile pasilla, dark, whole, dried	La Merced, Mexico	Mexico	3.01
Chili, Chile piquin, dried ground	—	Mexico	1.40
Chili, Chile, dried ground	La Anita, Mexico	Mexico	7.15
Chili, dried	Santa Maria, Sweden	Norway	7.63
Chili, dried ground	Spice Cargo	Mexico	12.21
Chili, dried ground	Rajah	Norway	7.87
Chili, dried ground	Engebretsen AS, Norway	Norway	12.15
Chili, dried ground	United States	United States	8.37
Chili, dried ground, hot	Hindu, Norway	Norway	5.96
Chili, dried ground, Mexican	Hindu, Norway	Norway	11.86
Chili, green, whole	Spain	Norway	2.33
Chili, red with seeds, dried	India	India	2.52
Chili, red, whole	Spain	Norway	2.92
Chili, red, whole	—	Norway	2.08
Chili, without seeds, dried	India	India	3.74
Chives, chopped, dried	Spice Cargo	Mexico	7.80

(*Continued*)

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TABLE 2.3 (*Continued*)
Antioxidants in Herbs and Spices

Product	Manufacturer/Product Label/ Country of Origin	Procured in	Antioxidant Content (mmol/100 g)
Chives, dried	Black Boy, Rieber og søn	Norway	7.11
Chives, dried	Natures Treats Australia PTY LTD, Australia	New Zealand	2.56
Chives, dried	Norsk Øko-Urt AB, Norway	Norway	11.14
Chives, fresh	BAMA gruppen, Norway	Norway	0.59
Chives, fresh	—	Norway	0.60
Cinnamon sticks, (Cassia vera indo)	Natures Treats Australia PTY LTD, Australia	New Zealand	6.84
Cinnamon, bark, whole	Mexico	Mexico	40.14
Cinnamon, bark, whole	Asian Bazaar	Mexico	32.61
Cinnamon, dried ground	India	India	31.64
Cinnamon, dried ground	Spice Cargo	Mexico	139.89
Cinnamon, dried ground	—	United States	17.65
Cinnamon, dried ground	Black Boy, Rieber og søn	Norway	53.04
Cinnamon, dried ground	Engebretsen AS, Norway	Norway	63.27
Cinnamon, dried ground	Santa Maria, Sweden	Norway	118.69
Cinnamon, dried ground	Canela Molida	Mexico	114.98
Cirsium heterophyllum, leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	38.18
Clove, dried ground	—	United States	125.55
Clove, dried ground	Black Boy, Rieber og søn	Norway	465.32
Clove, whole, dried	La Surtidora	Mexico	175.31
Clove, whole, dried	TRS Wholesale CO, England	Norway	317.96
Clove, whole, dried	Escosa, Mexico	Mexico	327.77
Clove, whole, dried	India	India	252.04
Club-moss, dried	The Norwegian Crop Research Institute, Norway	Norway	4.56
Coltsfoot, leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	61.32
Columbine, Granny's bonnet, dried	The Norwegian Crop Research Institute, Norway	Norway	3.96
Common alkanet, dried	The Norwegian Crop Research Institute, Norway	Norway	18.37
Common butterwort, leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	41.93
Common chickweed, dried	The Norwegian Crop Research Institute, Norway	Norway	5.17
Common elder, flower, dried	The Norwegian Crop Research Institute, Norway	Norway	24.13
Common elder, leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	20.36
Common fumitory, dried	The Norwegian Crop Research Institute, Norway	Norway	25.06
Common horsetail, dried	The Norwegian Crop Research Institute, Norway	Norway	12.17
Common mallow, flower and leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	9.06
Common mallow, flower, dried	Norsk Øko-Urt AB, Norway	Norway	24.63

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TABLE 2.3 (*Continued*)
Antioxidants in Herbs and Spices

Product	Manufacturer/Product Label/ Country of Origin	Procured in	Antioxidant Content (mmol/100 g)
Common mallow, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	9.20
Common nettle, stinging nettle, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	35.23
Common polypody, rhizome	The Norwegian Crop Research Institute, Norway	Norway	35.42
Common silver birch, leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	22.07
Common valerian, flower and leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	24.03
Condiment with red pepper, dried ground	Japan	Japan	5.23
Coriander, dried ground	Rajah	Norway	4.66
Coriander seeds	Onena Spices, Spain	Norway	0.28
Coriander seeds	Asian Bazaar	Mexico	1.26
Coriander, leaves, dried	Santa Maria, Sweden	Norway	2.84
Coriander, leaves, dried	Black Boy, Rieber og søn	Norway	2.10
Coriander, leaves, fresh	BAMA gruppen, Norway	Norway	1.20
Coriander, leaves, fresh	Norway	Norway	0.41
Coriander, seeds, green, dried	India	India	3.49
Cornflower, dried	The Norwegian Crop Research Institute, Norway	Norway	11.96
Cornflower, flower, dried	Norsk Øko-Urt AB, Norway	Norway	8.84
Creeping jenny (*Lysimachia nummularia*), leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	31.31
Cumin, dried ground	Mexico	Mexico	8.23
Cumin, dried ground	Black Boy, Rieber og søn	Norway	6.82
Cumin, dried ground	Spice Cargo	Mexico	9.14
Cumin, dried ground	Comino Molido	Mexico	10.30
Cumin, dried ground	Rajah	Norway	11.88
Cumin, seeds, whole, dried	Santa Maria, Sweden	Norway	2.54
Cumin, whole	Hindu, Norway	Norway	2.45
Curled parsley, fresh	Hafskjold Gartneri, Norway	Norway	0.34
Curry, powder	Japan	Japan	10.47
Curry, powder	Spice Cargo	Mexico	14.92
Curry, powder	United States	United States	9.98
Curry, powder	TRS Wholesale CO, England	Norway	10.93
Curry, powder	Black Boy, Rieber og søn	Norway	13.02
Curry, powder, Madras, hot, dried ground	Rajah	Norway	6.65
Curry, powder, Madras, mild, dried ground	Rajah	Norway	7.43
Curry, powder, mild, dried ground	Rajah	Norway	4.17
Dame's violet, dried	The Norwegian Crop Research Institute, Norway	Norway	22.63
Dandelion, flower, dried	Norsk Øko-Urt AB, Norway	Norway	12.72
Dandelion, leaves	Norway	Norway	6.89
Dandelion, leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	21.07

(*Continued*)

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TABLE 2.3 (*Continued*)
Antioxidants in Herbs and Spices

Product	Manufacturer/Product Label/ Country of Origin	Procured in	Antioxidant Content (mmol/100 g)
Dandelion, root	The Norwegian Crop Research Institute, Norway	Norway	4.91
Devil's bit, dried	The Norwegian Crop Research Institute, Norway	Norway	30.18
Dill, dried	Norsk Øko-Urt AB, Norway	Norway	20.23
Dill, dried	Goutess, GmbH	Norway	24.47
Dill, dried	Black Boy, Rieber og søn	Norway	15.94
Dill, dried	Hindu, Norway	Norway	2.79
Dill, dried	Santa Maria	Norway	2.68
Dill, fresh	Norway	Norway	1.39
Dill, fresh	—	Norway	2.18
Dill, seeds	The Norwegian Crop Research Institute, Norway	Norway	3.37
Dwarf birch, leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	86.22
English ivy, leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	27.98
Estragon, dried	Black Boy, Rieber og søn	Norway	43.31
Estragon, dried	Santa Maria	Norway	13.63
Estragon, French, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	43.22
Estragon, Russian, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	44.75
European golden rod, dried	The Norwegian Crop Research Institute, Norway	Norway	28.43
Fakouhoye leaves, dried	Mali	Mali	10.20
Fennel, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	18.91
Fennel, whole seeds, dried	TRS Wholesale, Co., England	Norway	5.84
Fenugreek, seeds	India	India	2.09
Fenugreek, whole	Asian Bazaar	Mexico	1.67
Field bindweed, dried	The Norwegian Crop Research Institute, Norway	Norway	17.51
Field forget-me-not, dried	The Norwegian Crop Research Institute, Norway	Norway	28.15
Field horsetail (*Equisetum arvense*), leaves, dried	Norsk Øko-Urt AB, Norway	Norway	9.41
Field rest-harrow (*Ononis arvensis*), root	The Norwegian Crop Research Institute, Norway	Norway	10.15
Figwort, dried	The Norwegian Crop Research Institute, Norway	Norway	8.69
Fir clubmoss, dried	The Norwegian Crop Research Institute, Norway	Norway	10.58
Garden catmint (*Nepeta x faassenii*), dried	The Norwegian Crop Research Institute, Norway	Norway	14.18
Garlic, dried ground	—	United States	0.80
Garlic, dried ground	Black Boy, Rieber og søn	Norway	2.13
Garlic, dried ground	Rajah	Norway	1.61
Garlic, dried ground	Spice Cargo	Mexico	0.78
Garlic, raw paste	Japan	Japan	0.08
Ginger		Norway	2.79

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TABLE 2.3 (*Continued*)
Antioxidants in Herbs and Spices

Product	Manufacturer/Product Label/ Country of Origin	Procured in	Antioxidant Content (mmol/100 g)
Ginger	Mali	Mali	3.93
Ginger, dried ground	—	Mexico	22.19
Ginger, dried	India	India	11.31
Ginger, dried ground	Santa Maria, Sweden	Norway	22.12
Ginger, dried ground	Northwest Delights, United States	Norway	0.86
Ginger, dried ground	Spice Cargo	Mexico	24.37
Ginger, dried ground	—	United States	21.57
Ginger, raw paste	Japan	Japan	5.33
Grass-of-Parnasuss (*Parnassia palustris*), dried	The Norwegian Crop Research Institute, Norway	Norway	52.27
Greater burdock, root	The Norwegian Crop Research Institute, Norway	Norway	14.26
Greater plantain, leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	22.03
Green mint, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	142.58
Grey alder (*Alnus incana*), leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	59.27
Ground-ivy (*Glechoma hederacea*), dried	The Norwegian Crop Research Institute, Norway	Norway	31.72
Hazel, leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	35.51
Heather, flower, dried	The Norwegian Crop Research Institute, Norway	Norway	56.98
Hoary plantain, leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	29.35
Hollyhock, flower and leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	10.16
Hop, cone	The Norwegian Crop Research Institute, Norway	Norway	30.96
Hops, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	35.28
Horehound (*Marrubium vulgare*), dried	The Norwegian Crop Research Institute, Norway	Norway	12.49
Hound's tongue, leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	32.65
Houseleek, dried	The Norwegian Crop Research Institute, Norway	Norway	5.24
Hyssop, flower, dried	Norsk Øko-Urt AB, Norway	Norway	52.29
Hyssop, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	44.90
Iceland moss (*Cetraria islandica*), dried	The Norwegian Crop Research Institute, Norway	Norway	0.71
Imperatoria ostruthium, rhizome	The Norwegian Crop Research Institute, Norway	Norway	27.56
Jalapeño pepper, dried	Black Boy, Rieber og søn	Norway	8.25
Japanese pepper, dried ground	Japan	Japan	36.92
Japanese rose, Ramanas rose, fruit shell, dried	The Norwegian Crop Research Institute, Norway	Norway	58.66

(*Continued*)

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TABLE 2.3 (*Continued*)
Antioxidants in Herbs and Spices

Product	Manufacturer/Product Label/ Country of Origin	Procured in	Antioxidant Content (mmol/100 g)
Juniper berries, blue, dried	The Norwegian Crop Research Institute, Norway	Norway	19.29
Juniper berries, coniferous litter, dried	The Norwegian Crop Research Institute, Norway	Norway	76.77
Juniper berries, dried	Norsk Øko-Urt AB, Norway	Norway	8.89
Juniper berries, dried	Black Boy, Rieber og søn	Norway	9.27
Juniper berries, green, dried	Norsk Øko-Urt BA, Norway	Norway	8.42
Juniper berries, green, dried	The Norwegian Crop Research Institute, Norway	Norway	80.26
Kaloonji, whole seeds, dried	Ashiq Cash&Carry, England	Norway	1.02
Knotgrass, dried	The Norwegian Crop Research Institute, Norway	Norway	16.62
Lady's bedstraw, dried	The Norwegian Crop Research Institute, Norway	Norway	9.90
Lady's mantle, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	43.31
Lady's mantle, leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	24.99
Lavender, leaves and flower, dried	Norsk Øko-Urt AB, Norway	Norway	29.61
Lemon balm (*Melissa officinalis*), leaves, fresh	Norway	Norway	1.32
Lemon balm, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	125.33
Lemon pepper	Santa Maria	Norway	0.66
Lemon pepper	Hindu, Norway	Norway	1.00
Lemon thyme, leaves and flower, dried	Norsk Øko-Urt AB, Norway	Norway	92.18
Lemon thyme, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	9.22
Liquorice, sweet-root, root and rhizome	The Norwegian Crop Research Institute, Norway	Norway	2.71
Lovage (*Levisticum officinale*), leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	23.70
Lovage (*Levisticum officinale*), leaves, dried	Norsk Øko-Urt AB, Norway	Norway	36.17
Maghaj, dried	India	India	0.27
Maral Root (*Leuzea carthamoides*), leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	69.57
Marigold (*Calendula officinalis*), flower and leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	9.83
Meadowsweet (*Filipendula ulmaria*), dried	The Norwegian Crop Research Institute, Norway	Norway	154.05
Meadowsweet, flower and leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	117.77
Meadowsweet, flower, dried	Norsk Øko-Urt AB, Norway	Norway	167.82
Meadowsweet, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	111.30
Merian, dried	Santa Maria, Sweden	Norway	53.92
Mint, dried	Onena Spices, Spain	Norway	71.95
Mint (*Mentha spicata*), fresh	—	Norway	1.27
Motherworth (*Leonurus cardiaca*), dried	The Norwegian Crop Research Institute, Norway	Norway	13.19

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TABLE 2.3 (*Continued*)
Antioxidants in Herbs and Spices

Product	Manufacturer/Product Label/ Country of Origin	Procured in	Antioxidant Content (mmol/100 g)
Mugwort, dried	The Norwegian Crop Research Institute, Norway	Norway	23.79
Mullein, flower, dried	The Norwegian Crop Research Institute, Norway	Norway	37.71
Mustard powder	Spice Cargo	Mexico	10.30
Mustard powder	Colman's	Norway	10.39
Mustard seed, yellow, ground	—	United States	10.53
Mustard seeds	India	India	3.78
Mustard seeds, brown, whole	Asian Bazaar	Mexico	6.70
Mustard seeds, ground	Mexico	Mexico	9.44
Mustard seeds, yellow, whole	Asian Bazaar	Mexico	7.52
Northern dock, dried	The Norwegian Crop Research Institute, Norway	Norway	43.61
Northern dock, root	The Norwegian Crop Research Institute, Norway	Norway	56.69
Nutmeg, dried	Roopaks, Ajmal Khan, New Delhi	India	19.42
Nutmeg, dried	India	India	33.00
Nutmeg, dried ground	Black Boy, Rieber og søn	Norway	20.32
Nutmeg, dried ground	Mexico	Mexico	43.52
Nutmeg, whole, dried	Jaifal, England	Norway	15.83
Onion, dried ground	—	United States	0.95
Oregano, dried	La Surtidora	Mexico	73.77
Oregano, dried	—	United States	40.30
Oregano, dried	Greece	Norway	45.58
Oregano, dried	Norsk Øko-Urt AB, Norway	Norway	89.51
Oregano, dried	Mexico	Mexico	47.64
Oregano, dried	Gökqehan, Turkey	Norway	96.64
Oregano, dried	Hindu, Norway	Norway	48.02
Oregano, dried	Black Boy, Rieber og søn	Norway	44.99
Oregano, dried	Santa Maria	Norway	21.42
Oregano, dried	McCormick	Mexico	82.61
Oregano, fresh	Norway	Norway	3.75
Oregano, fresh	Gartner, BAMA, Norway	Norway	3.81
Orpine (*Sedum telephium*), rhizome	The Norwegian Crop Research Institute, Norway	Norway	57.83
Paprika (powder), dried ground	Rajah	Norway	6.78
Paprika (powder), dried ground	—	United States	8.60
Paprika (powder), dried ground	Santa Maria, Sweden	Norway	5.93
Paprika (powder), dried ground	Paprika Molido	Mexico	7.44
Paprika (powder), dried ground	Engebretsen AS, Norway	Norway	8.08
Paprika, (powder), dried ground	Black Boy, Rieber og søn	Norway	5.59
Paprika, (powder), red, dried ground	Spice Cargo	Mexico	5.75
Parsely, big leaves, fresh	Linnes gård, Norway	Norway	1.93
Parsley	Mali	Mali	1.12
Parsley	Norway	Norway	0.86
Parsley	Sweden	Norway	1.22

(*Continued*)

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TABLE 2.3 (*Continued*)
Antioxidants in Herbs and Spices

Product	Manufacturer/Product Label/ Country of Origin	Procured in	Antioxidant Content (mmol/100 g)
Parsley	Lier	Norway	2.00
Parsley, big leaves, fresh	Sweden	Norway	2.03
Parsley, dried	Hindu, Norway	Norway	3.72
Parsley, dried	—	United States	7.43
Parsley, dried	Norsk Øko-Urt AB, Norway	Norway	10.09
Parsley, dried	Black Boy, Rieber og søn	Norway	3.64
Parsley, dried	Santa Maria	Norway	3.81
Parsley, dried	Spice Cargo	Mexico	8.23
Pepper, dried ground	McCormick	Mexico	50.96
Pepper, black, dried ground	La Surtidora	Mexico	5.08
Pepper, black, dried ground	Spice Cargo	Mexico	6.68
Pepper, black, dried ground	Rajah	Norway	6.65
Pepper, black, dried ground	Black Boy, Rieber og søn	Norway	8.71
Pepper, black, dried ground	—	United States	4.54
Pepper, black, whole, dried	India	India	4.15
Pepper, black, whole, dried	—	United States	4.34
Pepper, dark-green "berries" on the stem, fresh	Thailand	Norway	0.26
Pepper, green "berries" on the stem, fresh	Thailand	Norway	0.46
Pepper, white, dried ground	Santa Maria	Norway	3.92
Pepper, white, dried ground	Rajah	Norway	5.02
Pepper, white, whole	Roopaks, Ajmal Khan, New Delhi	India	3.49
Peppermint, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	160.82
Pepperwort, garden cress, fresh	—	Norway	2.42
Perforate St. John's wort, flower and leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	54.37
Piffi, dried ground	Engebretsen AS, Norway	Norway	0.61
Piri-piri, dried ground	Santa Maria	Norway	9.39
Piri-piri, dried	Black Boy, Rieber og søn	Norway	6.51
Pot marigold, flower, dried	Norsk Øko-Urt AB, Norway	Norway	11.47
Purple coneflower, flower and leaves, dried	Norsk Øko-Urt AB, Norway	Norway	16.09
Purple loosestrife (*Lythrum salicaria*), flower and leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	111.04
Quack grass, rootstock (*Elytrigia repens*), rhizome	The Norwegian Crop Research Institute, Norway	Norway	0.88
Rai, dried	India	India	2.84
Raspberry, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	46.89
Raspberry, leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	32.56
Raspberry, leaves, fresh	Norway	Norway	21.36
Red clover, flower, dried	Norsk Øko-Urt AB, Norway	Norway	39.92
Red wortleberries, leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	102.07
Red-berried elder, leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	56.66

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TABLE 2.3 (*Continued*)
Antioxidants in Herbs and Spices

Product	Manufacturer/Product Label/ Country of Origin	Procured in	Antioxidant Content (mmol/100 g)
Ribwort, leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	34.81
Rose, flower, dried	Norsk Øko-Urt AB, Norway	Norway	153.90
Rose-bay, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	101.33
Rose-bay, willow herb flower, dried	Norsk Øko-Urt AB, Norway	Norway	93.48
Rose-bay, willow herb, flower and leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	120.99
Rosemary, dried	Hindu, Norway	Norway	35.81
Rosemary, dried	Black Boy, Rieber og søn	Norway	66.92
Rosemary, fresh	Norway	Norway	5.64
Rosemary, fresh leaves	Gartner, BAMA, Norway	Norway	11.07
Rosemary, fresh leaves	BAMA gruppen, Norway	Norway	6.34
Rosemary, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	56.95
Rosemary, leaves, dried	Spice Cargo	Mexico	39.99
Rosemary, leaves, dried	Gökqehan, Turkey	Norway	24.34
Roseroot, fresh	The Norwegian Crop Research Institute	Norway	5.63
Saffron, dried ground	Delhi Keshar Co.	India	23.83
Saffron, dried ground	Roopaks, Ajmal Khan, New Delhi	India	61.72
Saffron, dried ground	Gaea, Greece	Norway	47.83
Saffron, stigma	Mexico	Mexico	7.02
Saffron, stigma	Carmencita, Spain	Norway	24.83
Saffron, stigma	Bahraman Saffron, Iran	Iran	20.58
Sage, dried	Hindu, Norway	Norway	34.88
Sage, dried	Spice Cargo	Mexico	58.80
Sage, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	39.36
Sanguisorba officinalis, dried	The Norwegian Crop Research Institute, Norway	Norway	33.37
Saunf, big, dried	India	India	7.09
Saunf, small, dried	India	India	6.46
Scented mayweed, flower, dried	Norsk Øko-Urt AB, Norway	Norway	16.63
Shah jerra, dried	Roopaks, Ajmal Khan, New Delhi	India	5.34
Shepherd's purse, dried	The Norwegian Crop Research Institute, Norway	Norway	5.52
Silverweed, dried	The Norwegian Crop Research Institute, Norway	Norway	35.79
Small-leaved lime, flower, dried	The Norwegian Crop Research Institute, Norway	Norway	34.83
Somage, dried ground	Iran	Iran	85.58
Sorrel, leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	19.52
Southernwood, flower, stem, and leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	34.88
Spanish chervil, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	54.96
Speedwell, dried	The Norwegian Crop Research Institute, Norway	Norway	94.79

(*Continued*)

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TABLE 2.3 (*Continued*)
Antioxidants in Herbs and Spices

Product	Manufacturer/Product Label/ Country of Origin	Procured in	Antioxidant Content (mmol/100 g)
Spice mix, Kjøkkensjef Natvigs	Black Boy, Rieber og søn	Norway	0.39
Spice mix, Aromat	Knorr	Norway	0.27
Spice mix, Gastromat	Gastromat A/S, Norway	Norway	0.60
Spice mix, taco	ICA, Sweden	Norway	2.63
Spice mix, taco	Santa Maria, Sweden	Norway	4.11
Spice mix, taco	Old El Paso	Norway	3.80
Spruce, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	29.31
St. John's wort, flower and leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	72.16
Star anise, dried	India	India	11.30
Stevia drypp, juice from fermented leaves	Medurt Pharma	Norway	3.28
Stevia rebaudiana, dried leaves	Medurt Pharma	Norway	63.55
Stevia rebaudiana, fermented leaves	Medurt Pharma	Norway	3.17
Stevia rebaudiana, leaves	Medurt Pharma	Norway	6.27
Stevia rebaudiana, leaves	—	Norway	14.25
Stinging nettle, dried	The Norwegian Crop Research Institute, Norway	Norway	13.09
Stinging nettle, leaves	Norway	Norway	3.66
Sugar kelp, dried, kombu royal	Iceland	Iceland	0.26
Summac, dried ground	Khater Spice, Lebanon	Norway	42.36
Summer savory, leaves and flower, dried	Norsk Øko-Urt AB, Norway	Norway	59.66
Sundew (*Drosera angelica*), dried	The Norwegian Crop Research Institute, Norway	Norway	79.02
Sundew (*Drosera rotundifolia*), dried	The Norwegian Crop Research Institute, Norway	Norway	85.97
Sweet marjoram, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	92.31
Tamarind	India	India	3.50
Tansy, flower, dried	The Norwegian Crop Research Institute, Norway	Norway	30.71
Tej pata (bay leaves), dried	India	India	18.54
Thribi, dried	Greece	Norway	42.56
Thyme, dried	Black Boy, Rieber og søn	Norway	63.75
Thyme, dried	Greece	Norway	42.00
Thyme, dried	Norsk Øko-Urt AB, Norway	Norway	63.13
Thyme, fresh	Norway	Norway	2.16
Thyme, fresh leaves	Gartner, BAMA, Norway	Norway	2.65
Thyme, fresh leaves	BAMA gruppen, Norway	Norway	1.46
Trembling poplar, aspen, leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	26.65
Turmeric, dried ground	—	United States	15.68
Turmeric, dried ground	Black Boy, Rieber og søn	Norway	10.25
Turmeric, dried ground	Rajah	Norway	10.92
Turmeric, dried ground	Unifood, India	Norway	10.55
Turmeric, dried ground	Spice Cargo	Mexico	15.63
Turmeric, dried ground	Rajah	Norway	11.83

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TABLE 2.3 (*Continued*)
Antioxidants in Herbs and Spices

Product	Manufacturer/Product Label/ Country of Origin	Procured in	Antioxidant Content (mmol/100 g)
Turmeric, whole, dried	India	India	13.60
Vanilla pod, seeds from pod	Onena Spices, Spain	Norway	3.73
Vanilla pod, seeds from pod	Tørsleffs, Haugen-gruppen	Norway	5.15
Vanilla pod, whole with seeds	Onena Spices, Spain	Norway	7.13
Vanilla pod, whole with seeds	Tørsleffs, Haugen-gruppen	Norway	7.38
Vanilla pod, whole with seeds	Black Boy, Rieber og søn	Norway	7.16
Vanilla pod, without seeds	Black Boy, Rieber og svn	Norway	10.09
Vanilla pod, without seeds	Onena Spices, Spain	Norway	8.69
Vanilla pod, without seeds	Tørsleffs, Haugen-gruppen	Norway	8.50
Vanilla, seeds from pod	Black Boy, Rieber og søn	Norway	2.59
Viola canina, leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	12.90
Wall germander (*Teucrium chamaedrys*), dried	The Norwegian Crop Research Institute, Norway	Norway	48.14
Wasabi, paste	Japan	Japan	0.11
White dead nettle, dried	The Norwegian Crop Research Institute, Norway	Norway	18.21
Wild marjoram, leaves, dried	Norsk Øko-Urt AB, Norway	Norway	131.92
Wild marjoram, leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	142.86
Wood sorrel (*Oxalis acetosella*), dried	The Norwegian Crop Research Institute, Norway	Norway	6.54
Woodland geranium (*Geranium sylvaticum*), dried	The Norwegian Crop Research Institute, Norway	Norway	113.27
Wormwood, absinth, dried	The Norwegian Crop Research Institute, Norway	Norway	10.42
Wych elm, leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	15.65
Yarrow, flower and leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	31.66
Yarrow, flower, dried	Norsk Øko-Urt AB, Norway	Norway	18.61
Yellow loosestrife (*Lysimacha vulgaris*), leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	72.96
Yellow sweet clover, flower and leaves, dried	The Norwegian Crop Research Institute, Norway	Norway	5.14

cytoprotective proteins in the liver (Sasaki et al. 2005). This is in consonance with a study by Kluth et al. (2007), in which extracts of thyme, allspice, or clove induced phase I- and/or phase II-related transcription in vitro (through the CYP3A4 promoter, and pregnane X receptor [PXR] and electrophile response element [EpRE]-dependent transcription).

Further underlining the potency of phytochemicals, we (Paur, Austenaa, and Blomhoff 2008) and others (Takada et al. 2004) have found several phytochemicals to be equally or even more efficient inhibitors of NF-κB as compared to classical anti-inflammatory drugs, such as ibuprofen and dexamethasone.

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Even though there is limited literature on the health effects of whole herbs or spices or extracts of whole herbs or spices, the number of studies investigating the possible health effects of single phytochemicals originating from herbs or spices is much higher. Resveratrol, curcumin, genistein, capsaicin, epigallocatechin gallate (EGCG), quercetin, β-carotene, and lycopene are among the most widely studied phytochemicals. Phytochemicals can alter the activity of several cell signaling pathways, which can lead to modulation of inflammatory processes, regulation of cytoprotective mechanism and regulation of cell growth and differentiation (extensively reviewed by Surh (2003) and Aggarwal and Shishodia (2006)). Most of the products categorized as herbal and traditional plant medicines are also based on antioxidant-rich dietary plants or isolated phytochemicals.

Herbal and traditional plant medicines emerged as many of the highest antioxidant-containing products in our study of various foods. We speculate that a highly inherent antioxidant property is an important contributor to an herb's medicinal qualities. In our study, we identified sangre de grado, the sap from the tree trunk of the species *Croton lechleri* sampled in Peru, to have exceptionally high antioxidant content. This tree sap has a long history of indigenous use in South America for healing wounds and as an antifungal, antiseptic, antiviral, and antihemorrhagic medicine. Proanthocyanidins are major constituents of this sap (Cai et al. 1991), and studies have shown that sangre de grado limits the transcription of a wide range of proinflammatory cytokines and mediators, accelerates the healing of stomach ulcers (Miller et al. 2000, 2001), and promotes apoptosis in cancer cells (Sandoval et al. 2002). Other herbal medicines that are extremely rich in antioxidants include triphala, an Indian Ayurvedic herbal formulation, that was shown to have anti-inflammatory activity (Rasool and Sabina 2007), antibacterial and wound-healing properties (Srikumar et al. 2007; Kumar et al. 2008), and cancer chemopreventive potential (Deep et al. 2005). Arjuna, another Ayurvedic formula, has been shown to have health benefits (Devi et al. 2007; Manna, Sinha, and Sil 2007), whereas goshuyu-tou, a traditional kampo medicine, has been shown to significantly lower the extracellular concentration of NO in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells (Okayasu et al. 2003).

The herbs Cinnamomi cortex and Scutellariae radix both contain very high levels of antioxidants (75 mmol/100 g). The herbal medicines saiko-keishi-to, juzaen-taiho-to, and hocyu-ekki-to, which are used for various kinds of inflammatory and infectious diseases, are all taken in a daily dose of 7.5 g, corresponding to 1.6, 1.1, and 0.7 mmol antioxidants per day, respectively. The antioxidant activity of the Japanese herbal medicine sho-saiko-to, which is composed of herbs tested in this study, was calculated to be about 1.3 mmol/7.5 g (the recommended daily dose of the medicine). This drug, which is commonly used to treat chronic hepatitis in Japan, may also inhibit the development of hepatocellular carcinoma (Oka et al. 1995) and decrease lipid peroxidation and hepatic fibrosis in experimental animals (Sakaida et al. 1998; Shimizu et al. 1999). The herbal medicine stronger neo-minophagen C, a glycyrrhizin preparation, has also been used extensively with considerable success in Japan for the treatment of chronic hepatitis in intravenous doses up to 100 mL/day (Kumada 2002). Our analyses reveal that this injection volume equals about 1 mmol antioxidants. Thus, such injections will boost the total antioxidant content within the body. It is tempting to speculate that several of the effects observed with these herbal medicines are mediated by their antioxidant activity.

It is not likely that all antioxidant-rich foods are good bioactive sources, or that all antioxidants provided in the diet are bioactive. Bioavailability differs greatly from one phytochemical to another, so the most antioxidant-rich foods in our diet are not necessarily those leading to the highest concentrations of active metabolites in target tissues. The antioxidants obtained from foods include many different molecular compounds and families with different chemical and biological properties including differences in absorption, transport and excretion, cellular uptake and metabolism, and eventually their effects on oxidative stress in various cellular compartments. Biochemically active phytochemicals found in plant-based foods also have many powerful biological properties that are not correlated with their antioxidant capacity. Thus, a food low in antioxidants may have beneficial health effects due to other food components or phytochemicals executing bioactivity through other mechanisms.

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Understanding the complex role of diet in chronic diseases is challenging since a typical diet provides more than 25,000 bioactive food constituents, many of which may modify a multitude of processes that are related to these diseases. Because of the complexity of this relationship, it is likely that a comprehensive understanding of the role of these bioactive food components is needed to assess the role of dietary plants in human health and disease development. We suggest that both their numerous individual functions and their combined additive or synergistic effects are crucial to their beneficial effects on human health, and thus a food-based research approach is likely to elucidate more health effects than the effects derived from each individual nutrient. The antioxidant food table is a valuable research contribution for plant-based nutritional research and may be utilized in epidemiological studies where reported food intakes can be assigned antioxidant values. It can also be used to test antioxidant effects and synergy in experimental animals, cell studies, or in human clinical trials. The ultimate goal of this research is to combine these strategies in order to understand the role of dietary phytochemical antioxidants in the prevention of chronic diseases related to oxidative stress.

2.8 CONCLUSIONS

The concepts discussed in this chapter can be summarized as follows:

- The total content of antioxidants has been assessed in more than 3500 foods; this provides a large database to support research into dietary antioxidants, health, and disease.
- The results show large variations both between food categories and within each category.
- Herbs and spices and composite herbal medicines are among the categories that contain the most antioxidants.
- Further research is needed to study the biological effects of antioxidant-rich herbs and spices on oxidative-stress-related diseases.

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3 Evaluation of the Nutritional and Metabolic Effects of *Aloe vera*

Meika Foster, Duncan Hunter, and Samir Samman

CONTENTS

3.1 Introduction 37
3.2 Taxonomy and Traditional Origins 38
3.3 Current Usage 38
3.4 Structure and Chemical Constituents 38
3.5 Effect of Cultivation and Processing 41
3.6 Health Effects: The Scientific Evidence 41
3.6.1 Topical Applications 41
3.6.1.1 Dermatological Conditions 41
3.6.1.2 Burn Injuries 44
3.6.1.3 Surgical Wound Healing 44
3.6.2 Oral Applications 44
3.6.2.1 Laxative 45
3.6.2.2 Diabetes Mellitus 45
3.6.2.3 Metastatic Cancer 46
3.6.2.4 Ulcers and Inflammation of the Gastrointestinal Tract 46
3.7 Active Ingredients and Mechanisms of Action 46
3.7.1 Active Latex Constituents 47
3.7.2 Active Gel Constituents 47
3.8 Safety and Efficacy 48
3.8.1 Toxicology 49
3.8.2 Carcinogenicity 49
3.8.3 Photoxicity 49
3.8.4 Adverse Effects 49
3.8.5 Contraindications and Drug Interactions 50
3.9 Drug/Vitamin Bioavailability 50
3.10 Research Needs 50
3.11 Conclusions 50
References 51

3.1 INTRODUCTION

Aloe vera has a long history of popular and traditional use. It is used in traditional Indian medicine for constipation, colic, skin diseases, worm infestation, and infections (Heber 2007). It is also used in Trinidad and Tobago for hypertension (Lans 2006) and among Mexican Americans for the treatment of type 2 diabetes mellitus (DM; Coronado et al. 2004). In Chinese medicine, it is often

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recommended in the treatment of fungal diseases (Heber 2007). In Western society, *Aloe vera* is one of the few herbal medicines in common usage, and it has found widespread use in the cosmetic, pharmaceutical, and food industries. In the case of health, the therapeutic claims for the topical and oral application of *Aloe vera* cover a wide range of conditions, but few claims have been the subject of robust clinical investigation. The conditions for which clinical trials of *Aloe vera* have been conducted include skin conditions, management of burn and wound healing, constipation, DM, and gastrointestinal disorders.

3.2 TAXONOMY AND TRADITIONAL ORIGINS

Aloe vera is one of approximately 420 species of the genus *Aloe* (Dagne et al. 2000), which is variously classified as belonging to the Asphodelaceae, Liliaceae, or Aloaceae families. Commonly known as *Aloe barbadensis* Miller, its legitimate name according to the international rules of botanical nomenclature is *A. vera (L.) Burm.f.* (Grindlay and Reynolds 1986). The geographic origin of *Aloe vera* is believed to be in Sudan, with the plant subsequently being introduced in the Mediterranean region and most other warm areas of the world (Grindlay and Reynolds 1986).

Aloe has been used extensively by the Egyptians, Assyrians, Mediterranean civilizations and in Biblical times (Grindlay and Reynolds 1986). The first authentic record of *Aloe* as a plant with healing properties is accredited to a Mesopotamian clay tablet dated at ca 2100 BCE. However, the first detailed depiction of the plant's medicinal value is found in the Papyrus Ebers, an Egyptian document dated at ca 1550 BCE, which sets out multiple *Aloe*-containing preparations for the treatment of external and internal ailments. The *Aloe vera* plant is described in detail in the Greek Herbal of Dioscorides (ca 70 AD), and its use promoted for the treatment of wounds, hair loss, genital ulcers, and hemorrhoids (Davis 1997). *Aloe vera* was officially listed as a purgative and skin protectant by the U.S. pharmacopoeia in 1820 (Park and Lee 2006) and was clinically used in the 1930s for the treatment of radiotherapy burns to the skin and mucous membranes (Collins and Collins 1935; Manderville 1939). Until today, *Aloe* is an important traditional medicine in many countries, including China, India, the West Indies, South Africa, and Japan (Grindlay and Reynolds 1986).

3.3 CURRENT USAGE

Aloe vera is one of the few herbal medicines widely used in Western society, with the manufacturing of *Aloe vera* extracts being one of the largest botanical industries worldwide (Grindlay and Reynolds 1986; Eshun and He 2004). In 2004, the value of the *Aloe* industry was estimated to be US$125 million for the cost of the raw *Aloe* material and US$110 billion for finished *Aloe*-containing products (International Aloe Science Council 2004). *Aloe vera* is used in the cosmetic, food, and pharmaceutical industries. In the cosmetic and toilet industry, it is used as a base material for skin moisturizers, soaps, shampoos, sun lotions, makeup creams, perfumes, shaving creams, bath aids, and many other products (Eshun and He 2004; Boudreau and Beland 2006). The food industry uses *Aloe* in the manufacture of functional foods, especially health drinks, and as a bitter agent (Saccu, Bogoni, and Procida 2001). Pharmaceutical products are available for topical applications (gels and ointments) and oral use (tablets and capsules; Hamman 2008).

3.4 STRUCTURE AND CHEMICAL CONSTITUENTS

Aloe vera is a perennial succulent xerophyte; it has elongated and pointed leaves that are joined at the stem in a rosette pattern and that grow to about 30–50 cm in length and 10 cm in breadth at the base in the adult plant (World Health Organization 1999). The leaf is protected by a thick, green epidermis layer (skin or rind), which surrounds the mesophyll. Immediately beneath the rind are located the vascular bundles, which are composed of three types of tubular structures: the xylem (transports water and minerals from roots to leaves), the phloem (transports starch and

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other synthesized products to the roots), and the large pericyclic tubules (contains the yellow leaf exudate commonly referred to as "aloes," "sap," or "latex"; Boudreau and Beland 2006). The pericyclic portion of the vascular bundle is adherent to the rind, whereas the remainder of the vascular bundle protrudes into the mesophyll layer (Danhof 1987). The mesophyll can be differentiated into chlorenchyma cells and thinner-walled parenchyma cells. The parenchyma (filet or pulp), which is the major part of the leaf by volume, contains a clear mucilaginous gel (known as *Aloe vera* gel; Femenia et al. 1999; Femenia et al. 2003).

Aloe vera is considered to be the most biologically active of the *Aloe* species (World Health Organization 1999). More than 75 potentially active constituents have been identified in the plant (Table 3.1) including vitamins, minerals, saccharides, amino acids, anthraquinones, enzymes, lignin, saponins, and salicylic acids. The leaf exudate contains anthraquinones, particularly barbaloin

TABLE 3.1
Classes and Selected Examples of Phytochemicals in *Aloe vera*

Class	Compound
Anthraquinones/anthrones	Aloe-emodin, aloetic-acid, anthranol, aloin A and B (or collectively known as barbaloin), isobarbaloin, emodin, and ester of cinnamic acid
Carbohydrates	Pure mannan, acetylated mannan, acetylated glucomannan, glucogalactomannan, galactan, galactogalacturan, arabinogalactan, galactoglucoarabinomannan, pectic substance, xylan, and cellulose
Chromones	8-C-glucosyl-(2'-O-cinnamoyl)-7-O-methylaloediol A, 8-C-glucosyl-(S)-aloesol, 8-C-glucosyl-7-O-methyl-(S)-aloesol, 8-C-glucosyl-7-O-methylaloediol, 8-C-glucosyl-noreugenin, isoaloeresin D, isorabaichromone, and neoaloesin A
Enzymes	Alkaline phosphatase, amylase, carboxypeptidase, catalase, cyclooxidase, cyclooxygenase, lipase, oxidase, phosphoenolpyruvate carboxylase, and superoxide dismutase
Inorganic minerals	Calcium, chlorine, chromium, copper, iron, magnesium, manganese, potassium, phosphorous, sodium, and zinc
Vitamins	B1, B2, B6, C, E, and folic acid
Miscellaneous, including organic compounds and lipids	Arachidonic acid, γ-linolenic acid, steroids (campesterol, cholesterol, β-sitosterol, triglycerides, triterpenoid, gibberellin, lignins, potassium sorbate, salicylic acid, uric acid, β-carotene, and choline
Amino acids	Alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tyrosine, and valine
Proteins	Lectins and lectin-like substance
Saccharides	Mannose, glucose, L-rhamnose, and aldopentose

Sources: Adapted from Vogler, B., and E. Ernst. 1999. *Br J Gen Pract* 49:823–28; Dagne et al. 2000. *Curr Org Chem* 4:1055–78; Choi, S., and M. H. Chung. 2003. *Semin Integr Med* 1:53–62; International Aloe Science Council. 2004. http://www.iasc.org/aloemarket.html (accessed August 18, 2009); Ni, Y., D. Turner, K. M. Yates, and I. Tizard. 2004. Isolation and characterization of structural components of Aloe vera L. leaf pulp. *Int immunopharmacol* 4:1745–55; Hamman, J. H. 2008. Composition and applications of *Aloe vera* leaf gel. *Molecules* 13:1599–616.

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(Figure 3.1), which appear to be responsible for its bitter taste and cathartic effect (Dagne et al. 2000; Boudreau and Beland 2006). Barbaloin and other products of the phenylpropanoid pathway are commonly referred to as polyphenolic compounds. These are derived from the precursor phenolic acids, and they may act as antioxidants to inhibit free radical–mediated cytotoxicity and lipid peroxidation (Cook and Samman 1996). *Aloe vera* also contains products of the isoprenoid pathway, including carotenoids, steroids, terpenes, and phytosterols (Samman 1998). Isoprenoids can be regarded as sensory molecules because they contribute to the color and fragrance of the products in which they exist.

Aloe vera gel is rich in polysaccharides, including acemannan (partially acetylated glucomannans; Figure 3.2), which has been reported as the primary active substance in the parenchyma (t'Hart et al. 1989). However, given the number of other potentially active compounds in the plant, it is possible that the biological activities of *Aloe vera* result from the synergistic action of a variety of compounds, rather than from a single defined component (Dagne et al. 2000; Hamman 2008). Equally, the potential for constituents to exhibit antagonistic and competitive activities also influences the overall biological activity of particular *Aloe vera* preparations (Hamman 2008).

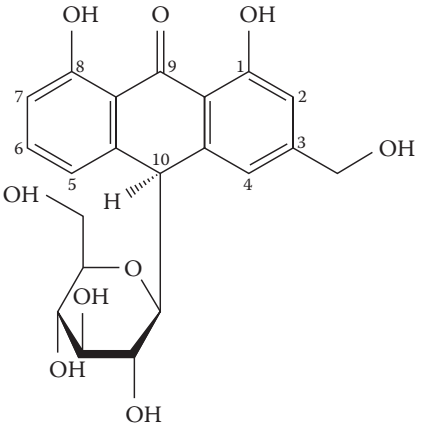


FIGURE 3.1 Structure of barbaloin (aloin), a glucoside of loe-emodin.

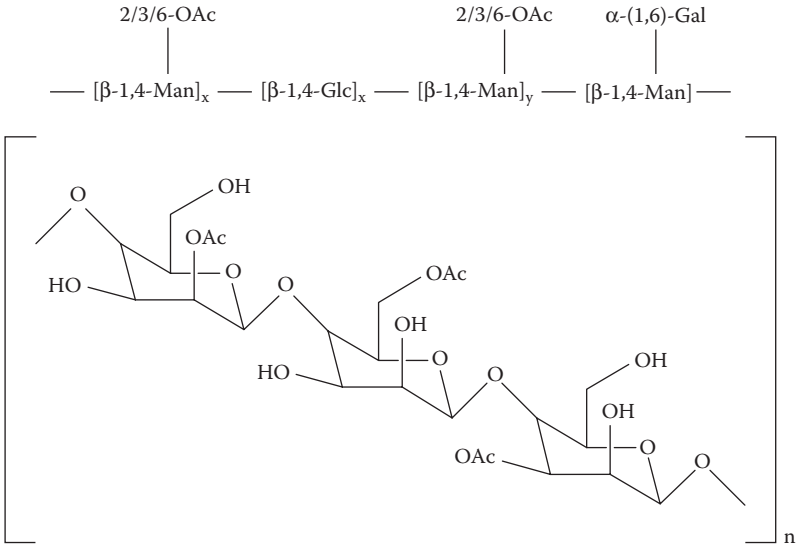


FIGURE 3.2 Structure of acemannan, a mucopolysaccharide that is extracted from *Aloe vera* leaves.

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3.5 EFFECT OF CULTIVATION AND PROCESSING

The composition of *Aloe vera* extracts differs according to the plant variety, climatic and seasonal variations, and the age of the plant (Eshun and He 2004). However, the processing method has the largest effect on the number and amount of active ingredients in a product (Wang and Strong 1995). The commercial production process of *Aloe vera* products typically involves crushing, grinding, or pressing of the whole *Aloe vera* leaf to produce juice, followed by various steps of filtration and stabilization to achieve the desired extract (Eshun and He 2004). This method provides ease of processing and higher efficiency in the recovery of the solids (Agarwala 1997), but it can result in a product that contains little or no active ingredients (Eshun and He 2004). In an analysis of 18 commercial *Aloe vera* products, only 9 exhibited quantifiable amounts of mucilaginous polysaccharide (Ross, Elsohly, and Wilkins 1997). Only three of the nine commercial *Aloe vera* gel powders sourced from leading international suppliers demonstrated satisfactory amounts of the polysaccharide Acemannan (Bozzi et al. 2007). Variable polysaccharide content in *Aloe vera* has been attributed particularly to heating the plant extract to >60°C, which results in significant changes in molecular weight (Turner et al. 2004). A further issue with the commercial production process is that during the commercial extraction of *Aloe vera* gel, it is virtually impossible to prevent the contamination by leaf exudates (Eshun and He 2004). Finally, the adulteration of *Aloe vera* products using fillers such as maltodextrin, glucose, glycerin, and malic acid represents a major concern for the *Aloe vera* market (Bozzi et al. 2007). As a counter to such misrepresentations in the industry, the International Aloe Science Council developed a certification program that validates the quality and quantity of *Aloe vera* in approved commercial products.

3.6 HEALTH EFFECTS: THE SCIENTIFIC EVIDENCE

The therapeutic claims for *Aloe vera* cover a broad range of conditions. It is commonly used topically in the treatment of dermatological and wound healing conditions. The oral application of the *Aloe vera* latex is promoted as a laxative, whereas gel and whole-leaf oral preparations have been variously recommended for use as an adjunct to chemotherapy treatment and to ameliorate diverse disorders such as DM, infectious diseases, metastatic cancer, and ulcerative colitis. The clinical use of *Aloe vera* is supported primarily by anecdotal evidence and case reports. The number of clinical trials exploring its effectiveness has begun to increase (Table 3.2); however, a standardization of methodological trial quality has yet to be achieved.

3.6.1 Topical Applications

The first case report of the beneficial effects of *Aloe vera* in the treatment of skin and wound healing was published in 1935, with fresh whole-leaf extract reported to provide rapid relief from the itching and burning associated with severe roentgen (radiation) dermatitis and complete skin regeneration (Collins and Collins 1935). Numerous subsequent reports have explored the role of topical *Aloe vera* administration in skin conditions and wound healing management, including psoriasis, dermatitis, oral mucositis, burn injuries, and surgical wounds.

3.6.1.1 Dermatological Conditions

Results of a number of clinical trials suggest that *Aloe vera* is positively indicated in the treatment of skin disorders. A trial of wound healing management after the full-faced dermabrasion of patients with acne vulgaris demonstrated that the saturation of a standard polyethylene wound gel dressing with *Aloe vera* significantly reduced time to reepithelization compared to use of the standard dressing alone (Fulton 1990). In a randomized, double-blind, controlled trial of *Aloe vera* or placebo cream in 60 patients with chronic psoriasis, the cure rate in the *Aloe vera* group was 83% (with no relapses at 12 months of follow-up) compared to only 7% in the placebo group (Syed et al. 1996).

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TABLE 3.2
Controlled Trials Investigating the Effectiveness of *Aloe vera* in the Treatment of Various Health Conditions in Humans

Health Condition	Study (Author, Year)	Treatment	Significant Effects of *Aloe vera* Treatment Compared to Control
		Gastrointestinal disorders	
Constipation	Odes and Madar 1991	*Aloe vera* + celandine + psyllium or placebo	Improved bowel movement frequency/consistency of stools/laxative dependence
	Chapman and Pittelli 1974	Aloin (from *Aloe vera* latex) or Phenolphthalein compared to placebo	Increased laxative effect
Irritable bowel syndrome	Davis et al. 2006	*Aloe vera* gel or placebo	No beneficial effect
Ulcerative colitis	Langmead et al. 2004	*Aloe vera* gel or placebo	No significant effects on clinical remission; improvements in the simple clinical colitis activity index and histological scores
Diabetes mellitus	Bunyapraphatsara et al. 1996	*Aloe vera* gel or placebo in combination with glibenclamide	Reductions in blood glucose and serum triglycerides (no effect on cholesterol)
	Yongchaiyudha et al. 1996	*Aloe vera gel* or placebo	Reductions in blood glucose and serum triglycerides (no effect on cholesterol)
	Nasiff, Fajardo, and Velez 1993	Whole-leaf *Aloe vera* extract or placebo	Reductions in total cholesterol, low-density lipoproteins, and triglycerides
		Disorders of the skin[a]	
Acne vulgaris	Fulton 1990	Polyethylene gel dressing + *Aloe vera*	Reduction in time to re-epithelization
Acute radiation dermatitis	Merchant et al. 2007	*Aloe vera* gel or a polar phospholipid-based cream	Less effective in reducing radiation-induced side effects
	Heggie et al. 2002	*Aloe vera* gel or aqueous cream	Less effective in reducing radiation-induced side effects
	Olsen et al. 2001	*Aloe vera* gel or placebo	No effect in reducing radiation-induced side effects
	Williams, Burk, and Loprinzi 1996	*Aloe vera* gel or placebo	No effect in reducing radiation-induced side effects
	Williams, Burk, and Loprinzi 1996	*Aloe vera* or no treatment	No effect in reducing radiation-induced side effects
Burn wounds	Maenthaisong et al. 2007[b]	Various treatments (including *Aloe vera* mucilage/cream; gauze + *Aloe vera* gel/powder)	Beneficial effect at a range of different doses in the treatment of burn wounds

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TABLE 3.2 (*Continued*)
Controlled Trials Investigating the Effectiveness of *Aloe vera* in the Treatment of Various Health Conditions in Humans

Health Condition	Study (Author, Year)	Treatment	Significant Effects of *Aloe vera* Treatment Compared to Control
Psoriasis	Heck et al. 1981	Gauze with *Aloe vera* or Silvadene cream	No significant reduction in healing time
	Syed et al. 1996	*Aloe vera* gel or placebo	Higher treatment success rate
	Paulsen, Korsholm, and Brandrup 2005	*Aloe vera* gel or placebo	Lower score of erythema + infiltration + desquamation
Radiation-related mucositis	Su et al. 2004	*Aloe vera* solution or conventional (baking soda mouth rinse, Benadryl + nystatin mouthwash)	No effect in reducing mucositis
Surgical wounds	Schmidt and Greenspoon 1991	*Aloe vera* gel or gauze dressing	Longer healing time than conventional treatment
Metastatic cancer	Lissoni et al. 1998	*Aloe vera* tincture (10% *Aloe vera*/90% alcohol) + melatonin or melatonin alone	Increased number of patients with stabilized disease and survival for 1 year

[a] *Aloe vera* treatments are applied topically for these conditions.
[b] This study was a meta-analysis of four controlled trials.

Converse results were reported in a later trial examining the efficacy of a commercial *Aloe vera* gel preparation in the treatment of slight to moderate psoriasis vulgaris. The *Aloe vera* or placebo gel was applied twice daily for 4 weeks to symmetrical test lesions using an intraindividual right/left comparison study design. The sum score of erythema, infiltration, and desquamation significantly favored the placebo treatment (Paulsen, Korsholm, and Brandrup 2005).

Further, despite case reports (Loveman 1937) and animal studies (Rowe 1940) to the contrary, *Aloe vera* extracts have either no effect or less effect than other topical treatments in acute radiation dermatitis. In the first of two randomized controlled trials in 194 women receiving radiation therapy for breast cancer, the topical self-administration of *Aloe vera* gel to radiation-exposed skin produced no difference in the severity of the dermatitis compared to a placebo gel. In the second study, the placebo group was replaced with a "no-treatment" group to account for any unintended beneficial effects of the inert carrier gel used as the placebo in the first trial. The results failed to show any benefit of the *Aloe vera* gel in preventing radiation-induced dermatitis (Williams, Burk, and Loprinzi 1996). Similarly, in 70 radiation therapy patients who were randomized to receive either commercially available *Aloe vera* gel or no treatment (other than mild soap), *Aloe vera* did not significantly protect against radiation-induced skin changes (Olsen et al. 2001). In a study involving 225 patients undergoing radiation therapy, the topical application of *Aloe vera* gel thrice a day throughout the treatment and for an additional 2 weeks after the completion of radiation therapy was significantly less efficacious in reducing the treatment-related side effects than aqueous cream (Heggie et al. 2002). In the pediatric setting, 45 patients undergoing radiation therapy for various diagnoses were treated with either an *Aloe vera*–based gel or a anionic polar phospholipid (APP)-based cream applied symmetrically within the irradiated field after each session. The authors reported statistically significant results favoring the APP-based cream on a number of skin assessment variables, including dryness, comfort, erythema, and peeling. The study was limited by a lack

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of description of randomization and blinding and the inclusion of patients with varied diagnoses, radiotherapy sites, and cancer treatment regimes (Merchant et al. 2007).

A 20 mL "swish and swallow" of *Aloe vera* solution (94.5% aloe juice) four times daily in addition to conventional treatment (baking soda mouth rinse, Benadryl and nystatin combination mouthwashes, and viscous lidocaine, as needed) did not improve radiation-related mucositis in patients with head-and-neck neoplasms. Study limitations included a small sample size, patient heterogeneity, a large distribution of primary cancer sites, and an inability to monitor compliance (Su et al. 2004).

3.6.1.2 Burn Injuries

Aloe vera has long been associated with the treatment of burns. With the advent of nuclear power, the U.S. government conducted research on the ability of *Aloe vera* to treat thermal and radiation burns with the aim of introducing its use into the military (Ashley et al. 1957). In 1959, the U.S. Food and Drug Administration approved the use of *Aloe vera* ointment as an over-the-counter medication for healing burns on the skin (Park and Lee 2006).

Heck et al. (1981) randomly assigned 18 patients with second-degree burns to be treated, after debridement, with gauze containing either *Aloe vera* cream or Silvadene ointment. The *Aloe vera* group had a mean healing time of 13 days compared to 16 days in the Silvadene group; however, the difference did not reach statistical significance. In a recent meta-analysis, a statistically significant benefit of *Aloe vera* for the treatment of burns was demonstrated. Using the duration of wound healing as an outcome measure, the meta-analysis of the efficacy of *Aloe vera* in burn wound healing concluded that *Aloe vera* treatments reduced healing time by approximately 9 days compared to conventional treatment groups ($p = .006$; Maenthaisong et al. 2007). Four controlled clinical trials (with a total of 371 subjects) met the inclusion criteria for the review. The four studies differed in their study design, intervention, and reported outcomes. The *Aloe vera* preparations included fresh *Aloe vera* mucilage (Thamlikitkul et al. 1991), gauze saturated with 85% *Aloe vera* gel (Visuthikosol et al. 1995), *Aloe vera* cream (Akhtar and Hatwar 1996), and 1% *Aloe vera* powder wrapped with Vaseline gauze (Sun et al. 1994). None of the studies standardized the amount of active *Aloe vera* ingredients administered. The outcomes measured were wound healing time, described as time to complete epithelization (Visuthikosol et al. 1995) or not defined (Akhtar and Hatwar 1996); the success rate of wound healing (Thamlikitkul et al. 1991); and epithelization rate (Sun et al. 1994). Maenthaisong et al. (2007) note that, due to differences in *Aloe vera* products and outcome measures used, it is difficult to draw a specific conclusion regarding the effect of *Aloe vera* on burn healing. Nonetheless, the results of the review combined with other evidence suggest that *Aloe vera* preparations at a range of different doses are beneficial in the treatment of burn wounds.

3.6.1.3 Surgical Wound Healing

Aloe vera has been reported to accelerate postoperative wound healing in periodontal flap surgery (Payne 1970). Conversely, a randomized controlled trial involving women with complications of wound healing after gynecological surgery found that the mean healing time in the conventional care group (53 days) was significantly shorter ($p < .003$) than in the *Aloe vera* gel group (83 days; Schmidt and Greenspoon 1991). The results of the trial must be interpreted with caution, as only 21 of 40 women completed the study and more patients were lost to follow-up from the gauze group ($n = 12$) than the *Aloe vera* group ($n = 5$). An intention-to-treat analysis was not performed (meaning that patients lost to follow-up were excluded from the analysis), which potentially introduces significant bias into the results.

3.6.2 Oral Applications

Therapeutic claims promote the use of oral *Aloe vera* in the treatment of a wide range of conditions, such as alopecia, Alzheimer's disease, congenital heart failure, depression, glaucoma, hemorrhoids, hepatitis, multiple sclerosis, and varicose veins; however, scientific investigations of such claims are

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limited. Claims that have been the subject of clinical trials include the oral application of *Aloe vera* preparations in the treatment of constipation, DM, metastatic cancer, and ulcers and inflammation of the gastrointestinal tract.

3.6.2.1 Laxative

Aloe vera latex is commonly used in the treatment of constipation (de Witte 1993); the laxative effect of the anthraquinone glycosides found in *Aloe vera* latex is well established (Ulbricht et al. 2008). In a double-blind, randomized, controlled trial of 28 healthy adults, aloin was reported to have a laxative effect compared to a placebo that was stronger than the stimulant laxative phenolphthalein (Chapman and Pittelli 1974). In subjects with chronic constipation, a novel preparation containing *Aloe vera*, celandine, and psyllium was found to improve a range of constipation indicators (bowel movement frequency, consistency of stools, and laxative dependence) in a 28-day double-blind trial; however, the effect of *Aloe vera* alone was not investigated in this study (Odes and Madar 1991). *Aloe vera* laxative preparations have been approved by the German Commission E governmental regulatory agency for use in the treatment of constipation as a second-line agent; however, *Aloe* latex is no longer recognized as an over-the-counter drug by the U.S. Food and Drug Administration due to a lack of sufficient data to establish its safety for use as a laxative.

3.6.2.2 Diabetes Mellitus

Aloe vera is a traditional remedy for diabetes mellitus (DM) in many parts of the world, including Latin America (Coronado et al. 2004) and the Arabian Peninsula (Yeh et al. 2003). Some evidence in humans and animals suggests that *Aloe vera* is able to alleviate the chronic hyperglycemia and perturbed lipid profile that are characteristic of DM, which are major risk factors for cardiovascular complications in the disease.

Agarwal (1985) reported hypoglycemic and hypolipidemic effects from the long-term dietary administration of 100 g of an *Aloe vera* gel preparation combined with 20 g of psyllium seed husks. The study involved 5000 patients aged 35–65 years with atheromatous heart disease, a population that included 3167 noninsulin-dependent diabetic patients. Marked reductions were noted in serum cholesterol, triglycerides, and total lipid levels, along with an increase in high-density lipoprotein (HDL) cholesterol. All but 177 of the diabetic patients demonstrated a normalization of fasting and postprandial blood glucose levels that necessitated the withdrawal of all oral hypoglycemic agents by the end of 2 months of therapy. A beneficial effect of *Aloe vera* gel alone on blood glucose and lipid parameters in diabetic subjects also has been demonstrated. In the first of two related clinical trials, 72 diabetic women without drug therapy were administered one tablespoon of *Aloe vera* gel or placebo for 6 weeks. Blood glucose and serum triglyceride levels were significantly decreased with *Aloe vera* treatment, although cholesterol concentrations were unaffected (Yongchaiyudha et al. 1996). In the second trial, the effects of *Aloe vera* gel or placebo in combination with glibenclamide (a commonly prescribed antidiabetic medication) were investigated, similarly resulting in significant reductions in blood glucose and serum triglyceride concentrations in the *Aloe vera* group (Bunyapraphatsara et al. 1996).

In addition to gel preparations, *Aloe vera* latex has been shown to lower fasting blood glucose levels in case studies of five patients with noninsulin-dependent DM (Ghannam et al. 1986). Further, the whole-leaf *Aloe vera* extract administered to 60 patients with hyperlipidemia in a 12-week controlled clinical trial resulted in significantly decreased levels of total serum cholesterol, triglycerides, and low-density lipoproteins (LDLs; Nasiff, Fajardo, and Velez 1993). However, although studies in humans provide promising preliminary data that denote a beneficial effect of *Aloe vera* in diabetes and associated cardiovascular complications, effects have yet to be confirmed by controlled clinical trials that are both randomized and blinded to subjects and investigators.

Animal studies exploring the effects of *Aloe vera* on blood glucose and lipids have demonstrated less consistent results likely due to different combinations of animal models and *Aloe vera* preparations used. In rodent models, both the chronic administration of *Aloe vera* latex to alloxan-induced

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diabetic mice (Ajabnoor 1990) and *Aloe vera* gel to streptozotocin (STZ)-induced diabetic rats (Rajasekaran et al. 2004) resulted in significant reductions in fasting blood glucose. Conversely, *Aloe vera* gel was reported to increase plasma glucose levels in alloxan-induced diabetic rats (Koo 1994). More recently, the antidiabetic effects of processed *Aloe vera* gel were investigated in mice exhibiting diet-induced obesity (DIO), an animal model that has been shown to demonstrate metabolic abnormalities that closely resemble those found in human noninsulin-dependent DM, including hyperglycemia, obesity, and insulin resistance (Kim et al. 2009). Oral administration of the gel reduced circulating blood glucose concentrations to a normal level, significantly decreased plasma insulin, and lowered triglyceride levels in the liver and plasma of the DIO mice. Similarly, *Aloe vera* gel extract has been shown to normalize the fasting blood glucose and plasma insulin levels and reduce the concentrations of cholesterol, triglycerides, and free fatty acids in the plasma, liver, and kidney of STZ-induced diabetic rats (Rajasekaran et al. 2006).

3.6.2.3 Metastatic Cancer

The concomitant oral administration of 1 mL twice a day of *Aloe vera* tincture (10% *Aloe vera* and 90% alcohol) and 20 mg/day of melatonin compared to melatonin alone was studied in 50 patients with locally advanced or metastatic solid tumors for whom no other effective standard therapy was available. In the group treated with *Aloe vera* and melatonin combined, 12 of 24 patients had their disease stabilized compared to only 7 of 26 patients in the melatonin-only group. In addition, the percentage of individuals surviving 1 year was significantly higher with *Aloe vera* plus melatonin compared with melatonin treatment alone (Lissoni et al. 1998).

3.6.2.4 Ulcers and Inflammation of the Gastrointestinal Tract

Aloe vera preparations are widely promoted for the treatment of gastrointestinal disorders, including ulcers and inflammatory bowel disease, but evidence of their effectiveness is inconsistent. In 1963, clinical evidence of the successful use of *Aloe vera* gel (administered in a heavy liquid petrolatum emulsion) was reported for the treatment of 12 patients with peptic ulcers (Blitz, Smith, and Gerard 1963). In a 3-month randomized controlled trial of 58 patients with irritable bowel syndrome, no evidence was found to suggest that *Aloe vera* has any beneficial effect (Davis et al. 2006).

A recent attempt to formally evaluate the efficacy and safety of *Aloe vera* gel in the treatment of ulcerative colitis produced encouraging, although not conclusive, results. In a randomized controlled trial of 44 subjects with mild to moderately active ulcerative colitis, the oral administration twice daily of 100 mL *Aloe vera* gel to 30 subjects for 4 weeks generated clinical remission and improvement more often than in the placebo group (14 subjects); however, despite positive trends the results failed to reach statistical significance. The simple clinical colitis activity index and histological scores showed small statistically significant improvements in the *Aloe vera* group. Six patients (20%) who were given *Aloe vera* gel and three patients (21%) who were given placebo withdrew from the study because of deterioration or a failure to improve sufficiently but were included in the statistical analyses. The *Aloe vera* preparation used in this study was reported to contain a high proportion (>95%) of *Aloe vera* pulp, and the dose administered was the maximum recommended by the manufacturers. No adverse effects were observed during the trial, and the authors note that a higher dose may have been more efficacious and suggest the need for further, larger controlled trials of *Aloe vera* gel in active ulcerative colitis and in the maintenance of remission (Langmead et al. 2004).

3.7 ACTIVE INGREDIENTS AND MECHANISMS OF ACTION

A large number of biological activities have been ascribed to *Aloe vera* to explain its purported health benefits, including antimicrobial, anti-inflammatory, lipid and glucose lowering, antiproliferative, immunostimulatory, and antioxidant functions. A number of potentially active ingredients in the latex and gel of *Aloe vera* have been identified; however, much has yet to be determined about

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their mechanisms of action. Further studies are also required to determine the active properties of numerous other *Aloe vera* constituents and to explore the competitive or synergistic actions of particular combinations of ingredients.

3.7.1 Active Latex Constituents

The major C-glycosides, barbaloin (Figure 3.1) and isobarbaloin, have been shown to be the principal agents responsible for cathartic and other effects of *Aloe vera* latex in humans and animals. Both barbaloin and isobarbaloin undergo decomposition in the large intestine to form the active metabolites aloe-emodin-9-anthrone and aloe-emodin (Figure 3.3), which induce laxation via multiple mechanisms. In vitro and in vivo studies in rats demonstrated that aloe-emodin-9-anthrones reduce the absorption of water from the intestinal lumen by inhibiting the activity of Na^+, K^+-adenosine triphosphatase (ATPase) and stimulate water secretion by increasing the paracellular permeability across the colonic mucosa (Ishii, Tanizawa, and Takino 1990). Secretion of water into the lumen by a prostaglandin-dependent mechanism has also been reported (Capasso et al. 1983). The net result is a reduction in water absorption and the formation of softer stools (Boudreau and Beland 2006). Aloe-emodin has been suggested to have antiangiogenic properties; it has been demonstrated to be a potent inhibitor of urokinase secretion and tubule formation of endothelial cells, both key events in angiogenesis (Cárdenas, Quesada, and Medina 2006).

3.7.2 Active Gel Constituents

Polysaccharides, particularly mannose-containing polysaccharides, cellulose, and pectic polysaccharides, comprise the major part of *Aloe vera* gel. Acetylated glucomannan is primarily responsible for the gel's mucilaginous properties (Hamman 2008) and has been found in vitro and in animal studies to modulate immune function (through macrophage activation and cytokine production) and accelerate wound healing (Ulbricht et al. 2008). Veracylglucan B and veracylglucan C (Figure 3.4), two maloyl glucans isolated from *Aloe vera* gel, have been demonstrated in vitro to have potent anti-inflammatory effects, although their effects on cell proliferation appear antagonistic (Esua and Rauwald 2006).

Among the nonpolysaccharide gel constituents, salicylic acid and other antiprostaglandin compounds may contribute to the local anti-inflammatory activity of *Aloe vera* via the inhibition of cyclooxygenase (Ulbricht et al. 2008). Potent antioxidant effects, including the ability to scavenge superoxide anions, have been attributed to the caffeoyl group of isorabaichromone, a derivative of

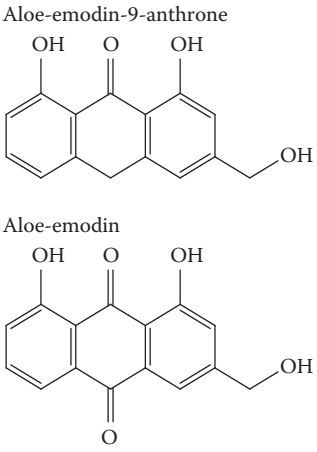


FIGURE 3.3 *Aloe*-emodins (anthraquinones) isolated from *Aloe vera*.

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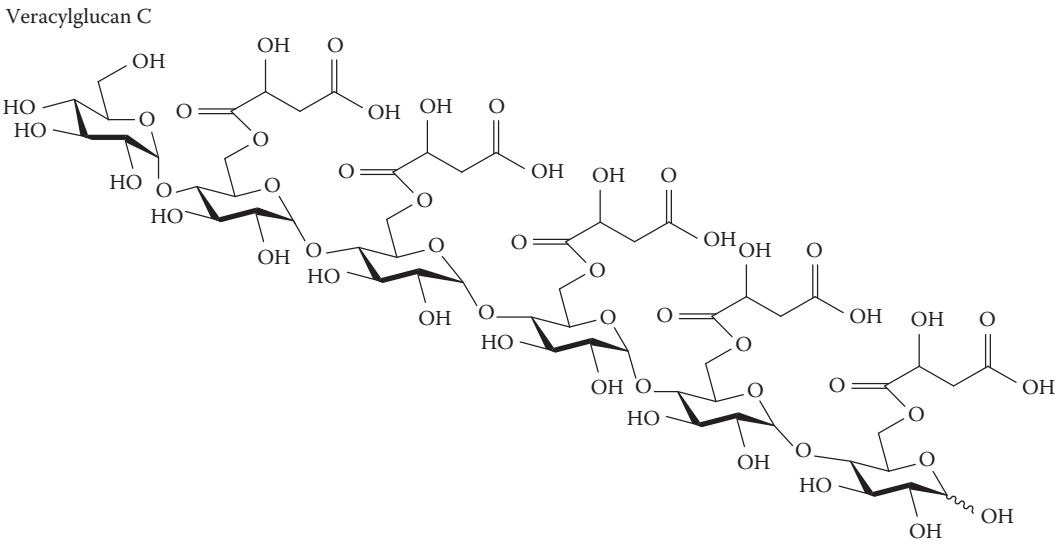


FIGURE 3.4 Veracylglucan C: a maloyl glucan isolated from *Aloe vera*.

aloesin (C-glycosylated 5-methylchromone). Five phytosterols have been isolated from *Aloe vera* gel based on their ability to decrease the HbA1c level in a mouse model (*db/db*) of type 2 DM. Each of the phytosterols, namely lophenol, 24-methyl-lophenol, 24-ethyl-lophenol, cycloartanol, and 24-methylene-cycloartanol, was shown to significantly decrease fasting blood glucose levels in the *db/db* mice compared to controls at a dose of 1 μg/day (Tanaka et al. 2006). Phytosterols are not extensively absorbed from the intestine but can bind cholesterol and prevent it from being absorbed (Ralph and Provan 2000). Phytosterols have been shown to lower plasma cholesterol concentrations, including the atherogenic LDL fraction (Moghadasian and Frohlich 1999). The mechanisms of action by which *Aloe vera* modulates blood glucose are unknown, but it has been suggested that it may interact with insulin. It has been hypothesized that *Aloe* stimulates insulin synthesis or its release from pancreatic β cells (Ajabnoor 1990). Processed *Aloe vera* gel was found to suppress the expression of the adipogenic genes *SREBP-1a*, *FAS*, and *GPAT*, suggesting that the gel improves insulin resistance by the reducing toxic effects of lipids in the liver (Kim et al. 2009).

3.8 SAFETY AND EFFICACY

Determining the safety and efficacy of *Aloe vera* is difficult due to the lack of standardization of commercially available *Aloe vera* preparations. Similarly, the need for a more detailed understanding of the plant's active components makes it difficult to evaluate the optimal doses of particular *Aloe vera* preparations for the treatment of specific disorders.

Despite these challenges, a recent systematic review of *Aloe vera* by the Natural Standard Research Collaboration concluded that topical application of *Aloe vera* gel or extract is safe for the treatment of mild to moderate skin conditions, burns, wounds, and inflammation (Ulbricht et al. 2008). In terms of efficacy, reasonable evidence in humans supports the topical use of *Aloe vera* for the treatment of burn wounds. Evidence for its use in psoriasis, dermatitis, and surgical wound healing is conflicting.

The Natural Standard Research Collaboration further concluded that the oral use of *Aloe vera* gel for its potential hypoglycemic effects and the short-term use of oral *Aloe* latex as a laxative are possibly safe; however, prolonged use of the latex is likely to be unsafe due to a theoretical risk of dehydration and electrolyte imbalance (Ulbricht et al. 2008). The cathartic properties of anthraquinone glycosides found in *Aloe vera* latex are well established. However, given the potential safety concerns with its use, there is a need for further clinical trials to investigate the benefits of latex

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administration over conventional laxative treatments. Although inconclusive, there is some preliminary evidence of a favorable effect of *Aloe vera* gel taken orally in type 2 DM, ulcerative colitis and the stabilization of metastatic cancer.

3.8.1 Toxicology

Until now there are no published controlled in vivo toxicology studies of *Aloe vera* in humans (Steenkamp and Stewart 2007). In animal studies, *Aloe vera*-derived ingredients were not found to be toxic in acute oral studies using mice and rats. In mice, the LD_{50} was >200 mg/kg and >80 mg/kg in parenteral and intravenous studies, respectively, whereas in rates the corresponding LD_{50} values were >50 mg/kg and >15 mg/kg, respectively. No significant toxicity was seen with acemannan given intravenously or intraperitoneally at 4-day intervals over 30 days at maximum dose levels of 200 mg/kg in mice and 50 mg/kg in rats (Cosmetic Ingredient Review Expert Panel 2007). The no observed adverse effect level (NOAEL) for whole-leaf *Aloe vera* powder was 87.7 and 109.7 mg/kg/day in male and female rats, respectively (Matsuda et al. 2007). Life-long *Aloe vera* gel ingestion (contributing 1% of total diet) in rats was demonstrated to produce no harmful effects or deleterious changes (Ikeno et al. 2002). In contrast, chronic ingestion of 100 mg/kg *Aloe vera* (extracted in ethanol) given orally in rats produced reproductive toxicity, significant sperm damage, inflammation, and mortality compared to control animals (Shah et al. 1989). In a recent safety assessment of *Aloe*, the Cosmetic Ingredient Review Expert Panel (2007) concluded that *Aloe* latex, but not the polysaccharide material derived from the inner gel, is cytotoxic.

3.8.2 Carcinogenicity

Tumor-promoting and antimutagenic activities have been ascribed to the latex of *Aloe vera* (Boudreau and Beland 2006). Multiple in vitro studies have demonstrated the potential genotoxicity of anthraquinones; however, anthraquinones in *Aloe vera* do not appear to be well absorbed, and four in vivo studies resulted in no genotoxicity from aloe-emodin and emodin (Brusick and Mengs 1997). Anthranoid-containing laxatives such as aloe-emodin have been suggested to cause colorectal cancer (Siegers et al. 1993); however, recent research has not shown any correlation. A 2-year carcinogenicity study in rats reported that whole-leaf *Aloe* powder was not carcinogenic at nontoxic dose levels in the colon (Yokohira et al. 2009). In many large epidemiological studies in humans, long-term laxative abuse has not been associated with colorectal cancer (Nusko et al. 2000; Park et al. 2009).

3.8.3 Photoxicity

Phototoxicity of aloe-emodin has been demonstrated in animal studies; however, phototoxicity was not observed in several clinical studies in humans using amounts of aloe-emodin that are commonly found in commercially available *Aloe vera* preparations (Cosmetic Ingredient Review Expert Panel 2007).

3.8.4 Adverse Effects

In the reviewed clinical trials, no serious adverse reactions were reported following *Aloe vera* administration. Three patients experienced allergic reactions after the topical application of an *Aloe vera* preparation (Williams, Burk, and Loprinzi 1996). In case reports, hypersensitivity and allergic responses to *Aloe vera* are the most commonly described adverse effects of *Aloe vera* use. The topical application of *Aloe vera* gel has resulted in contact dermatitis, and oral use may cause diarrhea or vomiting (Morrow, Rapaport, and Strick 1980; Ernst 2000; Wang et al. 2003; Chinnusamy et al. 2009). Many of these reactions appear to be associated with anthraquinone contaminants of the gel product.

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In rare cases, severe adverse effects have been associated with the oral application of *Aloe vera*. Induced acute toxic hepatitis has been observed in four instances of *Aloe vera* ingestion (Luyckx et al. 2002; Rabe et al. 2005; Kanat, Ozet, and Ataergin 2006). In one case, a 47-year-old man presented with acute oliguric renal failure and liver dysfunction after consuming high oral doses of *Aloe vera* (Luyckx et al. 2002). *Aloe vera* was also believed to be the cause of hypothyroidism in one female patient (Pigatto and Guzzi 2005) and Henoch–Schonlein purpura in another after an *Aloe vera* remedy juice was taken for back pain (Evangelos, Spyros, and Spyros 2005).

3.8.5 Contraindications and Drug Interactions

Allergy: Use of *Aloe vera* preparations should be avoided in individuals with a known allergy to plants of the Liliaceae family (garlic, onions, and tulips; Ulbricht et al. 2008).

Pregnancy: Use of *Aloe vera* as a laxative during pregnancy may pose potential teratogenic and toxicological effects on the embryo and fetus (World Health Organization 1999; Ulbricht et al. 2008).

Renal or cardiac disease: Prolonged use of *Aloe vera* latex has been associated with watery diarrhea resulting in electrolyte imbalance (Cooke 1981; Boudreau and Beland 2006), and anecdotal reports suggest that the increasing loss of potassium may lead to hypokalemia. Therefore, the *Aloe vera* latex is contraindicated in patients with a history of renal or cardiac disorders.

Drug interactions: Potential interactions have been suggested for *Aloe vera* and drugs that may alter electrolyte balance, such as thiazide diuretics and corticosteroids. Possible hypokalemia-related arrhythmia suggests a potential herb–drug interaction with cardiac glycosides. Caution is warranted in patients taking hypoglycemic agents as interactions with *Aloe vera* gel have been reported (Boudreau and Beland 2006). There exists a case report of a 35-year-old woman who lost 5 L of blood during surgery as a result of a possible herb–drug interaction between *Aloe vera* and sevoflurane, an inhibitor of thromboxane A_2 (Lee et al. 2004).

3.9 DRUG/VITAMIN BIOAVAILABILITY

Aloe vera gel has been shown to enhance vitamin C and E's bioavailability in a double-blind, randomized, controlled trial (Vinson, Al Kharrat, and Andreoli 2005). The authors suggest that *Aloe vera* gel protects against the degradation of vitamins in the intestinal tract and the gel polysaccharides may bind to vitamins and thereby slow down their absorption rate.

Aloe vera gel has been shown to significantly increase the transport of insulin in a cell model, and limited information suggests that if coadministered, it may also enhance the intestinal absorption of other poorly absorbed drugs (Hamman 2008).

3.10 RESEARCH NEEDS

Analysis of the potential efficacy of *Aloe vera* in the treatment of particular disorders is complicated by differences in *Aloe vera* preparations, their means of administration, and the animal model or study design employed in individual studies. Research on standardized methodological quality is, therefore, needed to identify which *Aloe vera* components, individually or in combination, exhibit therapeutic properties and the exact mechanisms by which they act. Controlled in vivo toxicology and safety studies of *Aloe vera* preparations in humans are also required.

3.11 CONCLUSIONS

Despite its long history of use, there remains a lack of consistent scientific evidence to support many of the therapeutic claims for *Aloe vera*. Evidence of efficacy is strongest for the laxative effects of *Aloe vera* latex, however, whether the latex is more efficacious than conventional laxative treatments

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has not yet been determined, and the anthraquinones in the latex are associated with considerable risks. The topical application of *Aloe vera* gel is likely safe and demonstrates overall efficacy in healing burn wounds, whereas some promising preliminary evidence suggests that the oral use of the gel may have beneficial effects in lowering blood glucose levels in type 2 DM, stabilizing metastatic cancer, and treating mild to moderate ulcerative colitis. Further research in humans is required to confirm these effects.

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4 Bilberry (*Vaccinium myrtillus* L.)

Wing-kwan Chu, Sabrina C. M. Cheung, Roxanna A. W. Lau, and Iris F. F. Benzie

CONTENTS

4.1 Introduction 55
4.2 Bilberry: Description, Usage, Source, and Composition 55
4.3 Anthocyanins 57
4.4 Bioavailability and Distribution of Anthocyanins 58
4.5 Health Effects of Bilberry 59
4.5.1 Antioxidant Effects 59
4.5.2 Genoprotective and Anticancer Effects 60
4.5.3 Cardioprotective Effects 62
4.5.4 Anti-Inflammatory Effects 63
4.5.5 Hypoglycemic Effects 63
4.5.6 Ocular Effects 65
4.5.7 Neuroprotective Effects 65
4.5.8 Antimicrobial Effects 66
4.6 Conclusion 67
References 67

4.1 INTRODUCTION

Bilberry (*Vaccinium myrtillus* L.) is one of the richest natural sources of anthocyanins. These polyphenolic components give bilberry its blue/black color and high antioxidant content, and they are believed to be the key bioactives responsible for the many reported health benefits of bilberry and other berry fruits. Although bilberry is promoted most commonly for improving vision, it has been reported to lower blood glucose, to have anti-inflammatory and lipid-lowering effects, and to promote antioxidant defense and lower oxidative stress. Therefore, bilberry is of potential value in the treatment or prevention of conditions associated with inflammation, dyslipidemia, hyperglycemia or increased oxidative stress, cardiovascular disease (CVD), cancer, diabetes, and dementia and other age-related diseases. There are also reports that bilberry has antimicrobial activity. In this chapter, bilberry and its components and characteristics are described, and evidence for the health benefits of bilberry is presented and discussed.

4.2 BILBERRY: DESCRIPTION, USAGE, SOURCE, AND COMPOSITION

The bilberry plant (Figure 4.1) is a low-growing shrub native to northern Europe, but is now also found in parts of North America and Asia. Bilberry is also known as European blueberry, whortleberry, huckleberry, and blaeberry. It belongs to a large genus (*Vaccinium*) of plants that also contains blueberry (*Vaccinium corymbosum*) and cranberry (*Vaccinium macrocarpon*; Upton 2001). Bilberry is sometimes called blueberry because both have similar appearance and are close relatives, but the true blueberry is native to the United States (National Institutes of Health 2010). Bilberry usually grows in heaths, meadows, and moist coniferous forests, and its growth is favored by moderate shade

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FIGURE 4.1 **(See color insert.)** Bilberry (*Vaccinium myrtillus* L.). (ukwildflowers.com. With permission.)

and moderately humid ground conditions. The bilberry is a small (5–9 mm in diameter) fruit, bluish black in color, with many seeds.

Bilberry is classified as a Class 1 herb by the American Herbal Products Association (Upton 2001), meaning it can be safely consumed when used appropriately. No mutagenic activity has been reported, and there are no cited contraindications to its use (Upton, 2001). Bilberry is sold as fresh, frozen, and dried whole berries, as well as in the form of preserves, jams, and juices, and, increasingly, liquid or powdered concentrates are sold as food supplements. Bilberry contains a variety of phenolic compounds, including flavonols (quercetin, catechins), tannins, ellagitannins, and phenolic acids, but anthocyanins make by far the largest contribution to its phytochemical mix (Upton 2001; Seeram 2008). These naturally occurring phenolic compounds are redox-active antioxidants as well as iron chelators (Benzie 2003; Zafra-Stone et al. 2007), and are found in red-, blue-, and purple-colored flowers, fruits, and vegetables. The usual daily dietary intake of anthocyanins is approximately 200 mg (Zafra-Stone et al. 2007). Bilberry has higher anthocyanin content compared to other types of berries, such as strawberry, cranberry, elderberry, sour cherry, and raspberry (Kowalczyk et al. 2003; Bagchi et al. 2004; Yildirim 2006; Cravotto et al. 2010). The total anthocyanin content of bilberry is generally in the range of 300–700 mg/100 g fresh fruit, although this range varies with cultivar, growing conditions, and degree of ripeness of the berry (Upton 2001; Burdulis et al. 2009). Along with anthocyanins, 100 g of fresh bilberry contains small quantities of vitamin C (3 mg), quercetin (3 mg), and catechin (20 mg; Upton 2001; Erlund et al. 2003).

Although most attention has been focused on the antioxidant properties of anthocyanins in relation to health benefits of bilberry, the effects are likely to extend beyond simple antioxidant action to involve cell-signaling pathways, gene expression, DNA repair, and cell adhesion, as well as antineoplastic and antimicrobial effects (Kowalczyk et al. 2003; Packer and Cadenas 2007; Zafra-Stone et al. 2007; Seeram 2008; Benzie and Wachel-Galor 2010). Commercial bilberry products are often standardized to a 25% anthocyanidin content (equivalent to 36% anthocyanins); but this content can

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vary greatly (Upton 2001; Lee 2008). Recommended daily dosages also vary greatly, for example, 20–60 g of dried berries and 160–480 mg of powdered extract (Upton 2001).

4.3 ANTHOCYANINS

Anthocyanins (from the Greek *anthos* for flower and *kyanose* for blue) are water-soluble polyphenolic flavonoid compounds (Clifford 2000; Ghosh and Konishi 2007; Yoshida, Mori, and Kondo 2009). Anthocyanins are responsible for the pink, red, blue, and purple color of plants (Upton 2001). The color is pH dependent; the color is red at pH < 2, changing to blue as pH increases and finally becoming colorless at high pH. The anthocyanin molecule consists of an anthocyanidin "core" with a sugar moiety attached. The sugar can be attached at various positions, and can be glucose, galactose, xylose, arabinose, or rhamnose (Clifford 2000; Kong et al. 2003; Prior and Wu 2006). Anthocyanins vary also in the number and position of hydroxyl and methoxyl groups attached to the core anthocyanidin structure. Therefore, although there are less than 20 naturally occurring anthocyanidins, there are many hundreds of different anthocyanins (Upton 2001; Prior and Wu 2006; Ghosh and Konishi 2007; Yoshida, Mori, and Kondo 2009). The most commonly found anthocyanidins in nature are cyanidin, delphinidin, petunidin, peonidin, pelargonidin, and malvidin, but these are very rarely found in their aglycone (nonsugar) forms (Kong et al. 2003; Prior and Wu 2006). The concentrations of the main anthocyanins found in bilberry are given in Table 4.1.

TABLE 4.1
Main Anthocyanins in Bilberry and Their Relative Concentrations

Anthocyanin	Mean Content (%)
Delphinidins (total)	15.17
Delphinidin-3-O-glucoside	5.81
Delphinidin-3-O-galactoside	5.04
Delphinidin-3-O-arabinoside	4.32
Cyanidins (total)	8.36
Cyanidin-3-O-glucoside	3.42
Cyanidin-3-O-galactoside	2.75
Cyanidin-3-O-arabinoside	2.19
Petunidins (total)	6.64
Petunidin-3-O-glucoside	3.67
Petunidin -3-O-galactoside	1.89
Petunidin-3-O-arabinoside	1.08
Malvidins (total)	5.43
Malvidin-3-O-glucoside	3.35
Mavlidin-3-O-galactoside	1.27
Malvidin-3-O-arabinoside	0.81
Peonidins (total)	1.87
Peonidin-3-O-glucoside	1.31
Peonidin-3-O-galactoside	0.34
Peonidin-3-O-arabinoside	0.22

Source: Adapted from Upton, R., ed. 2001. *Bilberry Fruit Vaccinium myrtillus L. Standards of Analysis, Quality Control, and Therapeutics.* Santa Cruz, CA: American Herbal Pharmacopoeia and Therapeutic Compendium.

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Although the function of anthocyanin pigments in plants is thought to be mainly that of attracting animals, insects, and birds for pollination or seed dispersal, anthocyanins may help in cold tolerance as well as antimicrobial and antioxidant defense of plant tissues (Benzie 2003; Kong et al. 2003). The anthocyanin content of berries varies across species and also depends on environmental factors, such as the amount of solar radiation, temperature, and soil content of nitrogen and phosphorus. The cultivation technique is also a factor affecting the total phenolic level of berries. Anthocyanins are found mainly in the skin of the berry, and damage to the fruit skin caused during harvesting decreases anthocyanin content (Upton 2001). Degrees of ripeness of berries also contribute to variation in total phenolic content—although the concentration of phenolic compounds is usually higher in unripe berries than in mature fruits, anthocyanins accumulate during bilberry maturation (Upton 2001). Anthocyanins have powerful antioxidant properties, and the content of anthocyanin pigment directly correlates with antioxidant activity of plants (Upton 2001; Bagchi et al. 2004; Yildirim 2006; Zafra-Stone et al. 2007). In addition to their antioxidant effects, anthocyanins have been reported to stabilize DNA, modify adipocyte gene expression, improve insulin secretion and sensitivity, and have antiapoptotic, anti-inflammatory, and antibacterial effects (Mas et al. 2000; Kong et al. 2003; Kowalczyk et al. 2003; Tsuda et al. 2005; Seeram 2008). These numerous and potentially highly beneficial effects of anthocyanins make foods rich in these compounds, such as bilberry, potential candidates as "functional foods" and phytotherapeutics (Cravotto et al. 2010).

4.4 BIOAVAILABILITY AND DISTRIBUTION OF ANTHOCYANINS

Unlike other polyphenolic flavonoids, anthocyanins can be absorbed intact, that is, without removing their sugar moiety. Absorption (and renal elimination) of anthocyanins is reported to be rapid, but of low efficiency (Prior and Wu 2006). In rats, absorption takes place in the stomach and small intestine, and varies depending on the structure of the anthocyanin. Absorption ranged from 11% for malvidin-3-glucoside to 22% for cyanidin-3-glucoside (Talavera et al. 2003). Plasma anthocyanins are found in plasma a few minutes after oral administration of berries or berry extracts, but are cleared within 6 hours. In humans, anthocyanin absorption from grapes, red wine, elderberry, blackcurrant, chokeberry, hibiscus, and raspberry has been studied (reviewed by Prior and Wu 2006). Most studies showed a maximal plasma concentration of anthocyanin at 1–2 hours postingestion (range 0.5–3.0 hours). Maximal plasma concentrations were not clearly related to dose and were generally in the 5–50 nmol/L range. The highest plasma concentration reported (99 nmol/L) occurred after a dose of red grape juice containing 266 μmol of malvidin-3-glucoside (Netzel et al. 2003). The major metabolites of anthocyanins recovered in urine are glucuronated and methylated conjugates (Prior and Wu 2006).

With respect to anthocyanins from bilberry, human data are lacking, although some animal studies have been performed (as described by Upton 2001). In rats, the plasma total anthocyanin concentration was 26 mg/L at 1 hour after intraperitoneal administration of bilberry anthocyanins. Anthocyanins were detectable also in tissues collected at the same time, at concentrations of 12–79 μg/g tissue. Kidney had three fold higher anthocyanin content than plasma, and skin had 1.5-fold higher content than plasma (Talavera et al. 2003, 2005). After oral administration (400 mg/kg) of bilberry anthocyanins to rats, a maximal plasma concentration of 2.47 mg/L was reported at 15 minutes, with bioavailability estimated at 1.2% (reported by Upton 2001). In a more recent animal study of distribution and excretion of bilberry anthocyanins (Sakakibari et al. 2009), the plasma concentration of mice was shown to peak at 15 minutes postingestion of an ethanol extract of fresh bilberries, showing a sharp decrease thereafter, and the renal excretion of anthocyanins represented 1.88% of the dose ingested. Thirteen anthocyanins were detected in the bilberry extract, and the main anthocyanins in plasma at 60 minutes postingestion were malvidin-3-glucoside and malvidin-3- galactoside (Sakakibari et al. 2009). In mice fed bilberry extract for 2 weeks, plasma levels reached a maximum of 0.26 μmol/L and anthocyanins were found only in liver, kidney, testes, and lung. No anthocyanins were found in

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brain, heart, muscle, eyes, or white fat. The researchers concluded that bilberry anthocyanins are absorbed but are taken up by specific organs (Sakakibari et al. 2009). However, there may be differences in the tissue distribution of anthocyanins across different species, as brain uptake of anthocyanins has been reported in a rat model (Talavera et al. 2005).

4.5 HEALTH EFFECTS OF BILBERRY

Besides its use as a delicacy, bilberry is widely used to improve night vision and to decrease vascular permeability and capillary fragility; moreover, the berry has various other reputed health benefits, although most interest has been focused on anthocyanin-related antioxidant effects (Camire 2000; Upton 2001; Mazza 2002; Park et al. 2007; Zafra-Stone et al. 2007). Although there are many studies that have investigated the antioxidant and other health-related effects of anthocyanins and anthocyanin-rich berries and extracts or juices, only a few have used bilberry itself, and data from controlled human trials are scarce. In Sections 4.5.1 through 4.5.8, studies that have used mixed berry juices or extracts including bilberry are discussed, as well as those that have used only bilberry fruit or extracts.

4.5.1 Antioxidant Effects

Anthocyanins are potent antioxidants that scavenge radicals and chelate metal ions (Pool-Zobel et al. 1999; Mazza et al. 2002; Prior and Wu 2006). Many herbs and berries have powerful antioxidant properties (refer to Chapter 2 on antioxidants in herbs and spices), and these properties are thought to underlie many of the various health effects of herbs and berries.

In primary cultures of rat hepatocytes, it was found that bilberry extract protected cells against oxidative damage (Valentova et al. 2006). As described by Upton (2001), bilberry or anthocyanin extracts of bilberry protects rat liver microsomes against oxidative damage and apolipoprotein B against ultraviolet (UV)-induced oxidative fragmentation. Animal studies show conflicting results. In a rat study, no significant change of urinary 8-OHdG (also known as 8-oxodG, and a biomarker of oxidative stress; Lee, Chung, and Benzie 2010), was reported after 14 weeks of supplementation with an anthocyanin-rich extract from mixed berries including bilberry (Lala et al. 2006). However, a significant decrease of malondialdehyde (a biomarker of lipid peroxidation; Benzie 1996) in brain was seen in OXYS rats fed with bilberry extract (2 g of dried aqueous extract including 0.35 g/kg diet; Kolosova et al. 2006). This strain of rats shows accelerated aging and higher oxidative stress compared to the Wistar rats and, interestingly, the Wistar rats did not show bilberry-related effects, suggesting that the antioxidant-effects of bilberry may be seen only in cases of elevated oxidative stress. A combination extract (OptiBerry) of six edible berries, including bilberry, was reported to show "unique antioxidant potential in a whole-body scenario" using an animal model exposed to hyperbaric oxygen (Bagchi et al. 2006). Rats given a bilberry extract (1% w/w in the diet) and subjected to doxorubicin (DOX) toxicity (induced with 15 mg/kg intraperitoneal injection of DOX) had lower lipid peroxides in serum and increased glutathione (GSH) in cardiac muscle cells compared to control animals, although no differences were seen in cardiac lipid peroxide levels (Choi et al. 2010). In mice stressed by restraint, marked increases in liver damage and reactive oxygen species (ROS) levels associated with this stress were restored to normal levels by administering a bilberry extract, and there was also enhanced mitochondrial complex II activity, elevated sodium/potassium ATPase activity, and elevated mitochondrial membrane potential with the bilberry treatment (Bao et al. 2010).

In contrast to the promising effects seen in animal studies, no effect on lipid peroxidation was seen in human volunteers after supplementation with mixed vegetables and fruits including bilberries (Freese et al. 2004). In general, most controlled human supplementation studies are performed on healthy subjects, and they have limited potential to respond in relation to biomarker changes. Better target groups for looking at antioxidant-related effects are those humans under higher

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oxidative stress, such as the elderly, those at elevated risk of heart disease, or diabetic patients. To date, there is only one published report of the effects of bilberry supplementation on antioxidant status and oxidative stress in human subjects (Karlsen et al. 2010). Subjects ($n = 31$) with at least one risk factor for CVD were supplemented with 330 mL/day bilberry juice (diluted to 1 L with water) for 4 weeks. However, results showed no significant changes in biomarkers of antioxidant status or oxidative stress in these subjects, compared to 31 control subjects (Karlsen et al. 2010).

Two points of caution are offered here with respect to antioxidant content and action of bilberry anthocyanins. First, many of the bilberry extracts used in trials have not been characterized or standardized to anthocyanin content. Variation in anthocyanin content affects the antioxidant content of extracts. In vitro testing of the antioxidant capacity of commercial bilberry products by our group showed surprising results (Lee 2008). Using the ferric reducing/antioxidant power ability (FRAP) assay (Benzie and Strain 1999), the total antioxidant capacity of 11 commercial powdered bilberry extracts from various countries of origin were tested. Values ranged from <20 to >2000 μmol/g (Lee 2008). There was a strong correlation between the FRAP value and the anthocyanin content ($r > .96$: $p < .001$). Interestingly, whereas the anthocyanin content of commercial bilberry products is assumed to be standardized to 250 mg/g, or at least to be high, only 5 of the 11 commercial products had any stated anthocyanin content and 3 of the 5 had stated values of <80 mg/g. Furthermore, the actual anthocyanin content of all but one product was <60 mg/g, with concomitantly low FRAP values (Lee 2008). In one product with a stated content of 250 mg/g anthocyanins, the measured content was <10 mg/g. Second, in vitro antioxidant activity may not reflect in vivo action (Pool-Zobel et al. 1999; Halliwell 2007). Molecular action of antioxidant phytochemicals may be independent of or indirectly related to antioxidant activity, may change with concentration, or may manifest only in the presence of other bioactives or under particular redox conditions (Halliwell and Gutteridge 2007; Packer and Cadenas 2007; Benzie and Wachtel-Galor 2010). Therefore, although anthocyanins are potent antioxidants in vitro, and fresh bilberry is a rich source of these, it cannot be assumed that all commercial bilberry products contain significant amounts of bilberry anthocyanins or that absorbed anthocyanins act directly as antioxidants in vivo. In the future, trials performed with bilberry extracts must use a standardized product of known anthocyanin content, and studies looking for health benefits should include a wide range of biomarkers of both antioxidant and nonantioxidant effects.

4.5.2 Genoprotective and Anticancer Effects

One in three people will receive a diagnosis of cancer at some point in their lives (WCRF 2007). Treatment of cancer is harsh and often unsuccessful. Cancer is a disease caused by mutations in key genes controlling cell division and growth. Damage to DNA increases the likelihood of such mutations, and any bioactive food or component that is found to protect DNA from damaging agents, lower baseline DNA damage, or increase DNA repair is a potential cancer-preventing agent (Collins 1999; Duthie 2007; Halliwell 2007; Kim et al. 2009). Although damage to DNA can be of many types and can result from many different processes and agents, oxidation-induced damage is thought to be a key factor. Chronic inflammation increases ROS load, and it is an important cancer risk factor (Aggarwal, Vijayalekshmi, and Sung 2009). Diet is an important modulator of risk (WCRF 2007; Benzie and Wachtel-Galor 2009; refer to Chapter 17 on herbs and spices in cancer prevention and treatment). Whereas most epidemiological data focus on the potential anticancer activity of mixed fruits and vegetables, few focus on types of individual berry. However, berries do have anticancer potential (Zafra-Stone et al. 2007; Seeram 2009), and there are some data from experimental studies that have used bilberry alone or in conjunction with other berries.

In vitro work and animal tumorigenic models have demonstrated that berry anthocyanins have cancer-preventive and -suppressive activity via antioxidant activity; antiproliferative, apoptotic, antiangiogenic, and anti-inflammatory effects; and induce the antioxidant response element (ARE) with consequent phase II enzyme induction and other cytoprotective effects (Hou 2003; Bagchi et al. 2004;

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Lala et al. 2006; Duthie 2007; Zafra-Stone et al. 2007; Wang and Stoner 2008; Seeram 2009; Benzie and Wachtel-Galor 2010; Matsunaga et al. 2010). Other mechanisms of genoprotection may involve direct interaction of anthocyanins with DNA. Natural anthocyanins have been reported to intercalate with DNA, forming a DNA copigmentation complex (Sharma and Sharma 1999; Mas et al. 2000; Kong et al. 2003). This triplex stabilization property suggests that anthocyanins could help regulate gene expression in addition to protecting DNA against oxidative damage (Sharma and Sharma 1999; Mas et al. 2000). Anthocyanins have been shown to protect DNA against oxidative stress induced by various agents (UV irradiation, hydrogen peroxide, *tert*-butyl hydroperoxide) in vitro (Lazzé et al. 2003; Seeram 2008; Svobodová, Zdařilová, and Vostálová 2009). However, it is noted that the doses used (100 μM or greater) for in vitro studies are much higher than values that can be reached in plasma through the dietary intake of anthocyanins. In animal feeding studies, cancer chemopreventive and therapeutic effects of anthocyanins and berries have been shown in various models (Hou 2003; Prior and Wu 2006; Choi et al. 2007; Zafra-Stone et al. 2007; Seeram 2008). Although convincing evidence from well-designed human experimental studies is lacking, there is sufficient robust evidence from in vitro and animal studies of anthocyanins to support clinical studies of cancer chemopreventive effects (Thomasset and Teller et al. 2009).

With reference to cancer-related studies that have investigated bilberry specifically, a hexane/chloroform extract was reported to induce a phase II detoxification enzyme (quinine reductase) in a cell-culture model (Upton 2001). Detoxification of xenobiotics is important for cancer prevention. The extract did not affect the activity of ornithine decarboxylase, an enzyme associated with progression of cancer; but it did inhibit growth of two breast cancer cell lines (Madhavi et al. 1998). Interestingly, a commercial anthocyanin-rich extract from bilberry was shown to inhibit growth of colon cancer cells but did not affect growth of normal colon cells, suggesting a possible specific action against cancer cells (Lala et al. 2006). Among the ethanolic extracts of 10 different berries tested for induction of apoptosis in human cancer cells (HL60 leukemia and HCT116 colon cancer cells), bilberry extract was reported to be the most effective. Interestingly, delphinidin and malvidin aglycone inhibited HL60 cells, whereas delphinidin and its glycoside, but not malvidin and its glycoside, inhibited HCT116 cells (Katsube et al. 2003). Delphinidin is the main anthocyanin in bilberry (Table 4.1).

In an animal study, the number of intestinal adenoma decreased significantly by 15–30% in rats with genetic colon adenoma that were fed a high dose of bilberry extract (10% w/w in diet, supplying approximately 5.5 g anthocyanin per kilogram per day (Mutanen et al. 2008). Similar findings were shown with a lower dose of a commercial bilberry extract (mirtoselect®, at a daily dose of 0.3% w/w diet, supplying approximately 0.5 g anthocyanin per kilogram per day; Cooke et al. 2006). This dosage is equal to approximately 740 g of fresh bilberries in terms of human intake. More recently, an extract of bilberry was shown to dose-dependently inhibit cell growth and promote induction of apoptosis in cultured breast cancer cells (MCF7-GFP-tubulin breast cancer cells; Nguyen et al. 2010). At higher doses (0.5–1.0 mg/mL), the bilberry extract arrested the cell-cycle at the G(2)/M phase and inhibited microtubule polymerization (Nguyen et al. 2010). In a DNA microarray study, an anti-inflammatory gene activation/inhibition profile was seen in macrophages treated with a bilberry extract (Chen et al. 2008). As noted earlier in this section, inflammation is an important risk factor for cancer. If the molecular evidence for an anti-inflammatory effect of bilberry is confirmed in clinical study, it would support the use of bilberry in cancer prevention. In a pilot study of 25 colorectal cancer patients, an anthocyanin-standardized extract of bilberry (mirtocyan®, supplying between 0.5 and 2 g of anthocyanins per day) was given for 7 days prior to surgery (Thomasset and Berry et al. 2009). After 7 days, bilberry anthocyanins and their glucuronide and methyl metabolites were detected in plasma and also in tumor tissue (179 ng/g tissue at the highest bilberry extract dose), and plasma levels were found to be related to dose. The tumor tissue showed a 7% decrease in proliferation compared to prebilberry values, and there was a small but significant decrease in plasma insulin-like growth factor-1 (IGF-1) seen with the lowest dose (Thomasset and Berry et al. 2009). These preliminary data from human study provide important and clear support for further clinical studies of bilberry anthocyanins in cancer chemoprevention.

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4.5.3 Cardioprotective Effects

All over the world, CVD is a leading cause of death. Major risk factors of CVD include central obesity, diabetes, hypertension, elevated levels of lipids, and high levels of uric acid. Increased oxidative stress may also contribute, and inflammation is a key factor. Atherosclerosis, the main underlying factor in CVD, is an inflammatory process associated with oxidative processes in and damage to the vascular endothelium (Libby, Ridker, and Maseri 2002). Therefore, the anti-inflammatory and antioxidant effects of anthocyanins are of relevance to potential cardioprotective effects of bilberry and other berries. Antihypertensive, lipid-lowering, hypoglycemic, and antiobesity effects would also be cardioprotective (Zafra-Stone et al. 2007; Erlund et al. 2008).

Gene expression in bilberry-treated macrophages stimulated with lipopolysaccharide (LPS) showed an anti-inflammatory profile (Chen et al. 2008). Furthermore, a controlled human supplementation trial showed decreased concentration of inflammatory biomarkers in the plasma of 31 subjects who took bilberry juice for 4 weeks (Karlsen et al. 2010) Most notably, significant decreases were seen in plasma levels of high-sensitivity C-reactive protein (hsCRP), a sensitive biomarker of subclinical inflammation and a predictor of CVD, and the proinflammatory cytokine interleukin 6 (IL-6). No significant effects were seen on plasma cholesterol, triglycerides, or uric acid concentrations (Karlsen et al. 2010). In an animal study, plasma triglycerides were decreased after feeding rats with an extract of bilberry leaves (3 g/kg/day) for 4 days (Cignarella et al. 1996). However, it is noted that the leaves and not the berries of *V. myrtillus* L. were used and the effects may not have been related to anthocyanins, which are found mainly in the deeply colored berries. In a controlled human study of 35 subjects who took 100 g of whole bilberries each day, platelet function, blood pressure, and high-density lipoprotein (HDL)-cholesterol were all improved (Erlund et al. 2008). However, in addition to the daily bilberry supplement, the subjects took a mixture of various berries during the study. A study of 23 healthy volunteers given a mixture of grape seed, pine bark, bilberry, and red wine for 4 weeks showed an insignificant decrease in acute impairment in endothelial function caused by a high-fat meal (Barringer, Hatcher, and Sasser 2008). In another human study, mixed anthocyanins from bilberry and blackcurrant (*Ribes nigram*) were given as an extract (320 mg/day) for 12 weeks to 60 middle-aged dyslipidemic Chinese subjects (Qin et al. 2009). Results showed significant improvements in low-density lipoprotein (LDL)-cholesterol (average decrease of approximately 14%) and HDL-cholesterol (average increase of approximately 14%). No significant changes were seen in plasma total cholesterol, triglycerides, or apolipoproteins, but the plasma concentration of cholesteryl ester transfer protein (CETP) was significantly decreased, and the change in CETP correlated with the changes in lipids. An in vitro study by the same group showed that cyanidin-3-O-glucoside lowered CETP activity in human HepG2 cells, and the researchers concluded that the bilberry and blackberry anthocyanin mixture improved lipids in their human subjects by inhibiting CETP activity and changing cellular cholesterol efflux (Qin et al. 2009).

Regarding blood pressure and vascular health, bilberry fruit anthocyanins have been reported (Upton 2001) to inhibit smooth muscle contraction and platelet aggregation. These are potentially antithrombotic and antihypertensive effects and have cardioprotective effects. Possible antihypertensive effects of bilberry are also suggested by the finding of inhibition of angiotensin-converting enzyme (ACE) activity in cells in vitro (Persson, Persson, and Andersson 2009). A significant, dose-dependent inhibition of ACE activity was seen in endothelial cells from human umbilical veins that had been incubated in bilberry extract (0.00625–0.1 mg/mL) for 10 minutes (Persson, Persson, and Andersson 2009). Interestingly, individual anthocyanidins (cyanidin, delphinidin, and malvidin) had no inhibitory effect, and the researchers concluded that ACE-inhibition seemed to be dependent on the specific mixture of anthocyanins in bilberry (Persson, Persson, and Andersson 2009). Anthocyanins from bilberry were reported to protect against ischemia reperfusion injury in an animal model, and to attenuate leucocyte adhesion and improve blood perfusion (Bertuglia, Malandrino, and Colantuoni 1995). In rats that were fed bilberry anthocyanins for 12 days prior

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to inducing hypertension, permeability of the blood–brain barrier was kept normal and there was limited increase in the vascular permeability of the skin and aorta wall (Detre et al. 1986).

Anthocyanins and bilberry have also been reported to have antiobesity and hypoglycemic effects, which would bring cardioprotective benefits. These will be discussed in Section 4.5.5.

4.5.4 Anti-Inflammatory Effects

Inflammation is a protective mechanism, but chronic inflammation increases oxidative stress and underlies many age-related diseases, including CVD and cancer (Libby, Ridker, and Maseri 2002; Halliwell and Gutteridge 2007; Aggarwal et al. 2009). Many studies suggest that anthocyanins, the predominant phenolic compounds found in bilberry, have anti-inflammatory effects (Karlsen et al. 2007; Chen et al. 2008; Dreiseitel et al. 2008; Kim et al. 2009). Suggested mechanisms include inhibiting proteasome activity, which controls the degradation of cellular proteins (Dreiseitel et al. 2008), and inhibiting nuclear factor κB (NF-κB) activation, which controls expression of genes involved in the inflammatory response (Karlsen et al. 2007; Chen et al. 2008). Supplementation with Medox (a commercial product of purified anthocyanins from bilberries supplying 300 mg of anthocyanins) by healthy subjects for 3 weeks was associated with a decrease in several NFκB-regulated proinflammatory chemokines and immunoregulatory cytokines (Karlsen et al. 2007), and a follow-up study showed decreased levels of hsCRP and inflammatory cytokines in plasma of 31 subjects who took 330 mL/day of bilberry juice for 4 weeks (Karlsen et al. 2010). As oxidative stress may mediate inflammation injury, the antioxidant properties of bilberry may be responsible for at least some of the anti-inflammatory effects reported. However, selective gene activation and inhibition by mechanisms other than direct antioxidant effects are likely (Chen et al. 2008; Benzie and Wachtel-Galor 2010).

4.5.5 Hypoglycemic Effects

The bilberry plant is reputed to possess antidiabetic properties, and its berries and leaves (as well as those of other *Vaccinium* species) have been used for centuries to ameliorate the symptoms of diabetes (Cignarella et al. 1996; Martineau et al. 2006; Ghosh and Konishi 2007; Cravotto et al. 2010). In a survey of 685 Italian herbalists, bilberry ranked fourth in a list of herbal remedies recommended for improvement of glycemic control (Cicero, Derosa, and Gaddi 2004). The reported hypoglycemic effect of bilberry is a desirable effect for helping to prevent or control type 2 diabetes, which is a highly prevalent condition caused by insulin resistance and B cell failure (ADA 2010). Type 2 diabetes is associated with increased oxidative stress, inflammation, and dyslipidemia, and is accompanied by an increased risk of CVD, cancer, and vision loss through cataract and retinopathy (Brownlee 2005; Jee et al. 2005; Choi et al. 2008; ADA 2010).

The hypoglycemic effect of bilberry may be mediated in part by interference with enzyme action, especially α-glucosidase activity (McDougall, Kulkarni, and Stewart 2008), and also by effects on insulin secretion and glucose transport. Anthocyanins were found to stimulate insulin secretion from cultured rodent pancreatic B cells, with cyanidins and delphinidins (the major anthocyanins in bilberry) showing the greatest effect among different anthocyanins tested (Jayaprakasam et al. 2005). In addition, low-bush blueberry, which belongs to the same family as bilberry, at 12.5 μg/mL was demonstrated to enhance glucose transport into muscle cells and adipocytes in the absence of insulin (Martineau et al. 2006).

In an animal study with a water–alcohol extract of bilberry leaves given to streptozotocin-induced diabetic mice (3 g/kg/day for 4 days), a significant decrease (26%) was seen in plasma glucose (Cignarella et al. 1996). Blood glucose was significantly decreased (by 33% and 51%, respectively) after administration of a phenolic-rich extract (containing approximately 287 mg/g anthocyanin) and an anthocyanin-enriched fraction (containing approximately 595 mg/g) from a *Vaccinium* blueberry extract at a dose 500 mg/kg to diabetic (C57b1/6J) mice (Grace et al. 2009). In gavage treatment

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with pure anthocyanins (300 mg/kg), malividin-3-O-glucoside was found to have a significant hypoglycemic effect in these animals, but delphinidin-3-O-glucoside did not (Grace et al. 2009). As shown in Table 4.1, the malvidin-3-O-glucoside concentration of bilberry is 3.35% (Upton 2001). Significant decreases in serum glucose and fructosamine were shown in alloxan-induced diabetic mice at, respectively, 120 minutes and 7 days after being given a 20 mg/kg dose of "antidiabetis," an herbal preparation that included bilberry (Petlevski et al. 2001).

Obesity is a strong predisposing factor for type 2 diabetes. Berry polyphenols may help prevent obesity by inhibiting digestive enzymes, such as lipase, thereby lowering fat absorption (McDougall, Kulkarni, and Stewart 2008). Cyanidin-3-glucoside has been shown to suppress the development of obesity in mice fed a high-fat diet and to regulate human adipocyte function (Tsuda 2008). Human preadipocytes were collected from subcutaneous adipose tissue, cultured, and differentiated into adipocytes before being treated with anthocyanins for 24 hours. Adiponectin, an anti-inflammatory cytokine, was upregulated, and there was downregulation of the proinflammatory cytokine IL-6 and also of the plasminogen activator inhibitor-1 (PAI-1); the anthocyanin treatment also activated adenosine monophosphate (AMP)-activated protein kinase (AMPK) in adipocytes without increasing the AMP/adenosine triphosphate (ATP) ratio (Tsuda 2008). Together, these changes indicate a role for anthocyanins in preventing metabolic syndrome, an increasingly common condition associated with insulin resistance, hypertension, and dyslipidemia that often progresses to type 2 diabetes. In a follow-up study, Tsuda and coworkers reported that a bilberry extract added to the diet of diabetic mice (27 g/kg diet, which gave an anthocyanin content of 10 g/kg diet) lowered serum glucose and improved insulin sensitivity (Takikawa et al. 2009). There were no differences in body weight or serum adiponectin levels between the bilberry-fed and the control animals, but the antidiabetic effects of the bilberry extract were associated with AMPK activation in white adipose tissue and skeletal muscle and liver, and were accompanied by increased glucose transporter 4 (GLUT 4) in white adipose and skeletal tissue and lower hepatic gluconeogenesis (Takikawa et al. 2009).

Although there are some published human studies of the hypoglycemic effects of berries (e.g., cranberry, chokeberry), strong evidence from human trials is lacking (Matsui et al. 2006; Helmstädter and Schuster 2010). To our knowledge, there are no published controlled human studies with bilberry on diabetes patients. The two published human supplementation studies with bilberry (Qin et al. 2009; Karlsen et al. 2010) studied subjects at elevated risk of CVD, but they were not diabetic. It is unlikely that significant effects of bilberry would be seen in subjects with normal glucose tolerance No differences were seen in plasma glucose levels in 60 nondiabetic dyslipidemic subjects who took a mixed bilberry and blackcurrant anthocyanins supplement (120 mg/day anthocyanins) for 12 weeks (Qin et al. 2009). In the study by Karlsen et al. (2010), which investigated the effect of 4 weeks of supplementation with 330 mL/day of bilberry juice in subjects with at least one risk factor for CVD, no glucose data were shown.

Although we lack human data on the antidiabetic effects of bilberry, numerous in vitro and animal studies provide good evidence of a role for bilberry in treating or preventing type 2 diabetes. This could be a very rewarding area of future research given the huge socioeconomic problem posed by this highly prevalent disease. In addition to the clear benefits that would come from increasing insulin secretion and glucose transport, other effects of bilberry, such as its antioxidant, anti-inflammatory, and lipid-lowering effects, would help delay the serious vascular complications of diabetes. Controlling obesity would help prevent many cases of type 2 diabetes. Also, there is increasing evidence of increased risk of cancer with hyperglycemia to hyperglycemia (Jee et al. 2005; Stocks et al. 2009). The Metabolic Syndrome and Cancer (Me-Can) Project is a large prospective study of six European cohorts, with a total of over 500,000 subjects, and after an average follow-up of 10.4 years, results strongly support high blood glucose as a risk factor for incident cancer at many specific sites and for cancer death (Stocks et al. 2009). Oxidative stress, inflammation, and increased amounts of growth factors, including IGF-1, also increase cancer risk and are high in those with type 2 diabetes. The combination of antioxidant, anti-inflammatory, and hypoglycemic effects of an

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herb or a functional food would bring significant long-term benefits, particularly to those with type 2 diabetes, and studies of bilberry focusing on these effects in this group are warranted.

4.5.6 Ocular Effects

Bilberry has a long history of use for eye disorders and in promoting vision. There have been numerous studies of the effects of bilberry on various aspects of vision and ocular disorders, including cataract, retinopathy, macular degeneration, and night vision (reviewed by Camire 2000; Upton 2001; Canter and Ernst 2004; Ghosh and Konishi 2007; Zafra-Stone et al. 2007). Many studies have shown positive effects, including improvement in retinal abnormalities, increased capillary resistance, slowing of progression of lens opacity and myopia, and improved dark adaptation. For example, in a study of 50 patients with mild senile cataract, 4 months of supplementation with bilberry anthocyanins plus vitamin E was reported to have a 97% success rate in preventing cataract progression (Bravetti, Fraboni, and Maccolini 1989). A double-blinded, placebo-controlled study reported that in six subjects who were given bilberry anthocyanins, dark adaptation at 1 hour and 3 hours postingestion was faster (6.5 minutes) compared to six control subjects (9 minutes; reviewed by Zafra-Stone et al. 2007). However these, and many other, studies, were small; used mixed supplements, such as bilberry plus other berries or bilberry combined with vitamin E; or were uncontrolled. Canter and Ernst (2004) reviewed 30 published trials of bilberry-extracted anthocyanins on lowered light and night vision. Only 12 of the 30 studies were placebo controlled, and the conclusion was that there was insufficient rigorous evidence to recommend the use of bilberry for improving night vision (Canter and Ernst 2004).

Nevertheless, there is supporting scientific evidence of beneficial effects of bilberry in relation to ocular disorders and vision loss. A study by Jang et al. (2005) showed that a bilberry extract (100 μM anthocyanins) protected against photooxidation of pyridinium disretinoid A2E, an autofluorescence pigment that mediates a detergent-like disturbance of cell membranes and light-induced damage to cells, and that the effects were due at least in part to the quenching of singlet oxygen. Milbury et al. (2007) showed that bilberry anthocyanins (1 mg/mL) modulated the oxidative stress defense enzymes heme oxygenase-1 (HO)-1 and glutathione-S-transferase-pi (GST-pi) in retinal pigment epithelial cells that were preincubated with anthocyanin extract before H_2O_2 challenge. Song et al. (2010) studied the effect of bilberry extract on cultured corneal limbal epithelial cells and showed that bilberry promoted physiological renewal and homeostasis of these cells. A randomized, double-blinded, placebo-controlled study showed that symptoms of asthenopia and contrast sensitivity for 22 of the 30 subjects studied (73%) improved significantly after 4 weeks of 100 mg/day of purified anthocyanin (85% anthocyanoside oligomers; Lee et al. 2005). In a study with cultured retinal ganglion cells, bilberry anthocyanosides inhibited chemical-induced cell damage and radical activation, and this neuroprotective effect, which may have been an antioxidant-related effect, was also seen in vivo when bilberry anthocyanosides (100 μg/eye) were injected into the vitreous of mice (Matsunaga et al. 2009).

Age-related vision loss, mainly due to senile cataract and macular degeneration, affects the quality of life of virtually all elderly persons. Diabetic retinopathy is highly prevalent in those who have had diabetes for 10 or more years and is a leading cause of blindness in developed countries. There is sufficient evidence from animal and cell-culture studies and small human trials to warrant more powerful, controlled human trials of bilberry in helping to address the huge clinical problem of age- and diabetes-related vision loss.

4.5.7 Neuroprotective Effects

The phenomenon of age-related degenerative diseases leading to cognitive decline is common and relentless. Stroke, whether triggered primarily by hypertension or thrombosis, is a major cause of death and disability. The vasodilatory and anti-inflammatory effects of bilberry can be expected to have significant effects in relation to the preservation of cognition and neuromotor function through

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lowered risk of both hemorrhagic and thrombotic strokes. Furthermore, neuronal tissue, including the retina, is rich in polyunsaturated fatty acid, and the antioxidant properties of anthocyanins may protect these oxidation-susceptible sites and, thereby, preserve brain and retinal function, although it is not yet clear whether anthocyanins are taken up by brain tissue. Berry fruits and fruit polyphenols have been reported to be neuroprotective, enhance dopamine release, and improve neuronal communication (Zafra-Stone et al. 2007; Matsunaga et al. 2009; Shukitt-Hale, Lau, and Joseph 2009). A commercial bilberry extract (Myrtocyan® at 200 mg/kg daily for 5 days by intraperitoneal administration) given to rats was reported to increase triiodothyronine transport to different regions of the brain, and bilberry is reported to promote short-term memory, vision, and control of sensory input in animals (Saija et al. 1990; Cignarella et al. 1996; Prior and Wu 2006; Zafra-Stone et al. 2007). However, most studies on the neuroprotective effects of berries and their constituents have been performed on cultured cells and in animals, and cognition-related studies using bilberry specifically are lacking.

4.5.8 Antimicrobial Effects

Antimicrobial effects of herbs and natural products can be via inhibition of bacterial binding (adhesion) to cell walls, direct antimicrobial killing, or by effects that potentiate antibiotics, as evidenced by lowered minimum inhibitory concentration (MIC) of antibiotics in the presence of an herb compared to that of the antibiotic alone. Several natural products have been found to have antimicrobial effects (Lee et al. 2006). Cranberry (*V. macrocarpon* Ait.) has powerful antiadhesion properties and is widely used to prevent infections of the urinary tract (see Chapter 6 on cranberry). Other researchers have reported the antiadhesion effects of berries on *Helicobacter pylori*, and also that berry extracts increase the antimicrobial effects of the antibiotic clarithromycin against *H. pylori* (Chatterjee et al. 2004).

Bilberry and other berry fruits, as well as purified berry phenolics, have been reported to show direct antimicrobial effects against human pathogens, including *Salmonella* and *Staphylococcus aureus* (Puupponen-Pimiä et al. 2005a,b). Interestingly, pure phenolic compounds (as opposed to berry extracts) were found to inhibit only gram-negative bacteria (which include *Salmonella* species and *Escherichia coli*), and the effects were related to the degree of hydroxylation of the pure phenolic compounds (Puupponen-Pimiä et al. 2005a,b). Berry extracts were found to inhibit those affected by the pure phenolic compounds; further, extracts inhibited the growth of not only *H. pylori* but also *Bacillus, Clostridium*, and *Staphylococcus,* which are gram-positive organisms. Therefore, whole berries or extracts of whole berries may be more effective as antimicrobials than purified extracts. The effects of various berries against various organisms are different. For example, cloudberry (*Rubus chamaemorus*) showed a bactericidal effect on *S. aureus*, whereas bilberry had a bacteriostatic effect (Puupponen-Pimiä et al. 2005a). The antimicrobial effects of bilberry against *Salmonella* and *Staphylococcus* were increased by enzymatic treatment of the fruit mash, which increased phenolic release into the berry juice; however, there may also have been structural changes to the berry components during treatment (Puupponen-Pimiä et al. 2005a,b). In a recent study of wild berries, bilberry juice inhibited adhesion of *Streptococcus pneumoniae* to human bronchial (Calu-3) cells, and bilberry juice also inhibited growth of *S. pneumoniae*, although the effects of bilberry were less than those of cranberry (Huttunen et al. 2010).

The findings of antimicrobial effects in herbs and other natural products is important and timely, not least because of the new infectious diseases arising in recent years. *S. pneumoniae* is the major cause of meningitis, otitis media, and pneumonia. The presence of various pathogens in food and water is common and their toxic consequences can be severe. Antibiotic resistance in microbes is a serious and increasing problem; moreover, the immune system declines with age and our populations are aging. Bilberry has a clear potential value as an antimicrobial agent. In a preliminary study conducted by our group, bilberry showed a direct effect against methicillin-resistant *S. aureas* (MRSA), with an MIC of 1.2 mg/mL for bilberry extract alone. Furthermore, the effect of vancomycin against MRSA strains was potentiated. In the presence of 0.6-mg/mL bilberry extract, the

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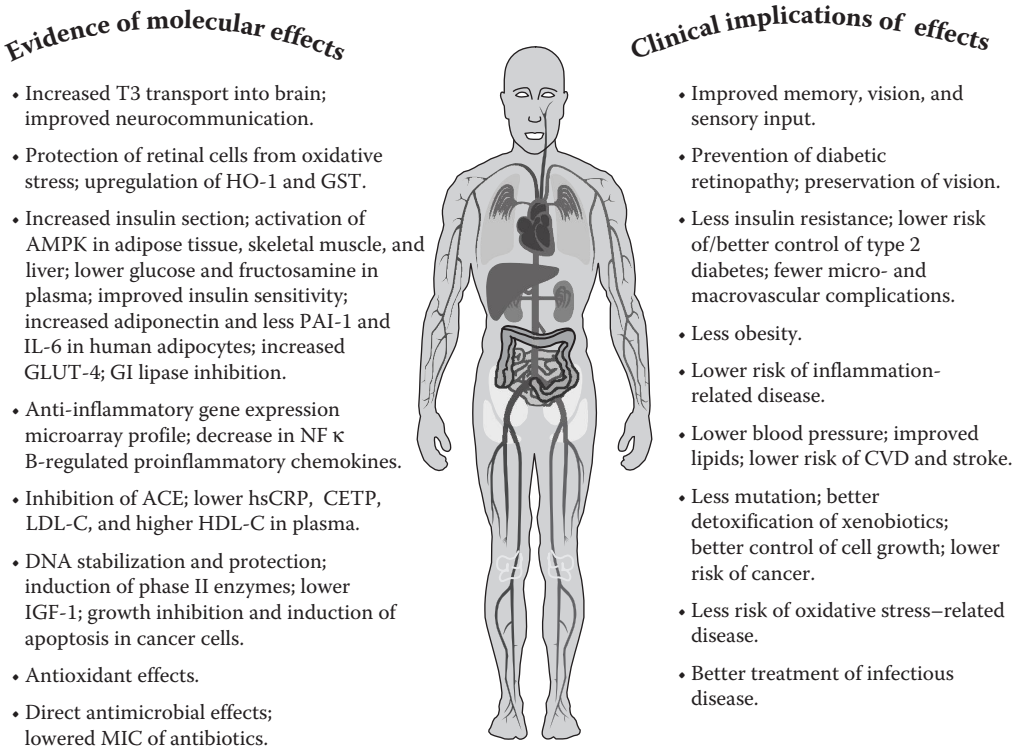


FIGURE 4.2 Molecular evidence of beneficial effects of bilberry and bilberry anthocyanins: There is a variety of molecular evidence of beneficial effects of bilberry and bilberry anthocyanins. These effects have important clinical implications for human health.

MIC for vancomycin was decreased from 1.8 to 0.7 μg/mL. These are interesting and potentially important findings for the use of bilberry in treating antibiotic-resistant organisms.

4.6 CONCLUSION

Throughout history, berries have been an important and valued part of the human diet. Berries contain many components, but anthocyanins, the phenolic compounds that give berries their red, blue, and purple colors, have been found to have a wide range of health-related properties, including antioxidant, antitumorigenic, anti-inflammatory, hypoglycemic, and antimicrobial effects. Bilberry is rich in anthocyanins, especially delphinidins and cyanidins. There is supporting evidence that these compounds are bioavailable and bioactive. Molecular effects that have been demonstrated in experimental studies and their clinical implications for human health are summarized in Figure 4.2. However, well-designed human trials using standardized extracts of bilberry are needed to provide the level and variety of clinical evidence that will translate current molecular insights and understanding into clear recommendations for bilberry as a possible tool to combat chronic and infectious diseases in our aging populations.

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5 *Cordyceps* as an Herbal Drug

Bao-qin Lin and Shao-ping Li

CONTENTS

5.1 Introduction 73
5.2 Antitumor Activity 75
5.3 Immunomodulating Effect 75
5.3.1 Potentiation Activity 76
5.3.2 Suppressive Effect 76
5.3.3 Effect on Gut Immune System 77
5.4 Antioxidant Activity 78
5.5 Antihyperglycemic Activity 78
5.6 Sexual and Reproductive Function Enhancement Activity 81
5.7 Antifatigue Activity 82
5.8 Protective Effect on the Kidney 83
5.9 Effect on the Liver 83
5.10 Active Components 84
5.10.1 Nucleosides and Their Activities 84
5.10.2 Sterols and Their Activities 96
5.10.3 Free Fatty Acids and Their Activities 96
5.10.4 Carbohydrates and Their Activities 96
5.11 Conclusions 97
References 98

5.1 INTRODUCTION

Cordyceps is the composite of a genus of fungus that grows on the larva of insects. To date, more than 350 *Cordyceps*-related species have been found worldwide based on fungus and/or insect host. However, since 1964, only *Cordyceps sinensis* has been recorded officially as an herbal drug in Chinese pharmacopoeia. *C. sinensis*, known as *Dongchongxiacao* (winter-worm summer-grass) in Chinese, is one of the most famous traditional Chinese medicines and medicinal mushrooms. The fungus attacks the larva of some species of insects (Fam. *Hepialidae*), and converts each larva to a sclerotium, from which the fruiting body grows (Figure 5.1).

According to the theory of Chinese medicine, *C. sinensis* is sweet in taste and neutral in nature, and it can replenish the kidney, soothe the lung, stop bleeding, and eliminate phlegm. The fungus *C. sinensis* has been used for the treatment of fatigue, cough, hyposexuality, asthenia after severe illness, renal dysfunction, and renal failure (State Pharmacopoeia Commission of PRC 2005). In China, it is found in the soil of prairies at elevations of 3500–5000 m, mainly in the provinces of Qinghai, Tibet, Sichuan, Yunnan, and Gansu. In China, *C. sinensis* has been known and used as a remedy for more than 300 years. It was first recorded in *Ben Cao Bei Yao* by Wang Ang in 1694, and the Italian scholar Saccardo named the *Cordyceps* found in China officially as *Cordyceps sinensis* (Berk.) Sacc. in 1878; this nomenclature has been used ever since.

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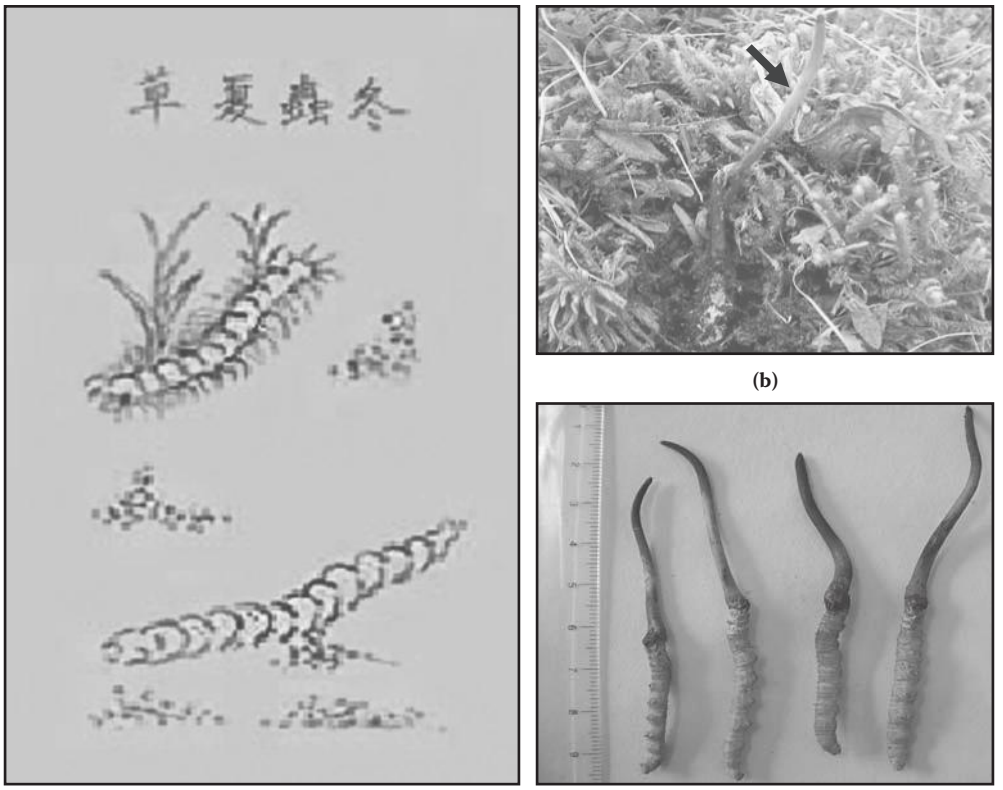


FIGURE 5.1 (See color insert.) (a) *Cordyceps sinensis*, illustrated in the book *Ben Cao Bei Yao* by Wang Ang, which was published in 1694; (b) *C. sinensis* found in the soil (arrowhead indicates *C. sinensis*); and (c) collected as raw materials.

The ecosystem of *C. sinensis* has been terribly affected by the restriction of habitat and overexploration. Although the Ordinance of Resources Protection on Wild Herbal Medicine was issued in 1987, the yield of natural *C. sinensis* is still decreasing. It was reported based on a survey conducted during June–July 2007 that the yield of natural *C. sinensis* decreased by more than 90% in the last 25 years. The price rocketed to more than 200,000 Renminbi (RMB)/kg (approximately US$25,000) in 2007 (Feng, Yang, and Li 2008), and its usage was limited during the past decade by its limited supply.

Due to the rarity and outstanding curative effects of *C. sinensis*, some natural substitutes such as *C. militaris*, *C. liangshanensis*, *C. gunnii*, and *C. cicadicola* have been sold in markets (Yang et al. 2009). In addition, several cultured mycelia of *C. sinensis* and *C. militaris* fungi have become the main substitutes of the natural species as commercial products, and 50 medicines and two dietary supplements related to cultured Cordyceps have been approved by the State Food and Drug Administration of China since 2002 (Feng, Yang, and Li 2008). For example, JinShuiBao capsule, the commercial product of Cs-4 (*Paecilomyces hepialid*, a standardized mycelium of *C. sinensis*), has been used in clinics throughout China. This product generates several million U.S. dollars every year. *Synnematum sinensis*, *Cephalosporium sinensis*, *Gliocladium roseum*, and *Mortierella hepialid*, the fungus strains isolated from natural *C. sinensis*, have also been subjected to large-scale fermentation and are used as commercial products (Cheung, Li, and Tsim 2005). Therefore, much effort has been invested in studying the evaluation of the quality, pharmacological activities, and clinical efficacies of natural and cultured cordyceps. In this chapter, we focus on the bioactivities, action mechanisms, and active ingredients of cordyceps, both natural and cultured.

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5.2 ANTITUMOR ACTIVITY

Cancer is the second leading cause of disease-related mortality throughout the world (Xiao and Zhong 2007). However, related therapy strategies are still limited to surgery, radiotherapy, and chemotherapy. Due to the limitations of surgery and radiotherapy and the side effects of chemotherapy, there is increasing interest in developing antitumor drugs from natural products. Studies have shown that cordyceps has antitumor activity in various cancers through several pathways. Both natural and cultured cordyceps have demonstrated antitumor effects (Feng, Yang, and Li 2008; Zhou et al. 2009a).

Studies in vivo showed cordyceps had an inhibitory effect on Ehrlich ascites carcinoma and meth-A fibrosarcoma (Ng and Wang 2005), EL-4 lymphoma (Yamaguchi et al. 1990), B16 melanoma (Wu, Zhang, and Leung 2007a), Lewis lung carcinoma (Nakamura et al. 1999), and H22 tumors (Chen et al. 2006) in mice. Furthermore, *C. sinensis* reversed the suppressive effect of Taxol-induced leukopenia in mice, which indicated that *C. sinensis* could be used with other chemotherapy methods for cancer treatment (Liu et al. 2008). Cordyceps exhibited direct cytotoxic activity against several kinds of tumor cells, including Lewis lung carcinoma, B16 melanoma, lymphocytic (Jurkat), prostate (PC3), breast (MCF7), hepatocellular (HepG2, Hep3B), colorectal (HT-29 and HCT 116), and HL-60 cells (Nakamura et al. 1999; Wang et al. 2005; Wu, Zhang, and Leung 2007a). Although cordyceps had a cytotoxic effect on tumor cells, it did not show any cytotoxicity against normal cells (Wu, Zhang, and Leung 2007a).

Several mechanisms contribute to the antitumor effect of cordyceps, such as direct cytotoxicity, immunopotentiation, apoptosis, selective inhibition of ribonucleic acid (RNA), and protein synthesis, as well as antioxidant, antiangiogenic, antimutagenic, antimetastatic, and antiviral activities (Xiao and Zhong 2007; Feng, Yang, and Li 2008; Zhou et al. 2009a). Of them, the apoptotic homeostasis regulated by cordyceps might be the most important (Buenz et al. 2005; Feng, Yang, and Li 2008) mechanism. The apoptotic molecular mechanism of cordyceps includes the activation of Bax, caspase-3 and/or -9, -8; inhibition of cyclooxygenase-2 (COX-2); and nuclear factor κB (NF-κB) protein expression and downregulation of Bcl-2 level (Xiao and Zhong 2007; Feng, Yang, and Li 2008). Besides, apoptosis of MDA-MB-231 human breast carcinoma cells induced by *C. militaris* aqueous extract (0.8 mg/mL) was also associated with loss of mitochondrial membrane permeability. In addition, the extract decreased Akt activation and reversed PI3K/Akt-pathway-enhanced apoptosis (Jin, Kim, and Choi 2008). Furthermore, the apoptotic events induced by the extract were also mediated by diminished telomerase activity (Park et al. 2009).

5.3 IMMUNOMODULATING EFFECT

The immune system protects human beings from infection with layered defenses of increasing specificity. First, physical barriers prevent pathogens from entering the body. If a pathogen breaks these barriers, the innate immune system provides an immediate, but nonspecific response. The human body possesses a third layer of protection, that is, the adaptive immune system. The adaptive immune response is activated by the response of the innate immune system. Cells of the innate system include phagocytes (macrophages, neutrophils, and dendritic cells), mast cells, eosinophils, basophils, and natural killer cells. In the adaptive system, B cells are involved in humoral immune response, whereas T cells contribute to cellular immune response. Immunopotentiating drugs are used to restore the immune system to normal and to reduce reoccurring and life-threatening infections. Immunosuppressive drugs are applied to control autoimmune disorders and inflammation when excessive tissue damage occurs, as well as to prevent transplant rejection after an organ transplant (Taylor, Watson, and Bradley 2005). Increasing evidence shows that cordyceps is a bidirectional modulator with both potentiating and suppressive effects on the immune system through regulating innate and adaptive immunity (Li and Tsim 2004; Ng and Wang 2005; Feng, Yang, and Li 2008; Zhou et al. 2009a).

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5.3.1 Potentiation Activity

Natural *C. sinensis* has a long history of use in the treatment of respiratory infections and cancer. It has been postulated that the responsible mechanism is related to immune activation, particularly the promotion of innate immunity. The oral administration of *C. sinensis* extract improved the phagocytosis of macrophages in resting and cyclophosphamide-treated C57BL/6 mice implanted subcutaneously with syngeneic EL-4 lymphoma cells (Yamaguchi et al. 1990). Cultured *C. sinensis* induced production of interleukin (IL)-1β, IL-6, IL-10, and tumor necrosis factor (TNF)-α; elevated phagocytosis of human peripheral blood mononuclear cells (HPBMC); and elevated macrophage phagocytosis and monocyte production of H_2O_2. However, it did not induce cytokine overliberation in mice (Ka et al. 2006). Further study showed that an aqueous extract of mycelia of *C. sinensis* enhanced the IL-6, TNF-α, and nitric oxide (NO) release from primary murine macrophages by inducing mitogen-activated protein kinase (MAPK) pathways characteristic of inflammatory stimuli. The extract also synergized with interferon-gamma (IFN-γ) to stimulate cytokine production from macrophages, and the extract-treated mice spleens showed decreased bacterial burden compared to vehicle control. The results indicate that *C. sinensis* mycelia protected the animals from proliferation of bacteria by activating the macrophages (Jordan, Sullivan, and Lee 2008a). The *C. sinensis* could also enhance the activity of natural killer cells (Ng and Wang 2005). A *C. militaris* water extract induced phenotypic and functional maturation of dendritic cells, which then initiated T-cell responses against microbial pathogens and tumors (Kim et al. 2006a).

Cordyceps also promotes the adaptive immune system, including cellular and humoral immunity. Although natural and cultured *C. sinensis* methanol extracts had no effect on the proliferation of splenocytes and cytokine liberation such as IL-2 in primary mouse splenocytes in vitro (Siu et al. 2004) or in BALB/c mice in vivo (Ka et al. 2006), the extracts enhanced concanavalin A–stimulated proliferation and IL-2 level of mouse splenocytes in vitro at 200 μg/mL (Siu et al. 2004) or ovalbumin-induced splenocyte proliferation and serum immunoglobulin (Ig) G, IgG1, and IgG2b levels in ovalbumin-immunized mice (Wu et al. 2006). Cultured *C. sinensis* increased CD25 expression on lymphocytes in vitro (Ka et al. 2006), augmented numbers of CD4+ and CD8+ cells, improved CD4+/CD8+ ratio, and reduced IgA and IgG levels in patients with posthepatic cirrhosis (Ng and Wang 2005). Moreover, cordyceps had a regulatory effect on bronchoalveolar lavage fluids (BALF) cells. The *C. sinensis* ethanol extract could enhance Th_1 immune response, such as IFN-γ and IL-12 production, which could then inhibit IL-10 release from Th_2 cells and finally reduce IgE production from B lymphocytes. Reduced production of IgE would attenuate the occurrence of asthma attacks (Kuo et al. 2001). Fruiting bodies of *C. militaris* water extract significantly upregulated IL-18 gene expression, an inducer of IFN-γ in T cells and NK cells, in C57BL/6 mouse brain and liver in vivo and in RAW264.7 cells in vitro (Kim et al. 2008). Fruiting bodies, but not caterpillars, of *C. cicadae* methanol extract enhanced proliferation and IL-2 and IFN-γ production in phytohemagglutinin (PHA)-stimulated HPBMC (Weng et al. 2002).

5.3.2 Suppressive Effect

Due to its inhibitory effect on the immune system, cordyceps can be used for treatment of autoimmune diseases and for immunosuppression after organ transplant. Early oral administration of *C. sinensis* (2.4 mg/g/day) induced the redistribution of HPBMC with reduced percentages of CD4+ T cells ($P < .05$), and attenuated the disease severity of lupus in (NZB/NZW) F1 mice with increased survival, decreased proteinuria, and reduced titers of anti-double-stranded DNA antibody (Chen et al. 2009). The administration of *C. sinensis* could augment the blocking effect of cyclosporin A on allogeneic graft rejection by reducing mononuclear cell infiltration in kidney grafts, CD4+ T cells in peripheral blood and serum IL-2, and IFN-γ production in an allograft kidney transplant rat model (Ding et al. 2009). Mycelia of *C. sinensis* water extract plus subtherapeutic

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cyclosporin A also decreased acute rejection in rats that had undergone heart transplant, completely ablated acute vasculopathy in mice at the dose of 50 mg/kg, and decreased IFN-γ release from mouse splenocytes and CD8+ T cells at 3.4 mg/mL in vitro (Jordan, Hirsch, and Lee 2008b).

Furthermore, cordyceps showed anti-inflammatory activity. Fruiting bodies of *C. sinensis* methanol extract inhibited PHA-stimulated lympho-proliferation and NK cells activity, and IL-2 and TNF-α release from HPBMC (Kuo et al. 1996). Chloroform and *n*-butanol fractions of the fruiting bodies of *C. sinensis* methanol extract inhibited the elevation of NO, inducible nitric oxide synthase (iNOS), TNF-α, and IL-12 in lipopolysaccharide (LPS)/IFN-γ-activated murine peritoneal macrophages in a dose-dependent manner in vitro (Rao, Fang, and Tzeng 2007). The administration of *C. militaris* decreased airway inflammation in ovalbumin-induced mice (Hsu et al. 2008), and had both anti-inflammatory activity on croton oil–induced mouse ear topical edema and carrageenan-induced rat hind acute edema (Won and Park 2005). The administration of *C. pruinosa* methanol extract inhibited the production of IL-1β, TNF-α, NO, and PGE_2 in LPS-stimulated macrophages at 10 μg/mL in vitro and LPS-administered mice at 5 mg/kg in vivo via the suppression of NF-κB activation (Kim et al. 2003). Methanol extract of caterpillars, but not fruiting bodies, of *C. cicadae* resulted in the suppression of proliferation of PHA-induced HPBMC and the lowering of IL-2, IL-4, IL-5, IFN-γ, and IL-12 release from PHA-stimulated HPBMC (Weng et al. 2002). So, different parts of cordyceps have different effects on immune response.

5.3.3 Effect on Gut Immune System

The gastrointestinal tract plays a dual role in human physiology, that is, digestion and uptake of nutrients, and maintenance of immune homeostasis. The gastrointestinal-associated lymphoid tissue (GALT) is composed of Peyer's patches and other GALT such as lymphoid aggregates in the appendix, large intestine, and esophagus, tonsils, and adenoids. There are macrophages, dendritic cells, B lymphocytes, and T lymphocytes in GALT. Both innate and adaptive responses collaborate in maintaining the immune balance of GALT (Huffnagle and Noverr 2008). Although *C. sinensis* hot water extract had no direct effect on the proliferation of *Salmonella* sp., *Escherichia coli*, and *Lactocbacillus* sp., it could significantly lower harmful bacteria populations (*Salmonella* sp. and *E. coli*.) and increase helpful bacteria numbers (*Lactocbacillus* sp.) in the small intestine of broiler chicks administered with 600 mg/kg/day for 35 days (Koh, Suh, and Ahn 2003a). The results indicate that *C. sinensis* regulates intestinal bacteria by improving GALT or systemic immunity or both. The oral administration of cultured mycelia of *C. sinensis* hot water extract at 1 g/kg/day for 7 days stimulated the activation of peritoneal macrophages and Peyer's patch cells with increase in granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-6 levels in ICR mice (Koh et al. 2002). Macrophages in GALT function as antibacterial guards, by phagocytosing and killing any microbes that penetrate the lamina propria (Macpherson, Marrinic, and Harris 2002). Therefore, *C. sinensis* was supposed to promote the activity of macrophages in GALT. The fact that cytokines such as GM-CSF and IL-6 play an important role in systemic immune cells suggests that *C. sinensis* modulates the systemic immune system partly through a mechanism mediated by Peyer's patch cell (Koh et al. 2002). In mesenteric lymph node (MLN) lymphocytes, *C. sinensis* aqueous extract enhanced the secretion of IL-2 and IFN-γ from Th1 cells. Besides, the extract improved IgA release from resting and concanavalin A–stimulated MLN lymphocytes, whereas increased production of IgA at mucosal surfaces could promote an anti-inflammatory environment by neutralizing antigens (Park et al. 2008). Furthermore, *C. sinensis* and *C. scarabaecola* showed intestinal immune system modulating activity by activation of T lymphocytes in Peyer's patch (Koh et al. 2002; Yu, Kim, and Suh 2003).

In summary, studies reveal that cordyceps has effects on both innate immunity and adaptive immunity. Furthermore, cordyceps also has a modulatory effect on gut immune system, which may further influence systemic immune function.

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5.4 ANTIOXIDANT ACTIVITY

Reactive oxygen species (ROS), including molecular oxygen (O_2), superoxide anion (O_2^-), H_2O_2, hydroxyl radical (OH), peroxynitrite ($ONOO^-$), and hypochlorous acid (HOCl; Zhou, Mrowietz, and Rostami-Yazdi 2009b), are well recognized as playing a dual role in biological systems, since they can be either beneficial or harmful to living systems (Valko et al. 2004). Normally, ROS form the natural by-products of aerobic metabolism and play a physiological role in cell signaling. However, the concentration of ROS can increase dramatically during times of environmental stress such as exposure to ultraviolet (UV) radiation or heat, causing damage to the lipids, proteins, and nucleic acids of cells. This injury to cell structures leads to several diseases, such as senescence, cancer, atherosclerosis and cardiovascular diseases, inflammatory lung diseases, immune dysfunction, and neurodegenerative disorders (Rahman 2003; Zhong 2006; Valko et al. 2007).

There is increasing evidence that cordyceps has antioxidant activity, which may be one of the mechanisms behind the antiaging, anticancer, anti-inflammatory, antiatherosclerosis, and immunomodulatory effects of cordyceps (Table 5.1). As far as different parts of *C. sinensis* are concerned, the fruiting bodies showed a similar potency with caterpillars in their antioxidant activities in xanthine oxidase assay, induction of hemolysis assay, and lipid-peroxidation assay (Li et al. 2002). The results also demonstrated that the caterpillar has a similar chemical composition to the fruiting body, which indicates that the function of the worm in cordyceps is to provide a growth medium for the fruiting body, and the caterpillar is eventually totally invaded by cordyceps mycelia (Li et al. 2002).

Both water (Li et al. 2001; Yu et al. 2006; Dong and Yao 2008) and ethanol (Wang et al. 2005; Won and Park 2005; Ra et al. 2008) extracts of cordyceps showed significant antioxidant activity in vitro. However, the water extract exhibited a stronger inhibitory effect on superoxide anions and hydroxyl radicals than the ethanol extract (Yamaguchi et al. 2000a). Furthermore, both natural *C. sinensis* and cultured cordyceps showed direct and potent antioxidant activities using in vitro assays, such as lipid-peroxidation assay, 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, and protein-peroxidation assay. Therefore, cultured cordyceps can be used for antioxidant activity, relieving human demands on natural *C. sinensis*, an endangered species (Li et al. 2001; Yu et al. 2006; Dong and Yao 2008).

5.5 ANTIHYPERGLYCEMIC ACTIVITY

Cordyceps has hypoglycemic activity in normal animals. The oral administration of a cordyceps carbohydrate extract "Cs-4" at 2 g/kg/day for 25 days increased insulin sensitivity, and the extract had potential beneficial effects by maintaining whole-body glucose disposal with a less-pronounced increase in insulin secretion after a carbohydrate challenge in rats (Balon, Jasman, and Zhu 2002). In another study, normal rats fed with Cs-4 at 250 or 500 mg/kg/day for 17 days showed significant decreases in fasting blood glucose level by 27% and 24%, respectively, and the fasting plasma insulin of rats in the 500-mg/kg group decreased by 37%. Furthermore, oral glucose tolerance tests demonstrated that the extract significantly improved glucose tolerance at 0.5, 1.0, and 2.0 hours after ingestion of glucose (Zhao et al. 2002).

Cordyceps also showed an antihyperglycemic effect in diabetic animals. Although fruiting bodies of natural *C. sinensis* (4 g/kg/day) had no effect on the fasting insulin level in diabetic rats (Lo et al. 2004), it improved the weight and attenuated water intake (day 15 to day 29), fasting blood glucose level (day 15 to day 26), and serum concentration of fructosamine (day 29) in diabetic rats. Fruiting bodies of *C. sinensis* also improved thymus weight and glucose tolerance (day 26; Lo et al. 2004 and 2006). However, in this experiment, *C. sinensis* had no effect on serum triglycerides and cholesterol concentrations of diabetic rats (Lo et al. 2004). Choi et al. (2004) also found that the aqueous extract of cultured *C. militaris* had no effect on fasting insulin level or glucose uptake

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TABLE 5.1
Antioxidant Activity In Vitro and In Vivo of Cordyceps

*Cordyceps	Model	Route	Daily Dose or Concentration	Study Duration	Effects	Treatment	References
C. sinensis hot water extract	Radiation-induced mice bone marrow and intestinal injuries	p.o.	50 mg/kg	7 days	Reduced lethality; protected against radiation-induced intestinal and bone marrow injury; accelerated the recovery of white blood cells	Radiation-induced gastrointestinal syndrome and bone marrow failure	Liu et al. 2006
	Radiation-stimulated bone marrow cells	CE	500 μg/mL	1 hour	Increased the survival of cells		
	Murine osteoblastic cells			24 hours	Reduced ROS level in the cells		
Fruiting bodies of cultured *C. sinensis* aqueous extract	D-galactose-induced aged mice	p.o.	1, 2, and 4 g/kg	6 weeks	Enhanced superoxide dismutase (SOD) activity of hepatic and brain and erythrocytes, blood GSH-Px and catalase activity; decreased MDA content in the brain and liver, and monoamine oxidase activity of brain; improved learning and memory	Antiaging	Ji et al. 2009
	Primary macrophages	CE	0.1–5.0 mg/mL	24 hours	Suppressed the elevation of lipid peroxide in LDL in a dose-dependent manner by scavenging free radicals; inhibited cholesteryl ester deposition	Antiatherosclerosis	Yamaguchi et al. 2000a

(*Continued*)

TABLE 5.1 (*Continued*)
Antioxidant Activity In Vitro and In Vivo of Cordyceps

*Cordyceps	Model	Route	Daily Dose or Concentration	Study Duration	Effects	Treatment	References
	Atherosclerotic mice	p.o.	50, 100, and 200 mg/kg/day	12 weeks	Inhibited LDL oxidation mediated by free radicals rather than by reduction in serum lipid level; suppressed the increased aortic cholesteryl ester level	Antiatherosclerosis	Yamaguchi et al. 2000b
Fruiting bodies of *C. militaris*	Gerbil with transient forebrain ischemia injury	p.o.	500 mg/kg	10 days	Reduced neuronal damage and gial activation via downregulation of lipid peroxidation in the ischemic CA1 region and decreased activation of astrocytes and microglia	Ischemic damage	Hwang et al. 2008
Mycelia of *C. ophioglossoides* MeOH extract	β-amyloid-induced Alzheimer disease rats	i.p.	100 mg/kg	31 days	Prevented β-amyloid-induced decrease in spatial memory and learning capacity	Alzheimer's disease	Jin et al. 2004
	β-amyloid stimulated SK-N-SH neuronal cells	CE	100 μg/mL	48 h	Reversed β-amyloid-induced cell death; suppressed β-amyloid-induced ROS release from cells		

Note: p.o.: per os, oral administration; i.p.: intraperitoneal administration; CE: cell experiments.
*Cordyceps extracts were incubated with cells.

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in the gastrointestinal tract. However, the extract was found to lower fasting serum glucose level, reduce triglycerides level in soleus muscles, increase the whole-body glucose disposal rate, as well as enhance glucose transporter 4 (GLUT 4) content and fraction velocity of glycogen synthase in the soleus and quadriceps muscles in 90% pancreatectomized rats that were fed *C. militaris* extract at 500 mg/kg/day for 8 weeks (Choi et al. 2004).

Interestingly, fruiting bodies, not caterpillars, of *C. sinensis* had an effect on lowering the fasting blood glucose level and increasing the thymus weight (Lo et al. 2004). However, fermented mycelia and broth of *C. sinensis* had similar antihyperglycemic effect with fruiting bodies in nicotinamide and streptozotocin-induced diabetic rats (Lo et al. 2006). Therefore, the fermented products of cordyceps can be developed as potential antidiabetic agents or functional foods for persons with a high risk of diabetes mellitus.

5.6 SEXUAL AND REPRODUCTIVE FUNCTION ENHANCEMENT ACTIVITY

Testosterone is necessary for normal sperm development. It activates genes in Sertoli cells, which promote differentiation of spermatogonia. Cordyceps has traditionally been used for the enhancement of sexual function in human beings. Evidence shows that *C. sinensis* and *C. militaris* can improve reproductive activity and restore impaired reproductive function (Table 5.2). The administration of *C. sinensis* enhanced libido and sexual activity, and restored impaired reproductive function in both sexes in human (Zhu, Halpern, and Jones 1998). Such effects are related to the

TABLE 5.2
Effect of *C. sinensis* and *C. militaris* on Sexual and Reproductive Function

Cordyceps		Model	Daily Dose or Concentration	Study Duration	Effects	References
Cultured *C. sinensis*	Fruiting bodies	Castrated rats	0.5–2.0 g/kg	21 days	Shortened penis erection latency and mount latency	Ji et al. 2009
	Mycelia	Male mice	0.02 or 0.2 mg/g	7 days	Promoted the production of plasma testosterone	Hsu et al. 2003b, Huang et al. 2004
		Primary mouse Leydig cells, and MA-10 mouse Leydig tumor cells	3 mg/mL	3 hours	Promoted testosterone release, and suppressed hCG and dbcAMP-stimulated testosterone productions	Huang et al. 2001a and 2001b; Hsu et al. 2003a and 2003b
Cultured *C. militaris*	Mycelia	Male rats	1% and 5% of diet	6 weeks	Improved sperm count and percentages of motile sperm cells; elevated plasma testosterone and estradiol-17 concentration	Chang et al. 2008a
		Subfertile boars	0.5% of diet (10 g/boar)	2 months	Improved sperm volume, morphology, and serum testosterone	Lin et al. 2007

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enhancement of testosterone release in plasma through cAMP (adenosine monophosphate)-protein kinase A signal pathway (Hsu et al. 2003a). Fractions of cultured mycelia of *C. sinensis* with water-soluble low-molecular-weight proteins and polysaccharides of relatively poor water solubility and protein, but not fractions with water-soluble low-molecular-weight polysaccharides, increased testosterone levels in mice (Hsu et al. 2003b; Huang et al. 2004). A protein in *C. sinensis* contributed to the observed hypotensive and vasorelaxant properties by improving the production of NO (Chiou et al. 2000); this protein might help the penis trap blood for erection, thereby improving sexual function (Drewes, George, and Khan 2003).

5.7 ANTIFATIGUE ACTIVITY

Fatigue is defined as difficulty in initiating or sustaining voluntary activity (Chaudhuri and Behan 2004), and can be classified into mental and physical fatigue (Mizuno et al. 2008). Fatigue is a common symptom in sickness and in health. Chronic fatigue can affect an individual's performance. In addition, long-term accumulated fatigue can lead to *karoshi* (a Japanese word meaning death as a result of overwork). In China, cordyceps is used to restore health after various diseases and to hasten recovery from exhaustion because of its adaptogenic (antistress) properties and ability to enhance endurance and strength (Bucci 2000).

Oral administration of *C. sinensis* mycelia water extract at 150 mg/kg/day for 7 days (Koh et al. 2003b) or ingestion of fruiting bodies of *C. militaris* at 500 mg/kg/day for 4 weeks (Jung, Kim, and Han 2004) significantly prolonged the swimming time of mice by about 20 and 24 minutes, respectively. This effect was related to the enhancement of immunity. The administration of *C. sinensis* at 150 mg/kg/day for 8 days inhibited the increase of total cholesterol and the decrease of alkaline phosphatase in rats, as well as significantly reversed the decreased weight of liver, adrenal gland, thymus, and thyroid (Koh et al. 2003b). The involvement of cordyceps in adenosine triphosphate (ATP) production also accounts for a decrease in physical fatigue when it is administered. The oral administration of cultured *C. sinensis* extract (200 mg/kg/day, p.o.) not only improved hepatic energy metabolism and blood flow in dietary hypoferric anemic mice for 4 weeks (Manabe et al. 2000) but also increased significantly the ATP/inorganic phosphate ratio in the liver of normal mice for 3 weeks (Manabe et al. 1996) or for 7 days (Dai et al. 2001) with no steatosis, necrosis, inflammation, or fibrosis in the liver specimens (Manabe et al. 1996 and 2000). Treatment with natural or cultured cordyceps extracts (1 g/kg/day, p.o.) for 3 days enhanced myocardial ATP generation capacity ex vivo in mice by 29% and 32%, respectively, which might be mediated by the enhancement of mitochondrial electron transport (Siu et al. 2004).

Patients having chronic fatigue syndrome often have depression. Around 30–70% of such patients show the features of major depression (Adler 2004). Supercritical fluid extract (SCCS, 2.5–10 mL/kg, p.o.), other than hot water extract (500–2000 mg/kg, p.o.), of *C. sinensi*s show significant antidepressant-like activity. After 5 days of administration, SCCS shortened immobility times in the mouse-tail suspension test, although it had no effect on locomotor activity in the mouse open field test. It was considered that SCCS played an antidepressant-like role by affecting the adrenergic and dopaminergic systems other than the serotonergic system (Nishizawa et al. 2007). In addition, cordyceps has a powerful antioxidant effect, which may eliminate the ROS produced in working muscles during exercise and help in relieving fatigue (Mizuno et al. 2008). Finally, *C. sinensis* induced a more efficient utilization and consumption of O_2, which resulted in a greater survival rate under a hypoxic environment (Lou, Liao, and Lu 1986) in mice. The results indicate a more efficient use of O_2 by cordyceps to support essential physiological activities of tissues and improve tolerance to hypoxia-induced acidosis. However, few clinical trials have been conducted on the antifatigue effect of cordyceps, and most of the conducted tests were methodologically flawed, especially in the inclusion of other drugs in the experiments. For instance, capsules containing Cs-4, *Rhodiaola rosea*, and other ingredients did not enhance muscle-tissue oxygen saturation (Colson et al. 2005) and cycling performance (Earnest et al. 2004) in healthy

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men. Similarly, ingestion of a supplement containing cultured *C. sinensis*, adenylpyrophosphoric acid, calcium pyruvate, and yohimbine hydrochloride once 1 hour before a sport activity showed no ergogenic effects in healthy men (Herda et al. 2008). One reason may be that the ingestion schema of *C. sinensis* was insufficient to elicit positive changes in humans. A 1-week loading phase followed by at least a 2–4-week maintenance phase may be needed to obtain the effect of promoting aerobic capacity and resistance to fatigue. Another reason may be that as these experiments were performed on healthy persons, there were fewer margins for physiological, health, and performance improvement than in diseased or elderly persons (Colson et al. 2005).

5.8 PROTECTIVE EFFECT ON THE KIDNEY

C. sinensis has been used for the treatment of renal diseases, such as chronic nephritis, chronic pyelonephritis, chronic renal dysfunction or failure, and nephritic syndrome (Feng, Yang, and Li 2008). The *C. sinensis* extract significantly improved renal function via antiapoptotic and anti-inflammatory activity in rats subjected to 60 minutes of ischemia and following 3 days of reperfusion of the kidneys. Downregulation of the apoptotic gene of caspase-3 accompanied the decreases in inflammatory genes such as MCP-1, TNF-α, and iNOS. The result indicates that *C. sinensis* plays a potential therapeutic role in renal transplantation (Shahed, Kim, and Shoskes 2001).

Another mechanism by which cordyceps protects the kidney is its inhibitory effect on mesangial cell proliferation. It has been suggested that glomerular sclerosis is preceded by proliferation of mesangial cells that exhibit smooth muscle cell features and accumulation of mesangial extracellular matrix. Both *C. sinensis* and *C. militaris*, at a concentration of 100 mg/mL, significantly reversed the proliferation of human mesangial cell stimulated by low-density lipoprotein (LDL; Wu, Wang, and Cheng 2000). Moreover, *C. sinensis* could protect the kidney from cyclosporine A–induced chronic nephrotoxicity, with lower blood urea nitrogen (BUN), interstitial edema and fibrosis, and bulbular necrosis (Wojcikowski, Johnson, and Gobë 2006). Furthermore, administration of a water extract of cordyceps had a protective effect in rats with acute renal failure induced by gentamicin. The possible mechanisms include protection of sodium pump activity, lowering of lipoperoxidation in tubular cells, and attenuation of lysosomal overactivity in tubular cells (Ng and Wang 2005; Li and Yang 2008a). In addition, *C. sinensis* enhanced cellular immunity in rats with chronic renal failure (Cheng 1992).

Clinical trials have also shown some evidence for the use of cordyceps as a renoprotectant remedy (Wojcikowski, Johnson, and Gobë 2004 and 2006). For example, Bailing capsule, a preparation made from *C. sinensis* mycelia, ameliorated the rejection of renal transplant, improved renal and liver function, regulated hypoproteinemia and hyperlipidemia, stimulated hemopoietic function, and decreased the incidence of infections in patients after renal transplant (Sun et al. 2004; Li et al. 2009). In patients with chronic renal failure, ingestion of another cordyceps product Cs-4, called JinShuiBao, also significantly promoted renal function, which decreased serum urea and creatinine and increased total blood protein and calcium (Feng, Yang, and Li 2008).

5.9 EFFECT ON THE LIVER

Cordyceps has been used in clinical practice for the treatment of chronic hepatitis and related diseases (Zhao 2000). There are several ways in which it contributes to the treatment of and protection against liver disease. First, cordyceps has a potential enhancing effect on the immunological function of patients suffering from chronic hepatitis B (Gong, Wang, and Tang 2000) and from posthepatic cirrhosis (Zhu and Liu 1992). Second, cordyceps was shown to inhibit and reverse liver fibrosis via degradation of collagen in rats with liver cirrhosis induced by dimethylnitrosamine (Li et al. 2006a; Wang, Liu, and Tang 2008), inhibit proliferation of hepatic stellate cells in vitro (Chor et al. 2005), downregulate intercellular adhesion molecule-I (an index of liver fibrogenesis)

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and CD126 in human fibroblasts (Li and Tsim 2004), and decrease expression of transforming growth factor-β and platelet-driven growth factor (Liu and Shen 2003). Third, cordyceps decreased lipid peroxide levels in serum and hepatic tissue, and lowered serum TNF-α in bacillus calmette guerin plus LPS-induced liver injury in mice (Zeng, Tang, and Yuan 2001).

5.10 ACTIVE COMPONENTS

Many active ingredients, such as cordycepin, polysaccharides, and ergosterol, have been isolated from various *Cordyceps* species and account for a range of bioactivities (Table 5.3).

5.10.1 Nucleosides and Their Activities

Nucleosides are one of the major ingredients in cordyceps. To date, more than 10 nucleosides and their related components, including adenine, adenosine, cytidine, cytosine, guanine, guanosine, uracil, uridine, hypoxanthine, inosine, thymine, thymidine, 2′-deoxyuridine, 2′-deoxyadenosine, cordycepin, N^6-methyladenosine, and 6-hydroxyethyl-adenosine, have been isolated and/or identified in cordyceps (Feng, Yang, and Li 2008). Adenosine A_1, A_{2A}, A_{2B}, and A_3 receptors are distributed in the brain, lung, heart, liver, and kidney, and are involved in central nervous system (CNS)-mediated events such as sleep, immunological response, respiratory regulation, cardiovascular function, and liver and kidney activity (Li and Yang 2008a). Interestingly, the pharmacological effects of cordyceps match well with the distribution and physiological roles of adenosine receptors, including anticancer, antiaging, antithrombosis, antiarrhythmias, and antihypertension; immunomodulatory activity; and protective effects on the kidney, liver, and lung (Li and Yang 2008a).

Macrophages express adenosine A_{2A}, A_{2B}, and A_3 receptors. Activation of these receptors results in the upregulation of IL-10; downregulation of IL-12 and TNF-α; and increase of vascular endothelial growth factor (VEGF), macrophage inflammatory protein (MIP)-1α, and NO, respectively (Kumar and Sharma 2009). The ratio of uridine:inosine:guanosine at 8:11:5, which is the ratio of natural *C. sinensis*, showed an enhancement effect on the release of NO, TNF-α, and IL-1 from resting primary mouse macrophages, whereas it had no effect on LPS-stimulated cells (Li and Yang 2008b). However, uridine:adenosine:guanosine at a ratio of 11:7:9, the ratio found in cultured *C. sinensis*, improved NO, TNF-α, and IL-1 production in resting macrophages while suppressing the release of cytokine from LPS-stimulated cells (Li and Yang 2008c). The results indicate that different components act on different subreceptors; therefore, different ratios of these nucleosides result in different immune responses in macrophages.

Cordycepin was isolated from cultured *C. militaris* in 1950 (Cunningham et al. 1950), and was identified as 3′-deoxyadenosine in 1964 (Kaczka et al. 1964). It mainly exists in cultured *C. militaris*, and there is little in natural and none in cultured *C. sinensis* (Feng, Yang, and Li 2008). Cordycepin possesses anticancer, immunomodulating, and antioxidant abilities. Ongoing phase I/II clinical trials are investigating cordycepin in the treatment of TdT-positive acute lymphocytic leukemia. A study demonstrated that cordycepin showed a cytotoxic effect on tumor cells. The growth of B16 cells was inhibited by 60 μM of cordycepin by 70.1% at 72 hours, and this effect was induced by stimulating adenosine A_3 receptors followed by the signaling pathway of GSK-3β activation and cyclin D1 inhibition (Yoshikawa et al. 2008). Furthermore, Won et al. found that cordycepin diminished the production of ROS (O_2^-, and H_2O_2) in platelet-derived growth factor-BB (PDGF-BB)–induced vascular smooth muscle cells in vitro, which helped inhibit the neointima formation and vascular sprout outgrowth in response to PDGF-BB. The A_1/A_2 adenosine-receptor antagonist dipropyl-8-sulphophenylxanthine (DPSPX; 10 nM, 60 minutes) reversed the inhibition of PDGF-BB–induced migration evoked by cordycepin. The A_1/A_2 receptors are widely expressed in vascular cells and exert cardioprotective effects. Therefore, cordycepin may act as an antiatherosclerotic agent by activating the A_1/A_2 receptor (Won et al. 2009).

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TABLE 5.3
Chemical Compounds in Cordyceps and Their Pharmacological Activities

Compound	Source	Bioactivity	Dosage	Effect	References
			Nucleosides		
Cordycepin	Cultured *C. militaris*	Anticancer	15 mg/kg/day (2 weeks), p.o.	Inhibited B16 tumor lump in mice	Yoshikawa et al. 2004
			100 μM (48 hours)	Inhibited the proliferation of B16 and HepG2 cells	Wu, Zhang, and Leung 2007a
			0.3, 1, 3 mg/mL	Antimetastatic action in a hematogenic lung metastatic mouse model in vivo and a chemoinvasion chamber model on B16 cells in vitro	Nakamura et al. 2005
			100 μM	Induced cell apoptosis in OEC-M1 human oral squamous cancer cells; human hepatocellular carcinoma BEL-7402 cells; MA-10 mouse Leydig tumor cells	Wu et al. 2007b; Shi et al. 2008; Jen et al. 2009
			10 μM	Resulted in cell death in multiple myeloma cells	Chen, Stellrecht, and Gandhi 2008
			67 μM	Modulated polyadenylate polymerase in HeLa and MCF-7 cells via cell cycle rather than apoptosis induction	Thomadaki, Tsiapalis, and Scorilas 2005
			20 μg/mL	Modulated polyadenylate polymerase in human T cells, acute lymphoblastic leukemia, and human Burkitt lymphoma cells	Thomadaki, Tsiapalis, and Scorilas 2008
		Antirestenosis	20 μM/day (21 days), i.p.	Inhibited neointima formation in balloon injury in rats, and 40% and 50% decrease in proliferation and migration of rat aortic vascular smooth muscle cells in vitro	Chang et al. 2008b

(*Continued*)

TABLE 5.3 (*Continued*)
Chemical Compounds in Cordyceps and Their Pharmacological Activities

Compound	Source	Bioactivity	Dosage	Effect	References
			Nucleosides		
		Immunomodulatory activity	0.75–240.0 μg/mL	Induced the production of IL-1β, IL-6, IL-10, and TNF-α from resting HPBMC and the proliferation of CD4+, CD8+, CD19+, CD56+, and CD 14+ cells; suppressed PHA-induced secretion of IL-2, IL-4, IL-5, IFN-γ, and IL-12 from HPBMC and reduced the proliferation of these cells	Zhou et al. 2002 and 2008
			5–30 μg/mL	Inhibited NO release from LPS-stimulated macrophages by downregulation of iNOS, COX-2 expression, and TNF-α through the suppression of NF-κB activation, Akt, and p38 phosphorylation	Kim et al. 2006b
			10–100 μM	Was a potent inhibitor of IL-1β-induced epithelial neutrophil-activating peptide and MMP-1 and 3 and strongly blocked the p38/JNK/AP-1 signaling pathway in rheumatoid arthritis synobial fibroblasts	Noh et al. 2009
		Antioxidant activity	10 mg/kg (10 days)	Reduced neuronal damage and gial activation via downregulation of lipid peroxidation in the ischemic CA1 region and decrease in activation of astrocytes and microglia	Hwang et al. 2008
		Antibacterial	10 μg/disk	Revealed potent growth-inhibiting activity toward *Clostridium paraputrificum* and *Clostridium perfringens*	Ahn et al. 2000
		Antithrombotic activity	10–500 μM	Inhibited 1 μM thapsigargin-induced, 5 μM U46619-induced, and 10 μg/mL collagen-induced platelet aggregation through $[Ca^{2+}]$i-regulating system	Cho et al. 2006, 2007a,b

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		Human African trypanosomiasis	0.1–100.0 μM (48 hours)	Reduced the growth of *Trypanosoma brucei* and *T. cruzi*, as well as *Leishmania major* and *L. amazonensis*	Rottenberg et al. 2005
			1 or 2 mg/kg (4 or 7 days)	Administration of cordycepin plus deoxycofomycin to *T. cruzi*-infected mice significantly reduced parasitemia	
		Insecticidal activity	25–500 mg/L (2–5 days)	Larvicidal activity against *Plutella xylostella*	Kim et al. 2002
Guanosine	Cultured *C. sinensis*		0.02 and 0.10 μM	Decreased NO release, but augmented TNF-α and IL-1β release from primary rat macrophages	Yu et al. 2007
Adenosine	Cultured *C. sinensis*		0.30 and 1.50 μM	Decreased NO release, but augmented IL-1β release from primary rat macrophages	Yu et al. 2007
Ergosterol	Cultivated mycelium of *C. sinensis*	Anticancer activity	100 μM (48 hours)	Showed cytotoxicity on MCF-7, B16, HL-60, and HepG2 in vitro	Wu, Zhang, and Leung 2007a
			10–40 μg/mL	Exhibited cytotoxicity activity on HL-60 cells with IC_{50} values of 23.3 μg/mL	Matsuda et al. 2009
Sterols					
β-Sitosterol		Anticancer activity	100 μM (48 hours)	Showed more potential cytotoxic effect than ergosterol on MCF-7, B16, HL-60, and HepG2 in vitro	Wu, Zhang, and Leung 2007a
			10–80 μg/mL	Exhibited cytotoxicity activity on HL-60 cells with IC_{50} values of 26.7 and 23.3 μg/mL	Matsuda et al. 2009
5α,8α-Epidioxy-24(*R*)-methylcholesta-6,22-dien-3β-D-glucopyranoside	*C. sinensis* methanol extract	Anticancer activity	10–200 μM (22 hours)	Showed significant inhibitory effects on K562, Jurkat, HL-60, WM1341, and RPMI 8226 cells	Bok et al. 1999
5α,8α-Epidioxy-24(*R*)-methylcholesta-6,22-dien-3β-ol	*C. sinensis* methanol extract	Anticancer activity	10–200 μM (22 hours)	Showed significant inhibitory effects on K562, Jurkat, HL-60, WM1341, and RPMI 8226 cells	Bok et al. 1999
5α,8α-Epidioxy-22E-ergosta-6,22-dien-3β-ol	Cultivated *C. sinensis* mycelium	Anticancer activity	1.9–10.0 μg/mL	Showed substantial cytotoxic activity on HL-60 cells with IC_{50} values of 7.8 μg/mL, and the apoptosis was related with the activation of caspases-3/7	Matsuda et al. 2009

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TABLE 5.3 (*Continued*)
Chemical Compounds in Cordyceps and Their Pharmacological Activities

Compound	Source	Bioactivity	Dosage	Effect	References
			Sterols		
5α,8α-Epidioxy-22E-ergosta-6,9(11),22-trien-3β-ol	Cultivated *C. sinensis* mycelium	Anticancer activity	1.9–10.0 μg/mL	Showed substantial cytotoxic activity on HL-60 cells with IC_{50} values of 7.5 μg/mL, and the apoptosis was related with the activation of caspases-3/7	Matsuda et al. 2009
5α,6α-Epoxy-5α-ergosta-7,22-dien-3β-ol	Cultivated *C. sinensis* mycelium	Anticancer activity	1.9–10.0 μg/mL	Showed substantial cytotoxic activity on HL-60 cells with IC_{50} values of 7.3 μg/mL, and the apoptosis was related with the activation of caspases-3/7	Matsuda et al. 2009
Ergosterol peroxide (C28H44O3; Cpd 6A)	Natural *C. cicadae* MeOH extract	Immunomodulatory activity	1.5–100 μM	Reversed PHA-induced increase of cyclin E, IL-2, IL-4, IL-10, and IFN-γ in primary human T cells	Kuo et al. 2003
H1-A	Natural *C. sinensis*	Treatment of kidney	40 μg/kg/day, p.o. (8 weeks)	Demonstrated significantly less proteinuria, lower serum creatinine level, and less renal mesangial proliferation in MRL lpr/lpr mice	Yang et al. 1999
			40 mM	Inhibited the proliferation of human mesangial cells stimulated by IL-1 plus IL-6 in vitro	Lin et al. 1999
			12.5 or 25 mM	Inhibited the cell proliferation and promoted the apoptosis of IL-1- and PDGF-BB-activated human renal mesangial cells	Yang et al. 2003
4β-acetoxy-scirpendiol	Fruiting bodies of *C. takaomantana*	Hypoglycemic activity	0.2–2 mM	Inhibited glucose uptake in HEK193 cells expressing recombinant Na^+/GLUT-1	Yoo and Lee 2006
			Polysaccharides		
Exopolysaccharide fraction	Cultured supernatant of *C. sinensis*	Anticancer activity	30–120 mg/kg /2 days (i.p., 28 days)	Inhibited the metastasis of B16 melanoma cells to lungs and livers and Bcl-2 level in the lungs of mice; enhanced the phagocytosis of peritoneal macrophages and proliferation of spleen lymphocytes	Zhang et al. 2005

			15–60 mg/kg (i.p., 7 days)	Inhibited the H22 tumor weight in ICR mice; enhanced the phagocytosis and cytokine release of peritoneal macrophages and proliferation and activity of spleen lymphocytes	Zhang et al. 2008
			30–120 mg/kg/2 day (28 days)	Lowered the c-Myc, c-Fos, and VEGF levels in the lungs and livers in B16 tumor-bearing mice	Yang et al. 2005
Exopolysaccharide-peptide complexes	Submerged mycelial culture of *C. sphecocephala*	Anticancer activity	0.1–1 mg/mL (48 h)	Inhibited the growth of HepG2 and SK-N-SH cells, and the apoptosis of both cancer cells was associated with DNA fragmentation, activation of caspase-3, and modulation of Bcl-2 and Bax	Oh et al. 2008
Mannoglucan	*C. sinensis* mycelium	Anticancer	N/A	Showed weak cytotoxicity activity against SPC-I cancer cells with IC_{50} of 63 μg/mL	Wu et al. 2007c
CPS-1 (23 kDa)	Aqueous extract of cultured *C. militaris*	Immunomodulatory activity	15 mg/kg (i.p.)	Suppressed the humoral immunity; inhibited croton oil-ear edema and acetic acid–vascular permeability in mice	Yu et al. 2004
Intracellular polysaccharides (83 kDa)	Mycelia of *C. sinensis*	Immunomodulatory activity	100–400 μg/mice on day 1 and 15 (s.c.)	Enhanced the serum IgG, IgG1, and IgG2b levels, but not splenocyte proliferation in ovalbumin-immunized mice	Wu et al. 2006
Polysaccharides	Submerged culture of *C. sinensis*	Immunomodulatory activity	0.025–0.1 mg/mL	Polysaccharides from culture filtrate, but not from mycelia, increased the cytokine production of TNF-α, IL-6, and IL-10 from HPBMC, and enhanced the CD11b expression and phagocytosis of HPBMC	Kuo et al. 2007
Polysaccharides	Mycelia of *C. gunnii*	Immunomodulatory activity	10, 100 mg/kg	Inhibited mice spleen lymphocytes proliferation, peritoneal macrophage phagocytosis, and cytocoxin T lymphocytes activity	Xiao et al. 2004
Cordysinocan (82 kDa)	UST 2000, a strain of *C. sinensis*	Immunopotentiating actvity	0–100 μg/mL	Induced the T lymphocyte proliferation and the secretion of IL-2, IL-6, and IL-8 in vitro; increased the phagocytosis and acid phosphatase activity of macrophages in vitro	Cheung et al. 2009
CS-OHFP	*C. sinensis*	Hypoglycemic activity	p.o.	Exerted hypoglycemic activity in normal mice	Kiho et al. 1993

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TABLE 5.3 (*Continued*)
Chemical Compounds in Cordyceps and Their Pharmacological Activities

Compound	Source	Bioactivity	Dosage	Effect	References
			Polysaccharides		
Polysaccharide-enriched fraction	Cultured *C. militaris*	Hypoglycemic activity	10–40 mg/kg/day (12 days, p.o.)	Showed antihyperglycemic activity in STZ-induced diabetic rats	Zhang et al. 2006
CSP-1 (molecular weight about 210 kDa)	Cultured Cordyceps mycelia from *Cephalosporium sinensis* Chen sp. nov.	Hypoglycemic activity	200 and 400 mg/kg/kg, p.o.	Displayed hypoglycemic effect in normal animals; reduced blood glucose level, but also significantly increased insulin levels in alloxan- and streptozotocin-diabetic mice	Li et al. 2006b
		Antioxidant activity	25–100 μg/mL	Reversed the viability, attenuated the GSH-Px and SOD, as well as reduced MDA level in PC12 cells treated with 200 μM H_2O_2	Li et al. 2003
CS-F30 (45 kDa)	Hot water extract of cultured mycelia of *C. sinensis*	Hypoglycemic activity	i.p.	Showed antihyperglycemic activity in genetic diabetic mice; lower plasma triglyceride level and cholesterol level	Kiho et al. 1996
			i.v.	Exerted hypoglycemic activity in normal and STZ-induced diabetic mice; lower plasma triglyceride level and cholesterol level	
CS-F10 (15 kDa)	Cultured mycelia of *C. sinensis*	Hypoglycemic activity	50 mg/kg, i.p.	Lowered the plasma glucose level in normal, streptozotocin-induced diabetic, and genetic diabetic mice; increased the activities of hepatic glucokinase in STZ-induced mice; decreased protein content of GLUT2 in rat liver	Kiho et al. 1999
CI-P	Caterpillar of *C. cidadae*	Hypoglycemic activity	N/A	Exerted hypoglycemic activity in normal mice	Kiho et al. 1990
CI-A	Caterpillar of *C. cidadae*	Hypoglycemic activity	N/A	Exerted hypoglycemic activity in normal mice	Kiho et al. 1990
Polysaccharides	Mycelia of *C. sinensis*	Antioxidant activity	100–200 mg/kg	Enhanced SOD activity of liver, brain, and serum as well as GSH-Px activity in liver and brain, reduced MDA level in liver and brain in H22 tumor-bearing mice	Chen et al. 2006
CBP-1 (17 kDa)	Cultured *C. militaris*	Antioxidant activity	0–2 mg/mL	Possessed hydroxyl radical–scavenging activity (IC_{50} = 0.638 mg/mL)	Yu et al. 2009

Polysaccharides	N/A	Protective effect on kidney	40–160 mg/kg/day (14 days)	Alleviated freezing-induced chronic renal failure in rats by inhibiting lipid oxidation	Lv et al. 2007
Polysaccharides	Cultured mycelia of *C. sinensis*	Protective effect on liver fibrosis	60 mg/kg/day (4 weeks)	Improved the hepatic function (ALT, AST, Alb, and Tbil), SOD, but decreased MDA, Hyp, and IV collagen content, and TMMP-2 level in liver tissue compared with dimethylnitrosamine-induced liver fibrosis in rats	Li et al. 2006c
Acidic polysaccharides	Cultured *C. militaris*	Anti-influenza virus activity	0.1 mg/15 μL/ mouse, intranasal treatment	Decreased virus titers in BALF and the lungs of mice infected with influenza A virus and increased survival rate; increased TNF-α and IFN-γ levels in mice	Ohta et al. 2007
			1–1000 μg/mL	Enhanced NO production, induced iNOS mRNA, iNOS protein, and IL-1β, IL-6, IL-10, and TNF-α mRNA in RAW 264.7 cells	
CO-N (33 kDa)	Polysaccharide fraction of *C. ophioglosoides*	Diagnosis of active Wegener's granulomatosis	N/A	Reacted with sera from patients with some collagen diseases	Ikeda et al. 1993
		Anticancer	N/A	Inhibited the proliferation of sarcoma 180 cells and the growth of a syngeneic solid tumor (MM46 mammary carcinoma) in vivo and exhibited cytotoxicity against IMC and P388D1 cells in vitro	Ohmori et al. 1989
Diphenyl Ethers					
Violaceol-I and -II	Cultured broth of *Cordyceps* sp. BCC 1861	Antitumor activity	N/A	Showed cytotoxicity against KB, BC, NCI-H187, and Vero cancer cells with IC_{50} of 6.36, 5.50, 3.70, and 1.3 μg/mL, respectively	Bunyapaiboonsri et al. 2007
		Antimicobacterial activity	N/A	Antimalarial with IC_{50} of 3.38 μg/mL; antituberculous activity with MIC of 200 μg/mL	
Cordyol C	Cultured broth of *Cordyceps* sp. BCC 1861	Antitumor activity	N/A	Showed cytotoxicity against BC, NCI-H187, and Vero cancer cells with IC_{50} of 8.65, 3.72, and 13.1 μg/mL, respectively	Bunyapaiboonsri et al. 2007

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TABLE 5.3 (*Continued*)
Chemical Compounds in Cordyceps and Their Pharmacological Activities

Compound	Source	Bioactivity	Dosage	Effect	References
		Diphenyl Ethers			
		Antimicobacterial activity	N/A	Antituberculous activity with MIC of 200 μg/mL; anti-HSV-1 with IC_{50} of 1.3 μg/mL	
Diocinol		Antitumor activity	N/A	Showed cytotoxicity against BC and Vero cancer cells with IC_{50} of 13.46 and 18.6 μg/mL, respectively	Bunyapaiboonsri et al. 2007
		Antimicobacterial activity	N/A	Antituberculous activity with MIC of 50 μg/mL	
Cordyol A		Antimicobacterial activity	N/A	Antituberculous activity with MIC of 100 μg/mL	Bunyapaiboonsri et al. 2007
		Alkaloids			
Cordyformamide	*C. brunearubra* BCC1395	Antimalarial activity	N/A	Against malarial parasite with an IC_{50} value of 18 μM	Isaka et al. 2007
		Anticancer activity	N/A	Against BC, KB, NCI-H187, and noncancerous Vero cells with IC_{50} of 39, 56, 56, and 140 μM, respectively	
Cordypyridone A	Mycelium of *C. nipponica* BCC 1389	Antimalarial activity	N/A	In vitro antimalarial activity with IC_{50} values of 0.066 μg/mL	Isaka et al. 2001
Cordypyridone B	Mycelium of *C. nipponica* BCC 1389	Antimalarial activity	N/A	In vitro antimalarial activity with IC_{50} values of 0.037 μg/mL	Isaka et al. 2001
Militarinone A	Mycelium of *Paecilomyces militaris* (anamorph of *C. militaris*)	Neurotrophic effect	10 μM	Had a pronounced neurotrophic effect in PC-12 cells	Schmidt et al. 2002
Militarinone B		Neurotrophic effect	N/A	Had a pronounced neurotrophic effect in PC-12 cells	Schmidt et al. 2003
Militarinone C		Neurotrophic effect	N/A	Had a pronounced neurotrophic effect in PC-12 cells	
Militarinone D		Anticancer activity	N/A	Had cytotoxicity in PC-12 cells	

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(+)-N-deoxymilitarinone A	*P. farinosus* RCEF 0097	Neurotrophic effect	33–100 μM	Induced neurite sprouting in PC-12 cells	Cheng et al. 2006
		Anticancer activity	100 μM	A cytotoxic effect was observed in human neurons (IMR-32)	
Farinosones A	*P. farinosus* RCEF 0101	Neurotrophic effect	50 μM	Induced neurite outgrowth in the PC-12 cell line	Cheng et al. 2004
Farinosones A		Neurotrophic effect	50 μM	Induced neurite outgrowth in the PC-12 cell line	
(3R,6R)-4-methyl-6-(1-methylethyl)-3-phenylmethylperhydro-1,4-oxazine-2,5-dione	Fruiting bodies of *Isaria japonica*	Anticancer activity	5–100 μg/mL	Induced apoptotic cell death of human leukemia cells (HL-60)	Oh et al. 2002
Proteins					
Hemagglutinin (30 kDa)	*C. militaris*	Anticancer and anti-HIV	0.0–1.2 mg/mL	Exhibited some antiproliferative activity to HepG2 cell; inhibited HIV-1 reverse transcriptase (IC_{50} = 10 μM)	Wong, Wang, and Ng 2009
CML (lectin, 31 kDa)	Ascomycete *C. militaris*	Hemagglutination and mitogenic activity	0.01–4.0 μM	Exhibited hemagglutination activity in mouse and rat erythrocytes, but not in human ABO erythrocytes; exhibited mitogenic activity against mouse splenocytes	Jung et al. 2007
Beauvericin	*C. dicadae, P.tenuipes, C. takaomanlana*	Anticancer activity	0.1–300.0 μM	Exerted cytotoxic effects on U937 and HL-60 cells	Cal⁄ et al. 2004
Cordycedipeptide A	Culture liquid of *C. sinensis* (Berk.) Sacc.	Anticancer activity	N/A	Had cytotoxic activities to L-929, A375, and Hela with IC_{50} values of 6.37, 4.69, and 12.71 μg/mL, respectively	Jia et al. 2005
Cordyheptapeptide A	*Cordyceps* sp. BCC 1788	Anticancer activity	N/A	Showed cytotoxicity against Vero cells	Rukachaisirikul et al. 2006
CSP (protease, 31 kDa)	Culture supernatant of *C. sinensis*	Fibrinolytic activity	0.5 mg/mL (0.2 μL)	Hydrolyzed fibrinogen, fibrin, and casein with a high efficiency, while hydrolyzing BSA and human serum albumin (HSA) to a lesser extent	Li et al. 2007
Enzyme (52 kDa)	Fruiting bodies of cultured *C. militaris*	Fibrinolytic activity	10 μL (0.64 mg/mL)	Hydrolyzed the fibrin α-chain followed by the γ-γ chains and the β-chain	Kim et al. 2006
			10 μg	Exhibited fibrinogenolytic activity by rapidly hydrolyzing the fibrinogen Aα, Bβ, and γ chains	

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TABLE 5.3 (*Continued*)
Chemical Compounds in Cordyceps and Their Pharmacological Activities

Compound	Source	Bioactivity	Dosage	Effect	References
			p-Terphenyls		
Gliocladinin A	Solid cultures of *Gliocladium* sp. that colonizes *C. sinensis*	Anticancer activity	N/A	Inhibited the proliferation of Hela and HCT116 with IC_{50} of 54 and more than 270 μM, respectively	Guo et al. 2007
		Antimicrobial activity	N/A	Showed activity against *Staphylococcus aureus* with MIC values of 270 μM	
Gliocladinin B	Solid cultures of *Gliocladium* sp. that colonizes *C. sinensis*	Anticancer activity	N/A	Inhibited the proliferation of Hela and HCT116 with IC_{50} of 40 and 80–100 μM, respectively	Guo et al. 2007
		Antimicrobial activity	N/A	Showed activity against *S. aureus* with MIC values of 130 μM	
			Naphthoquinones		
Erythrostominone	The culture of *C. unilateralis* BCC1869	Antimalarial activity	N/A	Showed antimalarial activity with IC_{50} of 4.0 μg/mL, and cytotoxic activity on BC, KB, and Vero cells with IC_{50} of 9.7, 23.0, and 15.0 μg/mL, respectively	Kittakoop et al. 1999
Deoxyerythrostominone				Showed antimalarial activity with IC_{50} of 7.5 μg/mL, and cytotoxic activity on BC, KB, and Vero cells with IC_{50} of 6.0, 12.4, and ca30 μg/mL, respectively	
4-O-methyl erythrostominone				Showed antimalarial activity with IC_{50} of 10.1 μg/mL, and cytotoxic activity on BC, KB, and Vero cells with IC_{50} of ca5, 24.0, and ca10 μg/mL, respectively	

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Epierythrostominol		Showed antimalarial activity with IC_{50} of 7.0 μg/mL, and cytotoxic activity on BC, KB, and Vero cells with IC_{50} of 4.2, 7.2, and 7.5 μg/mL, repectively
Deoxyerythrostominol		Showed antimalarial activity with IC_{50} of 8.5 μg/mL, and cytotoxic activity on BC, KB, and Vero cells with IC_{50} of ca10, 20, and 10 μg/mL, repectively
3,5,8-Trihydroxy-6-methoxy-2-(5-oxohexa-1,3-dienyl)-1,4-naphthoquinone	Antimalarial activity	Showed antimalarial activity with IC_{50} of 2.5 μg/mL

Note: p.o.: per os, oral administration; i.p.: intraperitoneal administration; i.v.: intravenous injection; s.c.: subcutaneous injection; N/A: unknown.

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5.10.2 Sterols and Their Activities

Several sterols, including ergosterol, H1-A, Δ^3ergosterol, ergosterol peroxide, ergosteryl-3-O-β-D-glucopyranoside, cereisterol, β-sitosterol, daucosterol, cholesterol, 22, 23-dihydroergosteryl-3-O-β-D-glucopyranoside, cholesteryl palmitate, campesterol, and dihydrobrassicasterol, have been identified in cordyceps (Feng, Yang, and Li 2008). Ergosterol exists in both free and combined forms in cordyceps, and the content of the free form is fairly high in both natural and cultured cordyceps (Yang et al. 2009). Ergosterol is a biological precursor of vitamin D_2, needed for bone development in humans. The sterol β-sitosterol is found mainly in natural cordyceps and commercial cultured *C. sinensis*, whereas it is lacking in commercial cultured *C. militaris* and cultured *C. sinensis* in Yang's laboratory (Yang et al. 2009). In Europe, β-sitosterol plays a major role in the treatment of benign prostatic hypertrophy (Wilt et al. 2000). Phytosterols, especially β-sitosterol, play a protective role against colon, prostate, and breast cancer (Awad et al. 2000). Moreover, phytosterols, mainly β-sitosterol, campesterol, and stigmasterol, decrease cholesterol absorption while being poorly absorbed themselves (Ostlund 2007). The bioactivities of sterols are helpful in elucidating some therapeutic indications of cordyceps such as in hyperlipidemia and cancer.

5.10.3 Free Fatty Acids and Their Activities

Ten free fatty acids (FFAs), that is, lauric acid, myristic acid, pentadecanoic acid, palmitoleic acid, palmitic acid, linoleic acid, oleic acid, stearic acid, docosanoic acid, and lignoceric acid, have been found in natural *C. sinensis*, *C. liangshanensis*, and *C. gunnii*, as well as in cultured *C. sinensis* and *C. militaris*. Among these FFAs, palmitic acid, linoleic acid, oleic acid, and stearic acid are the major components in natural and cultured cordyceps. Natural cordyceps contains more palmitic acid and oleic acid than cultured (Yang et al. 2009). The FFAs are not only essential nutritional compounds but also modulators of many cellular functions through their receptors. The FFA receptors are G-protein-coupled receptors, including G-protein receptor (GPR) 40, GPR41, GPR43, GPR120, and GPR84 (Rayasam et al. 2007; Hirasawa et al. 2008; Swaminath 2008). The activation of FFA receptors exhibits several physiological effects (Table 5.4); they, therefore, are purported to be novel therapeutic targets for diabetes, dyslipidemia, and immunomodulation, especially type 2 diabetes.

Pentadecanoic acid (C15) and palmitic acid (C16) are the most potent FFAs on GPR40, and can activate the GPR40 receptor and stimulate calcium release (Briscoe et al. 2003). This, in turn, triggers insulin release from the β-cells of the pancreas, thus producing a hypoglycemic effect. Both these FFAs exist in both wild and cultured cordyceps, palmitic acid being a main ingredient, and palmitic acid may be one of the active hypoglycemic components in cordyceps. On the other hand, FFAs in cordyceps may also indirectly promote glucose-stimulated insulin secretion and then inhibit plasma glucose level by activation of GPR120 in the intestinal tract (Hirasawa et al. 2008). The receptors GPR41, GPR43, and GPR84 are expressed on immune cells. Activation of these receptors by FFAs induces an immunomodulatory effect (Swaminath 2008) and cordyceps contains FFAs and possesses significant relevant activity, indicating that the FFAs in the cordyceps contribute to its immunomodulatory mechanisms.

5.10.4 Carbohydrates and Their Activities

Cordyceps not only contains a high amount of polysaccharides, ranging from 3–8% of the total dry weight, but also contains a high amount of D-mannitol. D-mannitol, also called cordycepic acid, was isolated from *C. sinensis* in 1957. It is one of the major compounds in natural and cultured cordyceps, which contributes over 3.4% (Li and Yang 2008a) and 2.4% (Feng, Yang, and Li 2008) of total dry weight, respectively. Due to its osmotic activity, D-mannitol has long been used for the treatment of cerebral edema and refractory intracranial hypertension in traumatic brain

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TABLE 5.4
Summary of Free Fatty Acid Receptor Ligands and Physiological Roles in Various Tissues

Subtype	Agonist	G-Protein Coupling	Tissue Distribution	Physiological Roles
GPR40	Medium-long chain, C12–C16	Gq/11	Pancreas, gastrointestinal tract, brain, monocytes	Glucose-dependent insulin release
GPR41	Short chain, C3–C5; equally activated by propionate, butyrate, and pentanonate	Gi/o	Immune cells, adipose tissue	Leptin production; anti-inflammatory response
GPR43	Short chain, C2–C3; prefer propionate	Gq/11, Gi/o	Immune cells (particularly in polymorphonuclear cells), spleen, bone marrow, adipose tissue	Inhibits lipolysis; immune function
GPR120	Medium-long chain, saturated FFAs C14–C18; unsaturated FFAs C16–C22	Gq/11	Intestinal tract, adipocytes, taste buds and lungs	Glucagon-like peptide-1 secretion; insulin secretion
GPR 84	Medium chain, C9–C14	Gi/o	Granulocytes, neutrophils, eosinophils, peripheral blood monocytes	Immune function

injury, subarachnoid hemorrhage, and stroke (Rangel-Castilla, Gopinath, and Robertson 2008), as well as in acute renal failure (Lameire, De Vriese, and Vanholder 2003). The inhalation of dry, powdered mannitol is a useful therapeutic agent for patients with cystic fibrosis (Jaques et al. 2008) and bronchiectasis (Ilowite, Spiegler, and Chawla 2008); the inhaled powder increases mucociliary clearance by rehydrating the airway. Mannitol is also used as a diagnostic test for airway hyper-responsivenes to help in the diagnosis of asthma (Anderson et al. 2009). These pharmacological effects of D-mannitol can thus be one important reason for cordyceps being used to treat some respiratory diseases such as asthma and chronic bronchitis, renal dysfunction and renal failure, and hypertension.

5.11 CONCLUSIONS

C. sinensis is a valued traditional Chinese medicine. Because it is rare and expensive, several other natural cordyceps, cultured mycelia, and fruiting bodies of cordyceps have become its main substitutes in commercial health food formulations. Experiments have shown that cordyceps has several bioactivities, such as antitumor, immunomodulatory, antioxidant, sexual and reproductive function enhancement, hypoglycemic, and antifatigue activities, and have a protective effect on the kidney and liver. Different compounds contribute to different bioactivities. Normally, the cultured mycelia of cordyceps are as effective as those found in natural cordyceps. Cordyceps is quite safe in the in vivo treatment of animals for up to 3 weeks. Fermented products of cordyceps, along with natural *C. sinensis*, could be potential agents or functional foods for maintaining human health.

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6 Cranberry

Catherine C. Neto and Joe A. Vinson

CONTENTS

6.1 Cranberry: Introduction and Traditional Origins 107
6.2 Production and Consumption 108
6.3 Phytochemical Composition 109
6.3.1 Anthocyanins 109
6.3.2 Flavonols 111
6.3.3 Flavan-3-ols (Catechins) 113
6.3.4 Proanthocyanidins 113
6.3.5 Benzoic and Phenolic Acids 113
6.3.6 Nonflavonoid Polyphenols 114
6.3.7 Terpenes and Sterols 114
6.4 Bioavailability of Cranberry Phytochemicals 114
6.5 Human Studies Relevant to Heart Disease and Diabetes 115
6.6 Investigation of Cranberry Constituents That Contribute to In Vitro Anticancer Activity 116
6.6.1 Ursolic Acid and Derivatives 116
6.6.2 Polyphenolics 117
6.6.3 Quercetin 117
6.6.4 Anthocyanins 117
6.6.5 Anticancer Activities of Cranberry Proanthocyanidins 118
6.7 Possible Chemopreventive Mechanisms and Effects 119
6.7.1 Apoptosis 119
6.7.2 Matrix Metalloproteinase Inhibition 119
6.7.3 Modulation of Ornithine Decarboxylase 120
6.7.4 *Helicobacter pylori* Inhibition 120
6.7.5 Evidence from Animal Models 120
6.7.6 Anti-Inflammatory Activities 121
6.8 Cranberries and Urinary Health 122
6.9 Future Directions for Research on Cranberries and Disease 123
Acknowledgments 124
References 124

6.1 CRANBERRY: INTRODUCTION AND TRADITIONAL ORIGINS

The name "cranberry" reportedly derives from the Pilgrim name for the fruit "craneberry," because the small, pink blossoms that appear in the spring resemble the head and bill of a Sandhill crane. Cranberries are unique among fruits. They can grow and survive only under a special combination of factors. They require acid peat soil, adequate freshwater supply, sand, and a growing season that stretches from April to November and is followed by a period of dormancy in the winter months that provides an extended cold period, necessary for fruiting buds to mature. Contrary to popular belief, cranberries do not grow in water. Instead, they grow on vines in impermeable beds that are layered

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with sand, peat, gravel, and clay. These beds, commonly known as "bogs," were originally made by glacial deposits, but now, they can be made by humans. Growers do not have to replant, since an undamaged cranberry vine will survive indefinitely. Some vines in Massachusetts are more than 150 years old. European settlers adopted the Native American uses of the fruit and found that cranberry is a valuable bartering tool. American whalers and mariners carried cranberries on their voyages to prevent scurvy. In 1816, Captain Henry Hall was the first to successfully cultivate cranberries (Cape Cod Cranberry Grower's Association, http://www.cranberries.org/cranberries/history.html). Today, cranberries are available in both fresh and processed forms. There are many varieties of cranberry fruit; white cranberry is an early harvest cranberry, which is picked 2–3 weeks prior to ripening.

There are two major species of cranberry: the American cranberry (*Vaccinium macrocarpon*) and the European cranberry (*V. oxycoccos*). The European cranberry fruit is smaller (0.6–1.2 cm) and only half the size of the American fruit. The American cranberry, which is frequently cultivated, is a member of the Ericaceae family, evergreens, creeping shrubs native to the cool, temperate, acidic soils and peat wetlands of Northeastern United States and southern Canada. Latvia, Belarus, Azerbaijan, and Ukraine are other cranberry-producing countries in Europe, with Turkey just beginning cranberry cultivation. The United States and Canada together account for more than 90% of the world's production (Zhao 2007). The forecast for U.S. cranberry production in 2010 is 735 million pounds up by 6% from 2009 figures and the second highest yearly production. Wisconsin is expected to lead all U.S. states in the production of cranberries, with 435 million pounds, followed by Massachusetts, with 195 million. New Jersey, Oregon, and Washington are also expected to have substantial production (Cranberries, National Agricultural Statistics Service (NASS), Agriculture Statistics Board, United States Department of Agriculture (USDA), August 17, 2010).

Cranberries are low-growing, woody, perennial vines with small, alternate, and ovate leaves. The plant produces stolons (horizontal stems) having a height of up to 6 feet (2 m). Short, vertical branches, or uprights, 2–8 inches (5–20 cm) in height, grow from buds on the stolons, and these can be either vegetative or fruiting. Each fruiting upright may contain as much as seven flowers. Pollination is primarily via domestic honeybees (Cranberry Institute, East Wareham, Massachusetts). Cranberries were first used by Native Americans, who discovered the wild berry's versatility. Native Americans used cranberries in a variety of foods, the most popular being pemmican, a high-protein combination of crushed cranberries, dried deer meat, and melted fat. They also used it as a medicine to treat arrow wounds and as a dye for rugs and blankets.

6.2 PRODUCTION AND CONSUMPTION

About 95% of the cranberries cultivated are processed into products such as juice drinks, sauce, and sweetened, dried cranberries. The remaining 5% are sold fresh to consumers. Cranberries used for processing are commonly frozen in bulk containers shortly after arriving at a receiving station. The primary method of harvesting cranberries takes advantage of the ability of cranberries to float. In this method, which is called wet harvesting, cranberry fields are flooded with water. After flooding, eggbeater-like devices stir up the water with sufficient force to dislodge the berries from the vines. When the berries float on the surface of water, they are pulled to the shore with hinged two-by-fours and loaded in trucks for delivery to the factory. Wet-harvested cranberries are generally processed for juice and sauce, because once a berry gets wet, there is increased chance of spoilage unless it is processed rapidly. Dry harvesting is the second method of harvesting cranberries. Because fully ripe berries are easily dislodged from their vines, this method employs a lawn mower–type machine that combs the berries from the vines. On arrival at the factory, dry-harvested cranberries are subjected to a unique test. The superior berries are sorted from those that are bruised, soft, or rotten by taking advantage of the fact that berries bounce when they are of good quality. Along a conveyor belt, each berry must successfully bounce over a series of wooden barriers. Berries that

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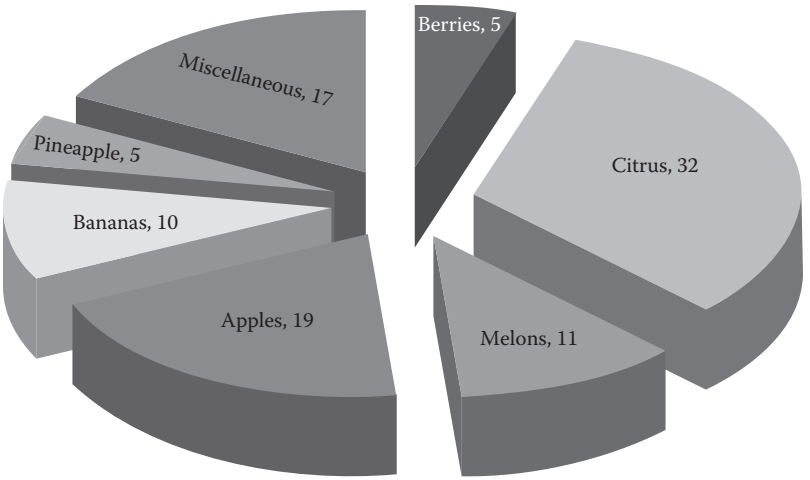


FIGURE 6.1 Per capita consumption of fruits in the United States in 2007. Economic Research Service (ERS), U.S. Department of Agriculture (USDA). Food Availability (Per Capita) Data System. http://www.ers.usda.gov/Data/FoodConsumption. Data last updated February 16, 2010.

fail this simple test fall into a disposal bin. The ones that pass the bounce test are sold as fresh fruit, typically packaged in plastic (MotherLindas.com, http://www.motherlindas.com/cranberries.htm).

There are approximately 450 cranberries in 1 pound, 4,400 cranberries in 1 gallon of juice, and 45,000 cranberries in a 100-pound barrel. It takes about 200 cranberries to make one can of cranberry sauce (foodreference.com, http://www.foodreference.com/html/fcranberries.html). The cranberry is an underconsumed fruit in the United States. Consumption of different types of fruits in the United States is shown in Figure 6.1. Americans eat on an average 326 g of fruit per day, and only 5.3% of this intake is berries (18 g). This translates to 2.8 g or about three cranberries per day. This is equivalent to drinking 0.25 mL of cranberry juice per day. The dominant cranberry product consumed in the United States is juice. In comparison, the per capita fruit consumption in the European Union in 2007 was considerably less, only 266 g/day (Freshfel Europe 2009). Average per capita consumption of berries in Norway was only 5 g/day (Ellingsen et al. 2008). Thus, Europeans on the average consume fewer berries than the U.S. population.

6.3 PHYTOCHEMICAL COMPOSITION

It is interesting to discover via a PubMed search that there are over 52,000 references for flavonoids (http://www.ncbi.nlm.nih.gov/sites/entrez, accessed Nov. 14, 2010). Among the subclasses, the order of references is flavonols > flavonones > anthocyanins > flavanols (catechins). Table 6.1 lists the potentially bioactive compounds identified and often quantified in cranberries. Table 6.2 lists the flavonoids quantified in cranberry as listed in the U.S. Department of Agriculture (USDA) database. Representative structures of the major constituents are shown in Figures 6.2 and 6.3.

6.3.1 Anthocyanins

When one thinks of cranberries, it is the color red that comes to mind. It is due to the presence of anthocyanins, which have been extensively studied for health benefits. There are six aglycones in the anthocyanin class, and the number of potential isomers is extremely large due to different positions of sugar attachment and the large number of different mono- and disaccharides (glycosides) and, the less common, but possible, phenolic acids and acyl groups that can be attached. Anthocyanin content has been reported to be as high as 91.5 mg/100 g ripe fruit at harvest depending on the cultivar

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TABLE 6.1
Potentially Bioactive Compounds Present in Cranberries and Cranberry Juice

Class of Compounds	Citation and Derivatives Identified
Anthocyanins	Functional group attached; Cunningham et al. 2003; Wu and Prior 2005
Cyanidin	Galactoside, arabinoside, and glucoside
Peonidin	Galactoside, arabinoside, glucoside, and digalactoside
Malvidin	Galactoside and arabinoside
Pelargonidin	Galactoside and arabinoside
Delphinidin	Arabinoside
Petunidin	Galactoside
Flavonols	Yan et al. 2002; Zheng and Wang 2003; Cunningham et al. 2003; Vvedenskaya et al. 2004
Quercetin	Galactoside (hyperin), rhamnoside (quercetin), xyloside (avicularin), and glucose (isoquercetin)
Kaempferol	Glucoside
Myrcetin	Xylopyranoside and arbinofuranoside
Catechins and Flavanols	Harnly et al. 2006
Epicatechin, catechin, epigallocatechin, epigallocatechin gallate, catechin gallate, and gallocatechin gallate	
Proanthocyanidins (dimers, trimers, and oligomers)	Prior et al. 2001; Gu et al. 2004
Procyanidin B2 EC-(4β→8)-EC Procyanidin A2 EC-(4β→8)-EC	
EC-(4β→6)-EC-(4β→8, 2β→*O*→7)-EC EC-(4β→8, 2β→O→7)-EC-(4β→8)-EC	
4–6mers	
7–10mers	
>10mers	
Benzoic and phenolic acids	Zuo, Wang, and Zhan 2002; Zheng and Wang 2003; Cunningham et al. 2003; Zhang and Zuo 2004; Mullen, Marks, and Crozier 2007
Benzoic acid; salicylic acid; *m*-hydroxybenzoic acid; *p*-hydroxybenzoic acid; 2,3-dihydroxybenzoic acid; 2,4-dihydroxybenzoic acid; 3,4-dihydroxybenzoic acid; *p*-hydroxyphenylacetic acid; vanillic acid; *trans*-cinnamic acid; *o*-hydroxycinnamic acid; *p*-coumaric acid; *o*-phthalic acid; caffeic acid; ferulic acid; sinapic acid; chlorogenic acid; and 5-*O*-caffeoylquinic acid	
Nonflavonoid polyphenols	Zafriri et al. 1989; Turner et al. 2005; Baur and Sinclair 2006; Turner et al. 2007
Phloretin-2-glucoside (phloridzin), ellagic acid, 2-*O*-(3,4-diydroxybenzoyl)-2,4,6-trihydroxyphenylmethylacetate, *cis*-resveratrol, *trans*-resveratrol, and secoisolariciresinol	
Terpenes and sterols	Jensen et al. 2002; Murphy et al. 2003; He and Liu 2006; Turner et al. 2007
Oleanolic acid, ursolic acid (UA), *cis*-3-*O*-*p*-hydroxy cinnamoyl UA, *cis*-3-*O*-*p*-hydroxy cinnamoyl UA, β-sitosterol, β-sitosterol-3-*O*-β-D-glucoside, monotropein, 6,7-dihydro monotropein, 10-*p*-*cis*-coumaroyl-1S-dihydro monotropein, and 10-*p*-*trans*-coumaroyl-1S-dihydro monotropein	

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TABLE 6.2
Content of Flavonoids in Cranberry Fruit by Flavonoid Class, as Reported by the USDA Databases on Nutrient Composition of Foods

Flavonoid	Milligrams per 100 Gram of Whole Cranberry Fruit
Flavonols, total[c]	21.96
Quercetin[a]	15.09 ± 1.06
Myricetin[a]	6.78 ± 1.67
Kaempferol[a]	0.09 ± 0.03
Anthocyanins, total[c]	91.57
Cyanidin[a]	41.81 ± 2.86
Peonidin[a]	42.10 ± 3.64
Delphinidin[a]	7.66 ± 1.93
Flavan-3-ol monomers[b]	7.26
(−)Epicatechin[a]	4.37 ± 0.93
(−)Epigallocatechin[a]	0.74 ± 0.28
(−)Epigallocatechin gallate[a]	0.97 ± 0.48
(+)–Catechin[a]	0.39 ± 0.16
PACs, total[c]	411.5
Dimers[b]	25.93 ± 6.12
Trimers[b]	18.93 ± 3.39
4–6mers[b]	70.27 ± 13.07
7–10mers[b]	62.90 ± 14.71
Polymers[b]	233.48 ± 49.08

[a] *Source:* USDA, 2007. Database for the Flavonoid Content of Selected Foods, Release 2.1, http://www.ars.usda.gov/nutrientdata.

[b] *Source:* USDA, 2004. Database for the Proanthocyanidin Content of Selected Foods, http://www.nal.usda.gov/fnic/foodcomp/Data/PA/PA.pdf.

[c] Sum of individual flavonoid contents in each class.

(Wang and Stretch 2001; Wu et al. 2006). Fruit of the early black cultivar tends to contain more anthocyanins and proanthocyanidins (PACs) than other cranberry cultivars (Wang and Stretch 2001; Vorsa and Howell 2003). Cranberry, compared to many other berries, has a very small number of anthocyanin isomers (n = 13), the major ones being galactosides and arabinosides of cyanidin and peonidin. A very similar anthocyanin profile exists in cranberry juice (Fuleki and Francis 1968; Ohnishi et al. 2006). Only the most recent references are listed in Table 6.1, since the newer technology liquid chromatography-mass spectrometry (LC-MS)/MS is better able to unequivocally identify compounds in mixtures than the classical methods of column chromatography followed by crystallization and spectral investigation. Whole cranberries contain a very broad concentration range of anthocyanins, 180–596 mg/kg fresh weight (fw; Bilyk and Sapers 1986), which also varies according to maturity from 0.8 to 111.0 mg/kg fw from the green to dark red stage (Celik et al. 2008). Cranberry juice contains much lower levels of anthocyanins, ranging from 1.3 mg/100 mL (Prior et al. 2001) to 2.5 mg/100 mL of juice (Cunningham et al. 2003).

6.3.2 Flavonols

The total amount of flavonols in cranberry ranges from 200 to 400 mg/kg (Zheng and Wang 2003; Cunningham et al. 2003; Vvedenskaya et al. 2004; Neto 2007b). Cranberry is the best source of flavonols among 30 flavonol-containing plant foods studied (Aherne and O'Brien 2002), and the

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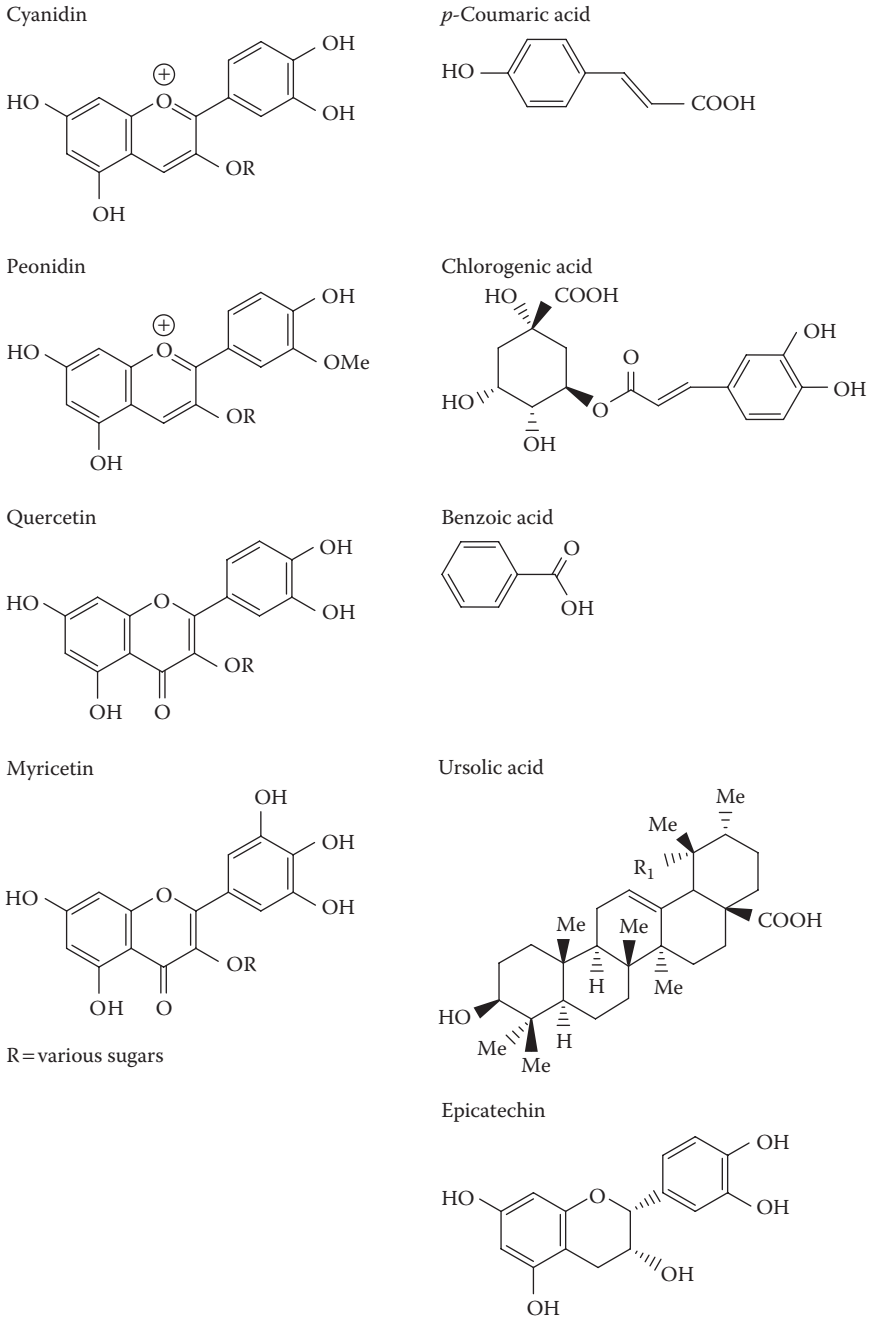


FIGURE 6.2 Structures of some of the major anthocyanins (cyanidin and peonidin), flavonols (quercetin and myricetin), phenolic and organic acids (*p*-coumaric acid, chlorogenic acid, and benzoic acid), flavan-3-ols (epicatechin), and terpenoids (ursolic acid) in cranberry fruit.

flavonol content of cranberry is almost twice as high as 12 other commonly consumed fruit juices, including pomegranate and grape (Mullen, Marks, and Crozier 2007). Quercetin is the most abundant flavonol in cranberry, and it varies from 11 to 25 mg/100 g, primarily as the 3-*o*-galactoside (Yan et al. 2002; Vvedenskaya et al. 2004). Cranberry is also the best source of quercetin (Manach et al. 2004). Myricetin is the second most abundant flavonol, followed by kaempferol (Vvedenskaya

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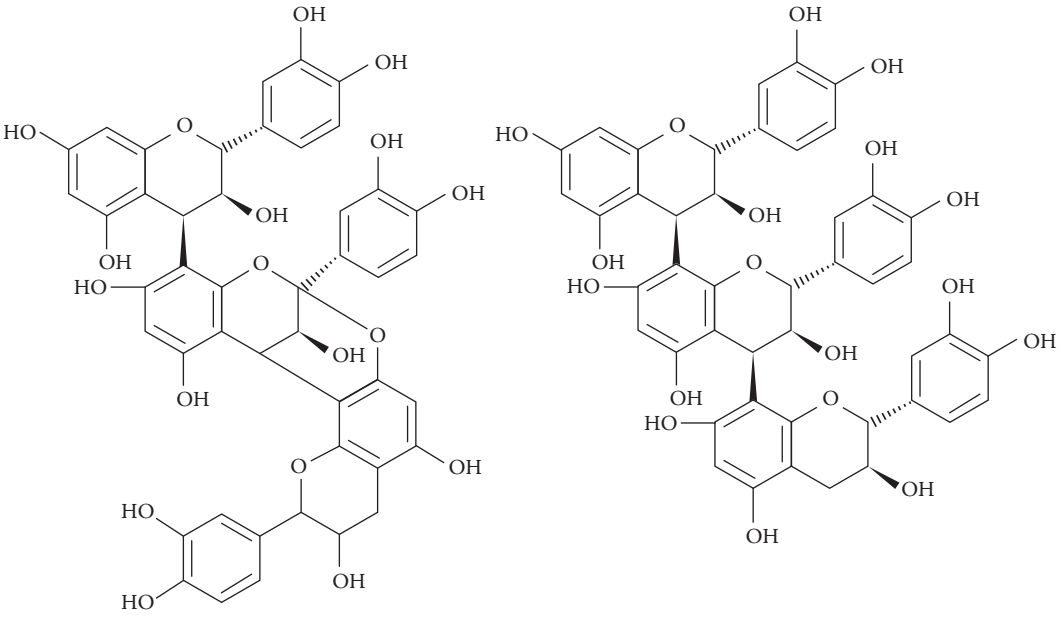


FIGURE 6.3 Cranberry uniquely contains A-type linkage (left), whereas other foods contain B-type linkage (right).

et al. 2004). These compounds are yellow in color, and there are 20 different flavonol glycosides in cranberry, as confirmed by another article (Vvedenskaya and Vorsa 2004).

6.3.3 Flavan-3-ols (Catechins)

Total catechins in raw cranberry average 17 mg/100 g, with epicatechin being the most abundant (Harnly et al. 2006). Berries have the highest catechin content among fruits. Cranberries have the highest levels of monomers (catechin + epicatechin) among berries, and monomer levels in cranberries are twice as high as those in blueberries (Gu et al. 2004). Cranberry juice contains 6 mg/l of catechins (Gu et al. 2004). One liter of cranberry juice has catechin and epicatechin monomers as 100 g of dark chocolate.

6.3.4 Proanthocyanidins

These compounds are the largest class of potential bioactives in cranberries. The PACs are also commonly called condensed tannins or poly flavan-3-ols, and are responsible for the bitter astringent taste of cranberries due to the binding of saliva proteins. Cranberries have the most dimers, trimers, 4–6mers, and 7–10mers compared with any other fruit studied (Gu et al. 2004). The most unique aspect of cranberry polyphenols is the occurrence of A-type linkages (C2→O→C7) between epicatechin units, as shown in Figure 6.3 (Foo et al. 2000b). These linkages are found elsewhere only in peanuts, blueberries, and plums (Gu et al. 2003). The average concentration of PACs is 419 mg/100 g (0.4%) by weight in cranberries and 231 mg/l in cranberry juice (Gu et al. 2004). These compounds exhibit potent in vitro antiadhesion bioactivity and are present in much higher concentrations in cranberries than in other foods.

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6.3.5 Benzoic and Phenolic Acids

Benzoic and phenolic acids represent 0.57% of the weight of fresh cranberries (Zuo, Wang, and Zhan 2002). Benzoic acid forms 80% of the total organic acids contained in cranberry juice. There exist 14 other benzoic and phenolic acids in cranberry juice, and *p*-coumaric acid is the most prevalent hydroxycinnamic acid. Many of these acids are bound to glucose and polysaccharides in cranberry (He and Liu 2006).

6.3.6 Nonflavonoid Polyphenols

Phloridzin is the most prevalent member of the class of dihydrochalcones present in cranberries at 120 mg/kg (Turner et al. 2007). This compound is present in very high quantities in apple, and is a dihydrochalcone with glucose-lowering activity in an animal model of diabetes (Masumoto et al. 2009). Resveratrols, one of the most studied polyphenolic compounds, with 2937 PubMed references, are found in cranberry juice at extremely low levels of 0.2 mg/l (Wang et al. 2002). This is much lower than the resveratrol content of red wine (14 mg/l; Baur and Sinclair 2006). Other compounds belonging to this stilbene class are present at very low levels in cranberry juice, and thus are probably nonbioactive.

6.3.7 Terpenes and Sterols

Terpenes include the volatile compounds that are responsible for the flavor and aroma of cranberries (Croteau and Fagerson 1968), as well as larger oily or waxy compounds. Cranberry fruit contains the triterpenoid ursolic acid (UA) in its peel (Figure 6.3), existing in aglycone form as well as in cis and trans *p*-hydroxycinnamate esters (Murphy et al. 2003). Quantitative analysis of cranberry fruit and products by LC-MS has determined the UA content of the whole cranberry fruit of different cultivars to be between 60 and 110 mg per 100 g of fresh fruit (Kondo 2006). UA has been reported to be a constituent of other fruits, including apples and highbush blueberries (Wang et al. 2000). The iridoid glycosides listed in Table 6.1 are believed to be unique to the *Vaccinium* species. Cranberries also contain the carotenoid lutein, as well as other carotenoids in lesser quantities. It is worth noting that although the compounds listed in Table 6.2 are the result of studies conducted on the American cranberry (*V. macrocarpon*), a similar variety of anthocyanins, flavonols, nonflavonoid polyphenols, and phenolic acids are found in the European cranberry (*V. oxycoccus*) even though this variety has not been studied as extensively as its American counterpart (Häkkinen et al. 1999; Kähkönen, Hopia, and Heinonen 2001).

6.4 BIOAVAILABILITY OF CRANBERRY PHYTOCHEMICALS

The first evidence of human absorption of cranberry compounds was demonstrated by a single subject who consumed 1800 mL of 27% commercial cranberry juice after an overnight fast (Zhang and Zuo 2004). The plasma was then extracted and derivatized prior to assay. Sixteen compounds were identified in plasma by GC-MS from blood samples drawn 45 and 270 minutes after consumption. After 45 minutes, five acids were identified: (1) benzoic acid, (2) *o*-hydroxybenzoic acid (salicylic acid), (3) *p*-hydroxyphenylacetic acid, (4) 2,3-dihydroxybenzoic acid, and (5) 2,4-dihydroxybenzoic acid. Phenylacetic acid and dihydroxybenzoic acid are not found in cranberry juice and, thus, they are the products of polyphenol breakdown or bacterial metabolism in the gut. At 270 minutes, the same five acids and ferulic acid plus sinapic acid were found. Anthocyanins were not analyzed in the study. In 2005, an early-morning urinary excretion study was conducted with 11 females who were administered a commercial cranberry juice three times a day (250 mL each) for 2 weeks (Duthie et al. 2005). High-performance liquid chromatography (HPLC) with electrochemical detection was used after urine hydrolysis with NaOH and extraction. After 1–2 weeks, the urinary

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level of salicyluric acid (metabolite of salicylic acid) significantly increased. This compound was also increased in the fasting plasma after 2 weeks of consumption of cranberry juice. A Japanese group gave 200 mL of 100% cranberry juice to 11 subjects, and urine was collected before administering the juice and again 24 hours afterwards (Ohnishi et al. 2006). Six of the 12 anthocyanin glycosides identified in cranberry juice by LC-MS/MS were found in the collected urine. Peonidin 3-*o*-galactoside, the second most plentiful anthocyanin in the juice, was the major anthocyanin in the urine. Over 5% of the consumed anthocyanins were found in the urine. This level of absorption is at least 10 times higher than that found for other berry juices, which ranges from 0.08% for blood orange juice (Giordano et al. 2007) to 0.2% for red wine and red grape juice (Frank et al. 2003) and 0.4% for black currant juice (Bitsch et al. 2004). A safety study in which a cranberry powder was given to 65 healthy women for 8 weeks at a dose of 1200 mg/day and the effects were compared with a placebo (Valentova et al. 2007) showed no toxicity for the powder. In decreasing order of content, hippuric acid, salicyluric acid, quercetin glucuronide, and dihydroxybenzoic acid isomers were found in the urine by LC-MS.

6.5 HUMAN STUDIES RELEVANT TO HEART DISEASE AND DIABETES

Growing evidence points to many potential pharmacological activities for cranberry and cranberry juice. Due to its high content of flavonoids and phenolic acids, cranberry ranks highly among fruits in both antioxidant quality and quantity (Vinson et al. 2001). In fact, cranberry has the highest level of fw polyphenols among a group of 22 fruits studied. These antioxidant properties are likely to contribute to cranberry's disease-fighting ability.

Cranberry compounds can cause an improvement in antioxidant status, which might be beneficial with respect to chronic diseases. The potential cardiovascular benefits have been reviewed by two groups of researchers (McKay and Blumberg 2007; Ruel and Couillard 2007). A single dose of 500 mL 27% cranberry administered to nine healthy women increased plasma antioxidant capacity against a sucrose control (Pedersen et al. 2000). The authors stated that this increase was due to the presence of ascorbic acid (vitamin C) in cranberry juice. Unfortunately, ascorbic acid was not included in the control sucrose solution. Another group found that cranberry juice relative to a control containing sugars and ascorbic acid significantly raised the plasma antioxidant capacity in normal subjects (Vinson et al. 2008). They also found that when cranberry juice was spiked on human plasma, the lipoproteins low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) were protected from oxidation in a dose-dependent manner. This second effect is directly related to atherosclerosis (Steinberg 2009).

The proof of benefit for disease prevention should be shown by a human supplementation study. There have been a few of these studies for cranberry, all done in the last 6 years. Cranberry powder equivalent to 240 mL of cranberry juice per day was given to 12 diabetic men and 15 diabetic women for 12 weeks (Chambers and Camire 2003). There was no effect on glucose control. According to the authors, no change was observed on glucose control because the basic cranberry powder might have undergone processing in its production from cranberry juice. In 2008, a Japanese group reported the effect of a cranberry powder and a placebo given to 30 type 2 diabetic subjects for 12 weeks (Lee et al. 2008). There was no effect of cranberry on fasting glucose or glycated hemoglobin levels. However, it was found that total cholesterol and LDL significantly decreased and the cholesterol/high-density lipoprotein (HDL) ratio significantly increased in the cranberry group. All the subjects were taking oral hypoglycemic drugs and, thus, their plasma glucose levels were under control. In another study, type 2 diabetics ($n = 12$) were given a low-calorie cranberry juice at a dose of 240 mL (Wilson et al. 2008). The juice was found to give a more favorable glucose and insulin response than a sugared water control.

Cranberry juice was given to 20 women at a dose of 750 mL/day for 2 weeks (Duthie et al. 2006). No effect on plasma lipids, antioxidant enzyme, or DNA oxidation was found, but plasma ascorbic acid was increased. Healthy men ($n = 21$) were given 7 mL/kg of cranberry juice for 14 days (Ruel et

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al. 2005). Although there was no effect on lipids, oxidized LDL was significantly decreased (10%) and plasma antioxidant capacity was significantly increased (7%) in the subjects. Inflammatory markers were not improved. In the latest study by the same group, men with elevated LDL and body mass index (BMI) were given low-calorie cranberry juice at doses of 125, 250, and 500 mL/day for three successive 4-week periods (Ruel et al. 2007). Significant decreases in oxidized LDL, systolic pressure, and the inflammatory markers ICAM-1 and VCAM-1 were recorded at the end of the study. All cranberry effects in this study were found to be beneficial with respect to heart disease.

6.6 INVESTIGATION OF CRANBERRY CONSTITUENTS THAT CONTRIBUTE TO IN VITRO ANTICANCER ACTIVITY

Cranberry's role as a potential chemopreventive agent is gradually emerging from in vitro model studies by various researchers. Cranberry compounds may act against cancers by inhibiting oxidative stress or by other pathways. Over the past 10 years, numerous in vitro studies have appeared addressing the effects of cranberry and its constituents against tumor cell proliferation and the possible mechanisms of action. Several reports of in vivo studies have appeared recently, lending support to the tumor-inhibiting potential of cranberry. These studies are discussed. A summary of in vitro and in vivo studies on the anticancer properties of cranberry, the constituents contributing to these properties, and some possible mechanisms of action are discussed.

The first in vitro report of anticancer activity in *Vaccinium* fruit appeared in 1996. A University of Illinois study reported that extracts of cranberry, bilberry, and other species inhibit ornithine decarboxylase (ODC) expression and induce the xenobiotic detoxification enzyme quinone reductase in vitro (Bomser et al. 1996). Some Canadian researchers reported that cranberry juice extracts inhibit breast tumor cell growth (Guthrie 2003), and later showed that an extract of cranberry press cake inhibits proliferation of MCF-7 and MDA-MB-435 breast cancer cells (Ferguson et al. 2004). Later studies by various researchers have focused on identifying the anticancer constituents of cranberry, which fall within several possible classes, including triterpenoids and flavonoids.

6.6.1 Ursolic Acid and Derivatives

A bioassay-guided fractionation approach was used to examine in vitro the antitumor activities of whole cranberry fruit and juice, extracts and fractions, and finally individual compounds or subfractions within structural classes. An ethyl-acetate extract of whole cranberry fruit inhibited growth of human tumor cell lines in vitro (Yan et al. 2002). From ethyl-acetate-soluble extracts, two hydroxycinnamate esters of UA were isolated and identified that inhibited the growth of several types of human tumor cells in vitro, including MCF-7 breast, HT-29 colon, DU145 prostate, H460 lung, ME180 cervical epidermoid, and K562 leukemia cell lines (Murphy et al. 2003). Growth-inhibitory concentrations (GI_{50}) for esters were 11–28 μg/mL, depending on the cell line. LC-MS analysis of cranberry fruit found that in addition to UA, its hydroxycinnamate esters are present in whole cranberry fruit in quantities averaging about 15–20 mg per 100 g of fresh fruit (Kondo 2006). UA isolated from cranberry fruit was also reported to inhibit the proliferation of HepG2 human liver cancer cells and MCF-7 cells (He and Liu 2006). It was also found to inhibit tumor colony formation in a dose-dependent manner in HT-29 and HCT116 colon tumor models over a 2-week period, based on clonogenic assays (Liberty, Hart, and Neto 2007).

UA has been reported as a constituent of herbal medicines marketed worldwide for treating inflammatory conditions (Kim et al. 2004), but bioavailability data on this compound is lacking. In vivo cancer studies of UA are scarce, but one mouse model study reported that a UA dose of 100 mg/kg inhibits murine fibrosarcoma FSaII growth (Lee et al. 2001). Numerous reports have appeared on the in vitro antitumor activity of UA (Novotny, Vachalkova, and Biggs 2001), and

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these suggest a variety of mechanisms of action, including early G1 cytostatic effect (Es-Saady et al. 1996), induction of apoptosis (Baek et al. 1997), enhancement of intracellular Ca^{2+} signaling (Novotny, Vachalkova, and Biggs 2001), enhanced release of cytochrome *c*, caspase activation (Andersson et al. 2003) and downregulation of inhibitor of apoptosis proteins (c-IAPs; Choi et al. 2000), increased expression of $p21_{WAF1}$ (Kim et al. 2000), and decreased matrix metalloproteinase (MMP)-9 expression (Cha et al. 1996). In addition to inhibiting the growth of several tumor cell lines, the hydroxycinnamoyl esters of UA were found to strongly inhibit expression of both MMP-2 and MMP-9 at micromolar concentrations in a DU-145 prostate tumor model (Kondo et al. 2004).

6.6.2 Polyphenolics

Polyphenolic compounds are expected to play a key role in chemoprevention; thus, many in vitro cranberry studies focus on the berry's polyphenolic constituents. Cranberry extracts containing PACs and other flavonoids were reported to inhibit ODC activity linked with cell proliferation in mouse epithelial (ME-308) cells (Kandil et al. 2002). Characterization of an active subfraction revealed the presence of dimers and oligomers of catechin/epicatechin, monomeric catechins, and quercetin glycosides. Water-soluble cranberry polyphenolic extracts from commercial cranberry powder were found to inhibit proliferation of several human tumor cell lines (Seeram et al. 2004) including two oral (CAL27 and KB), four colon (HT-29, HCT-116, SW480, and SW620), and three prostate (RWPE-1, RWPE-2, and 22Rv1) cancer cell lines. Although both anthocyanin and PAC subfractions inhibited proliferation, the total polyphenolic extract was found to be more effective in this study. The primary polyphenolic constituents of cranberry are flavonols, anthocyanins, and PACs. These will be considered in Sections 6.6.3 through 6.6.5.

6.6.3 Quercetin

Quercetin, a constituent of many fruits and vegetables, is widely reported to have antiproliferative and antineoplastic activities in vitro against a variety of cell lines (Ranelletti, Maggiano, and Serra 2000; Morrow et al. 2001; Sesink, O'Leary, and Hollman 2001; Lee, Huang, and Hwang 2002; Harris et al. 2005; Richter et al. 1999; Volate et al. 2005). Its mechanisms of action include induction of apoptosis, with cell-cycle arrest in G_1 phase (Richter, Ebermann, and Marian 1999; Choi et al. 2001); inhibition of epidermal growth factor (EGF) receptor expression and associated tyrosine kinase activity (Richter, Ebermann, and Marian 1999; Lee, Huang, and Hwang 2002); reduction of Ras protein expression (Ranelletti, Maggiano, and Serra 2000); increased expression of endogenous inhibitors of MMPs (Morrow et al. 2001); and phytoestrogenic interaction with estrogen receptors α and β (ERα and EBβ of human mammary MCF-7 cells; Harris et al. 2005). Quercetin is a constituent of an ethyl-acetate-soluble extract of cranberry fruit that inhibits tumor cell growth; it was nearly as effective as UA esters against the growth of MCF-7 breast adenocarcinoma, HT-29 colon adenocarcinoma, and K562 leukemia cell lines (Murphy et al. 2003). In a separate study, it was found to inhibit proliferation of HepG2 liver cancer cells (He and Liu 2006). Given the known properties of quercetin, it is a likely contributor to the observed in vitro anticancer activity of whole cranberry extracts. In vivo, quercetin glycosides are usually metabolized to sulfates or glucuronides (Sesink, O'Leary, and Hollman 2001). In a colon cancer study, a quercetin-enriched diet decreased formation of cancer precursor aberrant crypt foci fourfold in mice, and the evidence suggest that quercetin acted through induction of apoptosis via a mitochondrial pathway involving modulation of Bax and Bcl-2 protein expression (Volate et al. 2005).

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6.6.4 Anthocyanins

As powerful antioxidants, anthocyanins may be expected to inhibit oxidative processes linked with tumorigenesis. In vitro bioassays with cranberry anthocyanins show little direct antiproliferative or growth-inhibitory properties. Purified cyanidin-3-galactoside was evaluated in eight tumor cell lines in vitro using the sulforhodamine (SRB) assay and the highest concentration tested (250 μg/mL) showed less than 50% growth inhibition (Murphy et al. 2003). In another study, an anthocyanin subfraction from cranberry was found to limit growth in three prostate tumor lines (RWPE-1, RWPE-2, and 22Rv1) by 50–70%, but it did not significantly inhibit oral or colon tumor cell line proliferation (Seeram et al. 2004). Anthocyanins, including those from cranberry, have been implicated in the observed antiangiogenic properties of mixed berry extracts (Roy et al. 2002; Bagchi et al. 2004). Anthocyanin-rich extracts from a mixture of berry fruits were reported to inhibit tumor necrosis factor α (TNF-α)-induced vascular endothelial growth factor (VEGF) expression and to decrease hemangioma formation and tumor growth (Atalay et al. 2003). Anthocyanins, though not especially cytotoxic, may nonetheless play a role in limiting carcinogenesis by inhibiting other activities related to tumor formation.

6.6.5 Anticancer Activities of Cranberry Proanthocyanidins

Many studies report cranberry PACs are major contributors to anticancer activity (Kandil et al. 2002; Ferguson et al. 2004; Seeram et al. 2004). In fractionation studies of whole fruit, a PAC fraction selectively inhibited the proliferation of H460 human large cell lung carcinoma, HT-29 colon adenocarcinoma, and K562 chronic myelogenous leukemia cells in vitro. A subfraction retaining the activity in those three cell lines was isolated and characterized by matrix-assisted laser desorption/ionization (MALDI)-TOF (time-of-flight)-MS, and was found to contain PAC oligomers composed primarily of four to seven epicatechin units with at least one or two A-type linkages between the units (Neto et al. 2006). In clonogenic assays for tumor colony formation with HT-29 and HCT-116 colon tumor cell lines, a dose-dependent decrease in the number and size of tumor colonies was observed when cells were treated with a PAC fraction prepared from the early black variety of cranberry fruit (Liberty et al. 2009). The MALDI-TOF-MS characterization of this PAC fraction revealed that it was composed primarily of trimers through hexamers of epicatechin with both A- and B-type linkages. The fraction inhibited growth more effectively than the whole polyphenolic extract, with over 50% inhibition of colony formation in HCT116 observed at concentrations less than 10 μg/mL (Liberty, Hart, and Neto 2007).

The structures of PACs in cranberry fruit and cranberry products are complex, and further investigation is needed to determine any link between structure and activity of PAC. The MALDI-TOF-MS analysis found that PAC oligomer fractions from whole cranberry contained molecules up to 12 degrees of polymerization (DP) in size with as many as four A-type linkages. Although most fractions contained epicatechin units exclusively, some epigallocatechin unit masses were also detected (Neto et al. 2006). The effect of A-type linkages and oligomer size on the antitumor activity of PACs is the subject of ongoing studies. Earlier reports on PACs from various sources suggest that A-type linkages influence tumor-inhibitory and tumor-selectivity properties. A screening of small polyflavan-3-ols from different plants against GLC4 lung and COLO 320 colon carcinomas found that a trimer with an A-type linkage was more cytotoxic than dimers with A-type linkages and trimers with only B-type linkages (Kolodziej et al. 1995). In another study, wild blueberry PACs containing A-type linkages also showed selectivity, with a greater growth-inhibitory effect occurring with androgen-sensitive LNCaP prostate cancer cells than with androgen-insensitive DU145 cells (Schmidt, Erdman, and Lila 2006).

A recent study on cranberry PACs reports some promise for cranberry in alleviating cancer of the esophagus. A known risk factor for this type of cancer is acid reflux (Herulf et al. 1999). An in vitro model study employing SEG-1 human esophageal adenocarcinoma was used to investigate

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cranberry's effects on acid-induced cell proliferation. Cells were pretreated with PAC extract at concentrations of 25 or 50 μg/mL and then pulsed with acidified medium to simulate reflux. Maximum proliferation was induced 6 hours after treatment; further, proliferation was inhibited significantly in the PAC-treated cells. Annexin-V staining showed that apoptosis had occurred in treated cells, and flow cytometry experiments found these cells arrested at the G1 checkpoint (Kresty, Howell, and Baird 2008).

Recent findings suggest that cranberry PACs may work together with platinum drugs to limit the proliferation of ovarian cancer cells. In a study using a platinum-resistant SKOV-3 human ovarian adenocarcinoma cell line, treatment with a sublethal concentration of paraplatin together with an isolated cranberry PAC fraction was found to improve efficacy in decreasing cell proliferation as compared to treatment with PACs alone (Singh et al. 2009).

6.7 POSSIBLE CHEMOPREVENTIVE MECHANISMS AND EFFECTS

Berry bioactives may act individually, additively, or synergistically to prevent cancer proliferation (Seeram 2006). Inhibition of tumorigenesis by cranberry can involve complementary or synergistic activities between the flavonols, PACs, UA, and anthocyanins, since all these compounds have been reported as showing antiproliferative properties. Possible mechanisms of action for chemoprevention supported by in vitro evidence include induction of apoptosis, decreased invasion of surrounding tissue due to MMP inhibition, reduction of ODC expression and activity, and inhibition of oxidative or inflammatory processes.

6.7.1 Apoptosis

Induction of apoptosis is thought to play a role in the tumor-inhibitory activity of dietary phytochemicals including resveratrol (Joe et al. 2002) and epigallocatechin gallate (Yang et al. 1998). Recent reports suggest that apoptosis may play a key role in cranberry's ability to limit tumor cell growth. This activity may be associated with the presence of quercetin, UA, and/or PACs, which are all known to induce apoptosis (Baek et al. 1997; Choi et al. 2001). Dose-dependent induction of apoptosis by cranberry was observed in breast tumor models. An antiproliferative fraction from cranberry press cake also induced apoptosis in MDA-MB-435 breast tumor cells as determined by Annexin-V staining (Ferguson et al. 2004). An 80% aqueous acetone extract of whole cranberry fruit was reported to increase apoptosis in MCF-7 cells by 25% (Sun and Liu 2006), although at a concentration (50 mg/mL) much higher than would likely be encountered in vivo.

The whole polyphenolic extract of cranberry fruit was observed to increase apoptosis substantially in tumorigenic (MCF-7) breast cells as compared to nontumorigenic (MCF-10A) breast cells. At 250 μg/mL, the cranberry extract increased the baseline apoptosis rate to 92% in MCF-7 cells, without increasing apoptosis in MCF10A cells to a significant extent (Griffin et al. 2005). In colon tumor cell lines HCT116 and HT-29, treatment with UA or with cranberry PACs was found to induce significant rates of apoptosis at concentrations below 100 μg/mL (Liberty, Hart, and Neto 2007). Recent reports of the effects of cranberry PACs on human esophageal adenocarcinoma cells show that PACs induce apoptosis in this cell line (Kresty, Howell, and Baird 2008). Induction of apoptosis in SGC-7901 gastric cancer cells by an aqueous acetone extract of whole cranberry fruit was also reported (Liu et al. 2009). In a study of oral squamous cell carcinomas, a commercial cranberry powder reduced cell proliferation in SCC25 and CAL27 cell lines, and an upregulation of caspase-2 and caspase-8 messenger ribonucleic acid (mRNA) expression indicated that apoptosis played a role in reducing proliferation (Chatelain et al. 2008).

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6.7.2 Matrix Metalloproteinase Inhibition

Phytochemicals from whole cranberry fruit may act against cancers by limiting the processes involved in tumor invasion and metastasis, particularly the expression of MMPs involved in remodeling the extracellular matrix (Pupa et al. 2002). Both the whole cranberry polyphenolic extract and a cranberry PAC fraction were found to inhibit the expression of MMP-2 and MMP-9 in the DU145 prostate tumor cell line in a dose-dependent manner (Neto et al. 2006). Similar activity was reported for a flavonoid-rich extract of cranberry's close relative, the highbush blueberry (*V. angustifolium*; Matchett et al. 2006). Hydroxycinnamate esters of UA isolated from whole cranberry fruit were strong inhibitors of MMP-2 and MMP-9 protein expression, inhibiting expression significantly at concentrations of 10 μM or less (Kondo et al. 2004). This finding was consistent with the observed ability of UA to inhibit MMP expression in fibrosarcoma cells (Cha et al. 1996). Further studies are needed to determine the efficacy of cranberry and its PACs against tumor metastasis. Oligomers from grape seed have been found to possess antimetastasis activity both in vitro and in vivo (Mantena, Baliga, and Katiyar 2006).

6.7.3 Modulation of Ornithine Decarboxylase

The biosynthesis and metabolism of polyamines (spermidine and spermine) involved in cell proliferation is controlled by enzymes such as ODC and spermidine/spermine N1-acetyltransferase, and ODC can be affected by dietary polyphenolics (Singletary and Meline 2001). Overexpression of these enzymes is observed in models of cancer where ODC can play a regulatory role in transformation, invasion, and angiogenesis (Auvinen 1997), and can be induced by proinflammatory agents such as lipopolysaccharides (LPSs) or tumor-promoters such as 12-*o*-tetradecanoyl phorbol-13-acetate (TPA). A cranberry fruit flavonoid fraction inhibits the activity of ODC in an ME-308 cell line, as determined by an assay measuring the conversion of substrate (Kandil et al. 2002). Cranberry was also found to influence the expression of ODC induced by LPS in an H-ras transformed mouse fibroblast model (Matchett et al. 2005). A whole cranberry polyphenolic extract produced a dose-dependent inhibition of LPS-induced ODC expression, and induction by LPS was abolished at extract concentrations of 100 μg/mL or less (Matchett et al. 2005).

6.7.4 *Helicobacter pylori* Inhibition

H. pylori infection is positively associated with the incidence of gastric cancer (Uemura et al. 2001); thus, the prevention of this infection may reduce cancer risk. Antibacterial adhesion studies demonstrate that in addition to inhibiting *Escherichia coli* adhesion, cranberry components inhibit adhesion of *H. pylori* to human gastric mucus (Burger et al. 2000). A randomized, double-blind, placebo-controlled trial provided some clinical support for this finding, with significantly lower levels of *H. pylori* infection observed in adults consuming cranberry juice (Zhang et al. 2005). The cranberry polyphenolic extract and other polyphenol-rich juices had a bacteriostatic effect on the growth of *H. pylori* in vitro, with morphological changes in bacteria seen at concentrations of 1 mg/mL (Matsushima et al. 2008).

6.7.5 Evidence from Animal Models

Tumor growth inhibition by cranberry in an animal model was first reported in 2006 (Ferguson et al. 2006) by a group of Canadian researchers. In this study, explants of U87 glioblastoma, HT-29 colon carcinoma, or DU145 prostate carcinoma were established in female Balb/c mice. Mice in the treatment groups were intraperitoneally injected with either a flavonoid-rich aqueous extract from cranberry press cake or a PAC fraction prepared from the whole fruit extract. Dosages of 100 mg/kg body weight PAC fraction or 250 mg/kg press cake extract were administered 10 times

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over a period of 24 days. Both treatments resulted in up to 40% reduction in the time required for U87 glioblastoma tumors to reach milestone sizes. Flow cytometry experiments showed that the extracts arrest U87 cells in G1 phase after 24 hours, reducing the number of cells continuing to S phase. In mice given HT-29 tumor explants, the PAC treatment group exhibited significantly reduced tumor volume over the first 40 days compared to control. Proanthocyanidin treatment was also found to slow the growth of tumors in the DU145 group and induce complete regression of these tumors in the two treatment groups of mice (Ferguson et al. 2006).

Several animal studies have since appeared that examine the effects of cranberry treatment on models of cancer. A 2008 study used immune-competent syngeneic mice to investigate the effects of a nondialyzable (NDM) high-molecular-weight fraction from cranberry juice on the development of lymphoma (Hochman et al. 2008). The fraction was presumed to contain polyphenolic oligomers in the 12–30K weight range; therefore, exact structures could not be determined. Balb/c female mice were inoculated with Rev-2-T-6 lymphoma cells. The cranberry NDM fraction was injected into mice at nontoxic doses (2 or 4 mg) every 2 days for 2 weeks. At the end of the experiment, cranberry-treated mice showed no tumor development in comparison with the control group, in which 80% developed tumors within 3 weeks. The treated mice also showed an increase in the production of antibodies against lymphoma cells. In another study, Balb/c nu/nu mice were injected with human gastric cancer cell line SGC-7901 that had been pretreated with an acetone-soluble cranberry extract at doses ranging from 5 to 40 mg. After 4 weeks, the control group developed tumors (xenografts), whereas no tumors were observed in the highest cranberry dosage group and tumor size was reduced in mice receiving cranberry extract doses as low as 10 mg (Liu et al. 2009).

In a bladder cancer study that used Fischer-344 rats as a model, rats were treated with *N*-butyl-*N*-(4-hydroxybutylnitrosamine) to induce cancer. Rats received cranberry juice concentrate via gavage at doses of 1.0 or 0.5 mL/rat/day (nontoxic doses) for up to 6 months. Both treatment groups showed a significant decrease in the number of bladder lesions, particularly papillomas, and the higher dosage group showed a significant decrease in number of carcinomas and a 31% decrease in the total weight of bladder lesions (Prasain et al. 2008). Quercetin, a key constituent of cranberry juice, was not detected in the plasma; however, both quercetin and its metabolite methyl quercetin were detected in the urines of the treated mice.

6.7.6 Anti-Inflammatory Activities

The anti-inflammatory properties of phytochemicals may have an impact on many diseases including certain cancers. Cyclooxygenase (COX) is a key enzyme in the biosynthetic pathway to prostaglandins, which have many physiological roles, including the production of an inflammatory response. The COX-1 isozyme is expressed constitutively in all cells, whereas expression of COX-2 can be induced in response to inflammatory stimuli. The COX-2 is highly expressed in tumor tissues (Bottone et al. 2004), and studies show that nonsteroidal anti-inflammatory drugs (NSAIDs), for example, Sulindac, have a chemopreventive effect against colon cancer in cellular and animal models (Sheng et al. 1997; Fournier and Gordon 2000; Bottone et al. 2004). As COX-2 overexpression is thought to play a role in promoting certain cancers, inhibition of COX-2 activity or expression presents another potential route to chemoprevention. Several individual constituents of cranberry may have anti-inflammatory properties. Inhibition of COX activity by anthocyanins, including those found in cranberry, was reported (Seeram et al. 2001). Pure cyanidin is an effective COX-2 inhibitor, which reduces activity by 47%, with activity superior to other anthocyanins or catechins (Seeram, Zhang, and Nair 2003). The observed COX-1 inhibition may also be relevant to cancer, since some evidence suggests that COX-1 specific inhibitors are as effective as COX-2 specific inhibitors in decreasing events related to tumor development (Bottone et al. 2004).

The question of whether cranberry can decrease the expression of COX-1 or COX-2 in cellular models remains to be answered; to date, no published studies are available that evaluate the effects of cranberry on COX expression in cancer models. Inhibition of COX-2 expression, if observed,

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could contribute to anticancer activity. Considering the known effects of compounds found in cranberry fruit, modulation of COX-2 expression and associated pathways is likely to be beneficial. Both UA and quercetin are established inhibitors of cellular COX expression (Subbaramaiah et al. 2000; O'Leary et al. 2004). The anti-inflammatory actions of triterpenes including UA have been reviewed (Safayhi and Sailer 1997), and studies support an anti-inflammatory role both in vitro (Ringbom et al. 1998; Subbaramaiah et al. 2000) and in vivo (Recio et al. 1995; Baricevic et al. 2001). UA inhibited COX-2 transcription in a human mammary oncogenic epithelial cell line (184B5/HER) by a mechanism believed to involve the protein kinase C signal transduction pathway (Subbaramaiah et al. 2000). Several anti-inflammatory activities of quercetin have also been reported. Quercetin reduced COX-2 mRNA expression in Caco-2 colon cancer cells, and both quercetin and its metabolite quercetin-3'-sulfate inhibited COX-2 activity (O'Leary et al. 2004). In a rat model of colitis, quercetin delivered in the form of rutin inhibited the TNF-α-dependent activation of nuclear factor κB (NF-κB), a transcription factor involved in the control of cell proliferation and inflammation, in a dose-dependent manner (Kim et al. 2005). Similarly, UA has been reported to suppress NF-κB activation (Shishodia et al. 2003). Quercetin has been reported to inhibit expression of inducible nitric oxide synthase, a promoter of inflammation that has also been linked to tumor angiogenesis, in cellular models (García-Mediavilla et al. 2007).

6.8 CRANBERRIES AND URINARY HEALTH

The use of cranberry juice to prevent UTIs has a long history that was for many years supported mainly by anecdotal evidence. This is no longer the case: A body of scientific evidence has accumulated to support the use of cranberry in the maintenance of urinary tract health. Studies started appearing in the 1980s demonstrating the ability of cranberry juice to prevent adherence of *E. coli* bacteria to uroepithelial cells and other eukaryotic cells (Sobota 1984; Zafriri et al. 1989; Ofek et al. 1991). As type 1–fimbriated bacteria were susceptible to the fructose in citrus fruit juices as well, the effect on type P-fimbriated *E. coli* was observed to be specific to cranberry (Zafriri et al. 1989) and other *Vaccinium*. During the mid-1990s, a clinical study conducted by Avorn et al. (1994) on the female residents of a long-term care facility found a significant decrease in bacteria in the urine after 1 month of cranberry juice consumption. Since then, at least 15 clinical trials have evaluated the prophylactic effects of cranberry against urinary infections in a variety of populations. These studies are the subject of several detailed review articles (Howell 2002; Jepson and Craig 2007; Guay 2009).

For many years, scientists and health practitioners believed that the antibacterial effects of cranberry juice were due to acidification of urine by hippuric acid produced by the metabolism of the quinic acid in cranberries (Blatherwick 1914; Bodel, Cotran, and Kass 1959). However, this claim was never substantiated. Studies correlating urinary pH with cranberry juice consumption show either no significant change in urine acidity or only a slight reduction in pH, which is insufficient to cause a bacteriostatic effect (Howell 2002). Researchers began to examine other possible mechanisms of action, which led to the discovery of bacterial antiadhesion properties (Sobota 1984). Studies on antiadherence of uropathogenic P-fimbriated *E. coli* (UPEC) responsible for the majority of UTIs found that a high-molecular-weight NDM from cranberry inhibited adhesion (Ofek et al. 1991). Bioassay-guided fractionation of cranberry targeting the active compounds found that cranberry tannins (Howell et al. 1998) blocked adherence of P-fimbriated *E. coli* to uroepithelial cells. The structures of these compounds were determined by nuclear magnetic resonance (NMR) analysis to be polyflavan-3-ol or PAC trimers composed of epicatechin units with an A-type linkage (Foo et al. 2000). The A-type linkage between monomer units (Figure 6.3), which features two linkage sites between the units (4β→8 and 2β→*O*→7 interflavanoid bonds), is a structural feature common to PACs from *Vaccinium* fruit. Proanthocyanidins from most other sources including cocoa and grape seeds contain primarily B-type (4β→8) linkages (Neto 2007), as shown in Figure 6.3. Cranberry PACs are primarily dimers, trimers, and larger oligomers of epicatechin units containing

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both A- and B-type linkages; therefore, their three-dimensional structures are diverse. Although cranberry NDM has been cited more in recent studies for its ability to inhibit cellular adhesion of *H. pylori* (Burger et al. 2000) and the development of lymphoma (Hochman et al. 2008), no information is available on the molecular structures of its components.

The A-type linkage may hold the key to the antiadhesion activity of cranberry PACs. A comparison study of PACs isolated from several food sources, including cranberry, apple, grape, green tea, and chocolate, found that cranberry PACs prevented *E. coli* adhesion at the lowest concentration tested (60 μg/mL). Among the others, grape PACs showed antiadherence properties, but only at a much higher dose (1200 μg/mL), and the other food sources showed no activity (Howell et al. 2005). This study further found antiadhesion activity in human urine following consumption of cranberry juice cocktail, but not after consumption of other PAC sources. A randomized, double-blind, placebo-controlled crossover trial with 20 healthy volunteers was conducted to determine whether urine collected after cranberry consumption inhibited UPEC adherence to uroepithelial bladder cells. Subjects received a single dose of cranberry juice (750 or 250 mL mixed with 500 mL water) or placebo at night, and urine was collected in the morning and screened for antiadhesion against six UPEC strains. A significant and dose-dependent decrease in adherence of bacteria was observed in the urine of subjects who consumed cranberry (Di Martino et al. 2006).

Several studies have examined the phenomenon of antibacterial adhesion by cranberry constituents in an attempt to better understand what happens at a submicroscopic level. Atomic force microscopy was used to measure the effect of cranberry juice exposure on bacterial surface characteristics and adhesion forces in a P-fimbriae-expressing *E. coli* (HB101pDC1) and a nonfimbriated strain. A decrease in fimbrial length was measured after a short exposure to cranberry juice, with a greater biopolymer density recorded near the cell wall (Liu et al. 2006). Adhesion forces decreased in proportion to cranberry juice concentration. No significant effect of cranberry on surface polymers or adhesive forces was observed with nonfimbriated *E. coli*. Later studies by this group showed that the effects of culturing the bacteria in cranberry juice or PAC extract on bacterial adhesion forces were reversible (Pinzon-Arango, Liu, and Camesano 2009). Interestingly, a more marked decrease in adhesive forces was observed with the juice cocktail than with the PAC fraction, suggesting that other components in the juice play a role in reducing bacterial adhesion. Another study found that morphological changes occurred when *E. coli* bacteria were grown in the presence of cranberry juice or PAC extract (Johnson et al. 2008). The authors observed a decrease in visible P-fimbriae and downregulation of gene expression associated with flagellar basal body rod and motor proteins.

Clinical studies have demonstrated the efficacy of consuming cranberry juice or solids in UTI prevention for various populations, including women with recurrent UTIs (Walker et al. 1997; Stothers 2002; Bailey et al. 2007), pregnant women, (Wing et al. 2008), the elderly (Avorn et al. 1994; McMurdo et al. 2005; McMurdo et al. 2009), and children (Ferrara et al. 2009). A randomized, double-blind, placebo-controlled study of women aged 28–44 years with recurring UTIs using 400 mg of cranberry solids or placebo for 3 months found that 70% of the subjects had fewer UTIs while on cranberry (Walker et al. 1997). In a study of women aged 25–70 years with a history of high UTI recurrence (six or more in the previous year), it was found that consumption of 200 mg of concentrated cranberry extract standardized to 30% phenolics twice a day for 12 weeks prevented UTI recurrence in all subjects for the duration of the study (Bailey et al. 2007). A follow-up study of these subjects 2 years later found that those who continued to take cranberry remained free of infection. In pregnant women, for which asymptomatic bacteriuria can cause adverse perinatal outcomes if not detected and treated, consumption of 27% cranberry juice cocktail (240 mL, thrice daily) resulted in 57% reduction in asymptomatic bacteriuria and 41% reduction in UTI (Wing et al. 2008).

In addition to Avorn's landmark study on elderly women (Avorn et al. 1994), a randomized, double-blind, placebo-controlled study of hospitalized patients over 60 years of age found that the

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group consuming 25% cranberry juice cocktail (150 mL twice daily for 35 days or until discharge) had approximately half the occurrence of symptomatic UTI (McMurdo et al. 2005). A follow-up study by this group comparing treatment with 500 mg of cranberry extract to that with low doses of the antibiotic trimethoprim in older women found that both treatments reduced recurrent UTI. Trimethoprim treatment had only a limited advantage in this regard, and the cranberry group reported less adverse effects (McMurdo et al. 2009). Further clinical studies in such populations continue to provide information for health practitioners on the most effective forms and dosing regimens of cranberry against UTI.

6.9 FUTURE DIRECTIONS FOR RESEARCH ON CRANBERRIES AND DISEASE

Evidence from in vitro, in vivo, and clinical studies suggests that cranberry and its phytochemicals may have a mitigating effect on UTIs, cardiovascular diseases, and cancer. Mechanisms of action of cranberry range from antioxidative and anti-inflammatory actions to induction of cellular apoptosis; modulation of protein synthesis and gene expression involved in cell proliferation; prevention of bacterial adhesion and formation of biofilms that lead to infection; and effects on plasma lipoprotein levels, antioxidant status, and glucose metabolism. Future research should continue to examine cranberry's role in regulating these processes and address how the unique blend of phytochemicals found in cranberry fruit and juice works together. Cranberry's efficacy depends largely on the bioavailability of its phytochemicals to various tissues, which is another topic for further research.

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7 The Amazing and Mighty Ginger

Ann M. Bode and Zigang Dong

CONTENTS

7.1 Introduction 131
7.2 History and Origin of Ginger 132
7.3 Usage, Preparation, and Processing 132
7.4 Bioactive Components of Ginger 133
7.5 Metabolism of Ginger 133
7.6 Health Effects: The Scientific Evidence 134
7.6.1 General Antioxidant Properties of Ginger 135
7.6.2 Anti-Inflammatory Effects of Ginger 135
7.6.3 Ginger as an Antinausea Agent 137
7.6.4 Anticarcinogenic Activities of Ginger 139
7.6.5 Cardiovascular and Other Disease-Preventive Effects of Ginger 144
7.7 Safety, Efficacy, and Contraindications 146
7.8 Summary and Conclusions 146
Acknowledgments 147
References 147

7.1 INTRODUCTION

The use of "natural" or alternative medicines has increased markedly over the last few years. More and more older adults (i.e., baby boomers) are using complementary and alternative medicine dietary supplements and herbal remedies without advice from a physician on the assumption that these substances will have a beneficial effect (Cohen, Ek, and Pan 2002). However, this might not be a safe or advisable practice. For example, at least one recent survey revealed a significant problem with herb–chemotherapeutic drug interactions in cancer patients and, notably, at least half of the herbal remedies taken by these patients lacked research data documenting their potential interactions (Engdal, Klepp, and Nilsen 2009). Regrettably, a great deal of the information regarding the effectiveness and safety of these remedies has been garnered from anecdotal or historical accounts, and much of the information offered is generally misleading and might even be detrimental (Ernst and Schmidt 2002).

Ginger (*Zingiber officinale* Roscoe, Zingiberaceae) is one of the most commonly consumed dietary condiments in the world (Surh et al. 1999). The oleoresin (i.e., oily resin) from the rhizomes (i.e., roots) of ginger contains many bioactive components, such as [6]-gingerol (1-[4′-hydroxy-3′-methoxyphenyl]-5-hydroxy-3-decanone; Figure 7.1), which is the primary pungent ingredient that is believed to exert a variety of remarkable pharmacological and physiological activities. Although ginger is generally considered to be safe (Kaul and Joshi 2001), the lack of a complete understanding of its mechanisms of action suggests caution in its therapeutic use (Wilkinson 2000a). Previous reviews (Barrett, Kiefer, and Rabago 1999; Ness, Sherman, and Pan 1999; Talalay and Talalay 2001)

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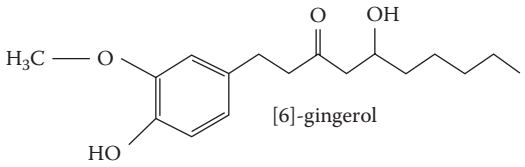


FIGURE 7.1 Structure of [6]-gingerol, believed to be the most abundant bioactive component of ginger root.

have emphasized the importance of careful scientific research in establishing the safety and efficacy of potential therapeutic plant remedies and in defining the risks and benefits of herbal medicine. Ginger has been used for thousands of years for the treatment of numerous ailments, such as colds, nausea, arthritis, migraines, and hypertension. The medicinal, chemical, and pharmacological properties of ginger have been extensively reviewed (Surh, Lee, and Lee 1998; Ernst and Pittler 2000; Afzal et al. 2001; Bode and Dong 2004; Boone and Shields 2005; Borrelli et al. 2005; Chrubasik and Pittler 2005; Chrubasik, Pittler, and Roufogalis 2005; Grzanna, Lindmark, and Frondoza 2005; Thompson and Potter 2006; Eliopoulos 2007; Shukla and Singh 2007; White 2007; Ali et al. 2008; Nicoll and Henein 2009). Over the last few years, interest in ginger or its various components as valid preventive or therapeutic agents has increased markedly, and scientific studies focusing on verification of ginger's pharmacological and physiological actions have likewise increased (Ali et al. 2008). The primary purpose of this chapter is to comprehensively examine the available scientific evidence regarding ginger's proven effectiveness in preventing or treating a variety of pathologic conditions.

7.2 HISTORY AND ORIGIN OF GINGER

Ginger is a member of a plant family that includes cardamom and turmeric. Its spicy aroma is mainly due to presence of ketones, especially the gingerols, which appear to be the primary component of ginger studied in much of the health-related scientific research. The rhizome, which is the horizontal stem from which the roots grow, is the main portion of ginger that is consumed. Ginger's current name comes from the Middle English *gingivere*, but this spice dates back over 3000 years to the Sanskrit word *srngaveram*, meaning "horn root," based on its appearance. In Greek, it was called *ziggiberis*, and in Latin, *zinziberi*. Interestingly, ginger does not grow in the wild and its actual origins are uncertain.

Indians and Chinese are believed to have produced ginger as a tonic root for over 5000 years to treat many ailments, and this plant is now cultivated throughout the humid tropics, with India being the largest producer. Ginger was used as a flavoring agent long before history was formally recorded. It was an exceedingly important article of trade and was exported from India to the Roman Empire over 2000 years ago, where it was especially valued for its medicinal properties. Ginger continued to be a highly sought after commodity in Europe even after the fall of the Roman Empire, with Arab merchants controlling the trade in ginger and other spices for centuries. In the thirteenth and fourteenth centuries, the value of a pound of ginger was equivalent to the cost of a sheep. By medieval times, it was being imported in preserved form to be used in sweets. Queen Elizabeth I of England is credited with the invention of the gingerbread man, which became a popular Christmas treat.

7.3 USAGE, PREPARATION, AND PROCESSING

Ginger is used in numerous forms, including fresh, dried, pickled, preserved, crystallized, candied, and powdered or ground. The flavor is somewhat peppery and slightly sweet, with a strong and spicy aroma. The concentration of essential oils increases as ginger ages and, therefore, the

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intended use of the rhizome determines the time when it is harvested. If extracting the oil is the main purpose, then ginger can be harvested at 9 months or longer. Ginger is commonly pickled in sweet vinegar, which turns it a pink color; this form is popular with sushi. Ginger harvested at 8–9 months has a tough skin that must be removed before eating, and the root is more pungent and is used dried or pulverized into ground ginger. This is the form most commonly found in our spice racks and used in cookies, cakes, and curry mixes. Candied or crystallized ginger is cooked in sugar syrup and coated with granulated sugar. Ginger harvested at 5 months is not yet mature and has a very thin skin, and the rhizomes are tender with a mild flavor and are best used in fresh or preserved forms.

7.4 BIOACTIVE COMPONENTS OF GINGER

At least 115 constituents in fresh and dried ginger varieties have been identified by a variety of analytical processes. Gingerols are the major constituents of fresh ginger and are found slightly reduced in dry ginger, whereas the concentrations of shogaols, which are the major gingerol dehydration products, are more abundant (Jolad et al. 2005) in dry ginger than in fresh ginger. At least 31 gingerol-related compounds have been identified from the methanolic crude extracts of fresh ginger rhizome (Jiang, Solyom et al. 2005). Ginger has been fractionated into at least 14 bioactive compounds, including [4]-gingerol, [6]-gingerol, [8]-gingerol, [10]-gingerol, [6]-paradol, [14]-shogaol, [6]-shogaol, 1-dehydro-[10]-gingerdione, [10]-gingerdione, hexahydrocurcumin, tetrahydrocurcumin, gingerenone A, 1,7-bis-(4′ hydroxyl-3′ methoxyphenyl)-5-methoxyhepthan-3-one, and methoxy-[10]-gingerol (Koh et al. 2009). The proportion of each individual component in a sample of ginger depends on country of origin, commercial processor, and whether the ginger is fresh, dried, or processed (Schwertner, Rios, and Pascoe 2006). Of the bioactive pungent components of Jamaican ginger, including [6]-, [8]-, and [10]-gingerols and [6]-shogaol, [6]-gingerol appears to be the most abundant pungent bioactive compound in most of the oleoresin samples studied (Bailey-Shaw et al. 2008). Although phylogenetic analysis has showed that all ginger samples from widely different geographical origins are genetically indistinguishable, metabolic profiling showed some quantitative differences in the contents of [6]-, [8]-, and [10]-gingerols (Jiang et al. 2006). An examination of the concentrations of [6]-, [8]-, and [10]-gingerols and [6]-shogaol in 10 different ginger-root dietary supplements purchased randomly from a variety of pharmacies and health food stores yielded some disconcerting results (Schwertner, Rios, and Pascoe 2006). Perhaps not surprisingly, the content of these active components was found to vary extensively from none or very minute amounts to several milligrams per gram. In addition, the suggested serving size ranged from about 250 mg to 4.8 g/day (Schwertner, Rios, and Pascoe 2006). The basis for the wide range of dosing is not clear. These studies suggest that ginger contains a variety of bioactive compounds and standardization of contents is critically lacking.

7.5 METABOLISM OF GINGER

Although ginger is one of the most widely consumed spices in the world, not a great deal is known regarding its metabolism or metabolites. Evaluating the bioactivity of ginger is necessary for completely understanding its mechanism of action and potential therapeutic effects. Although many food-derived supplements are consumed today with little knowledge of their activity or safety, more attention is beginning to be given to addressing these issues. The most well-studied bioactive component of ginger is probably [6]-gingerol (Surh et al. 1999). The careful isolation of several metabolites of [6]-gingerol following its oral administration (50 mg/kg) to rats was reported (Nakazawa and Ohsawa 2002). A primary metabolite, (S)-[6]-gingerol-4′-*O*-β-glucuronide, was detected in the bile and several minor metabolites were found in β-glucuronidase-treated urine, suggesting that [6]-gingerol undergoes conjugation and oxidation of its phenolic side chain (Nakazawa and Ohsawa 2002). Gingerol is rapidly cleared from rat plasma following intravenous administration (3 mg/kg;

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Ding et al. 1991), and it was reported to be metabolized enzymatically in a stereospecific reduction to gingerdiol (Surh and Lee 1994).

A method has been developed for the simultaneous quantification of [6]-, [8]-, and [10]-gingerol and [6]-shogaol in rat plasma in pharmacokinetic studies after oral administration of ginger oleoresin (Wang et al. 2009b). The investigators were able to identify a glucuronide of [6]-gingerol after hydrolysis of β-glucuronidase, and the intestinal glucuronidation was further confirmed by comparing plasma samples of hepatic portal vein and femoral vein (Wang et al. 2009b). This method was also used to obtain pharmacokinetics, tissue distribution, and excretion studies of 6-gingerol after oral or intraperitoneal administration in rats (Wang et al. 2009a). In a study in which a ginger extract (approximately 53% [6]-gingerol) was administered to rats by oral ingestion, [6]-gingerol was absorbed rapidly into the plasma, with a maximal concentration (4.23 μg/mL) being reached after 10 minutes (Jiang, Wang, and Mi 2008). The [6]-gingerol was distributed to various tissues and the most concentration was found in the gastrointestinal tract. Peak concentrations of [6]-gingerol were reached in most tissues at about 30 minutes, and the concentration in tissues was higher than that in plasma (Jiang, Wang, and Mi 2008).

At least one clinical trial focused on the pharmacokinetics of [6]-, [8]-, and [10]-gingerols and [6]-shogaol along with their respective conjugate metabolites (Zick et al. 2008). In this case, human volunteers were given ginger at doses ranging from 100 mg to 2 g and blood samples were taken at 15 minutes to 72 hours after a single oral dose. Results indicated that the free forms of [6]-, [8]-, and [10]-gingerols or [6]-shogaol were not detectable, whereas the respective glucuronide of each compound was detected, suggesting that these ginger components are readily absorbed after oral consumption and can be detected as glucuronide conjugates (Zick et al. 2008). Although progress in determining the active components and metabolites of ginger and understanding their pharmacokinetics has been made, more work is clearly needed.

7.6 HEALTH EFFECTS: THE SCIENTIFIC EVIDENCE

Because ginger and its metabolites appear to accumulate in the gastrointestinal tract, the consistent observations of ginger exerting many of its effects in this area are not surprising. Ginger has been purported to exert a variety of powerful therapeutic and preventive effects and has been used for thousands of years for the treatment of hundreds of ailments from colds to cancer. Like many medicinal herbs, much of the information has been handed down by word of mouth with little controlled scientific evidence to support the numerous claims. However, in the last few years, more organized scientific investigations have focused on the mechanisms and targets of ginger and its various components. In Sections 7.6.1 through 7.6.5, the evidence for the effectiveness of ginger as an antioxidant, anti-inflammatory agent, antinausea compound, and anticancer agent as well as the protective effect of ginger against other disease conditions are reviewed (Figure 7.2).

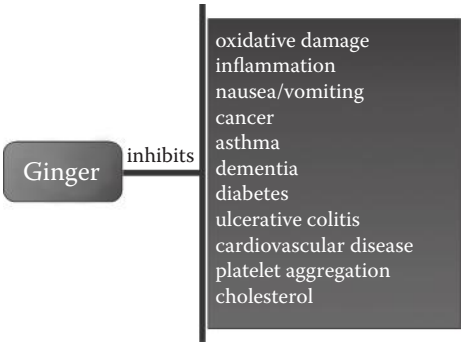


FIGURE 7.2 The variety of protective effects wielded by ginger.

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7.6.1 General Antioxidant Properties of Ginger

The presence of oxidative stress is associated with numerous diseases and a common mechanism often put forth to explain the actions and health benefits of ginger is associated with its antioxidant properties (Aeschbach et al. 1994; Ahmad, Katiyar, and Mukhtar 2001). Ginger was reported to decrease age-related oxidative stress markers (Topic et al. 2002) and was suggested to guard against ethanol-induced hepatotoxicity by suppressing oxidative consequences in rats treated with ethanol (Mallikarjuna et al. 2008). Ginger root contains a very high level (3.85 mmol/100 g) of total antioxidants, surpassed only by pomegranate and some types of berries (Halvorsen et al. 2002). The phorbol ester, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), promotes oxidative stress by activating the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system or the xanthine oxidase system or both. Ginger was reported to suppress TPA-induced oxidative stress in human promyelocytic leukemia (HL)-60 cells and Chinese hamster ovary AS52 cells (Kim et al. 2002). Others have shown that ginger compounds effectively inhibit superoxide production (Krishnakantha and Lokesh 1993). Several reports indicate that ginger suppresses lipid peroxidation and protects the levels of reduced glutathione (GSH; Reddy and Lokesh 1992; Ahmed, Seth, and Banerjee 2000; Ahmed, Seth, Pasha, and Banerjee 2000; Shobana and Naidu 2000; Ahmed et al. 2008; El-Sharaky et al. 2009).

Reactive nitrogen species, such as nitric oxide (NO), influence signal transduction and cause DNA damage, which contributes to disease processes. Nitric oxide is produced by inducible nitric oxide synthase (iNOS), which is stimulated in response to various stresses. [6]-gingerol was reported to dose-dependently inhibit NO production and reduce iNOS in lipopolysaccharide (LPS)-stimulated mouse macrophages (Ippoushi et al. 2003). [6]-gingerol also effectively suppressed peroxynitrite-mediated oxidative damage (Ippoushi et al. 2003). Ippoushi et al. (2003) later proposed that [6]-gingerol and peroxynitrite form a symmetric dimer with [6]-gingerol covalently linked at the aromatic ring of peroxynitrite, attenuating peroxynitrite-induced oxidation and nitration reactions (Ippoushi et al. 2005). [6]-shogaol, 1-dehydro-[10]-gingerdione, and [10]-gingerdione also decreased LPS-induced NO production, and [6]-shogaol and 1-dehydro-[10]-gingerdione were reported to effectively reduce iNOS expression (Koh et al. 2009). In the bromobenzene (BB)-induced hepatotoxicity model, orally given ginger extract (100 mg/kg body weight [BW]) normalized NO levels and total and reduced glutathione levels, and also decreased the level of lipid peroxidation (El-Sharaky et al. 2009). Ginger consumption has also been reported to decrease lipid peroxidation and normalize the activities of superoxide dismutase and catalase, as well as GSH and glutathione peroxidase, glutathione reductase, and glutathione-S-transferase, in rats (Ahmed et al. 2008). Ginger supplementation before ischemia/reperfusion resulted in a higher total antioxidant capacity (i.e., normalized glutathione peroxidase and superoxide dismutase activities) and lower total oxidant (lower tissue malondialdehyde, NO, and protein carbonyl contents) status levels compared to an untreated group of Wistar albino rats (Uz et al. 2009). Overall, the rats fed ginger (5%) experienced less kidney damage due to oxidative stress induced by ischemia/reperfusion (Uz et al. 2009).

Ginger extract has been reported to exert radioprotective effects in mice exposed to gamma radiation (Jagetia et al. 2003), and the effect was associated with decreased lipid peroxidation and protection of GSH levels (Jagetia, Baliga, and Venkatesh 2004). [6]-gingerol pretreatment also decreased oxidative stress induced by ultraviolet B (UVB) and activated caspase-3, -8, -9, and Fas expression (Kim et al. 2007). Evidence does seem to suggest that ginger and some of its components are effective antioxidants in vitro. However, whether the physiological activity occurs in humans in vivo is not clear, and the specific mechanism and cellular targets are still to be determined.

7.6.2 Anti-Inflammatory Effects of Ginger

One of the many health claims attributed to ginger is its purported ability to decrease inflammation, swelling, and pain. [6]-gingerol (Young et al. 2005), a dried ginger extract, and a dried gingerol-enriched extract (Minghetti et al. 2007) were each reported to exhibit analgesic and

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potent anti-inflammatory effects. Earlier animal studies suggest that rat hind limbs perfused with [6]-gingerol showed increased heat production that was associated with increased oxygen consumption and lactate efflux (Eldershaw et al. 1992). The thermogenesis was at least partly associated with vasoconstriction independent of adrenergic receptors or secondary catecholamine release. In contrast, larger doses of ginger components inhibited oxygen consumption, which was attributed to disruption of mitochondrial function (Eldershaw et al. 1992). These results were supported in a later study in which rats that were given a single intraperitoneal injection of [6]-gingerol (2.5 or 25 mg/kg) exhibited a rapid, marked drop in body temperature and a significant decrease in metabolic rate (Ueki et al. 2008).

Data suggest that ginger may exhibit anti-inflammatory effects through the modulation of calcium levels mediated through transient receptor potential vanilloid subtype 1 (TRPV1), which is a heat- and pain-sensitive receptor that can interact with [6]-gingerol (Dedov et al. 2002). [6]-gingerol has been reported to induce a substantial rise in intracellular calcium levels in Madin–Darby canine kidney renal tubular cells by stimulating both extracellular calcium influx and thapsigargin (an endoplasmic reticulum Ca^{2+} pump inhibitor)-sensitive intracellular calcium release (Chen et al. 2008). The gingerols are known to be TRPV1 agonists (Dedov et al. 2002), and the [6,8,10]-gingerols and [6,8,10]-shogaols can increase the intracellular calcium concentration in TRPV1-expressing HEK293 cells through TRPV1 (Iwasaki et al. 2006). Shogaols appear to be more potent than the gingerols, and most of the compounds cause aversive or nociceptive responses mediated by TRPV1 when applied to the eye or following subcutaneous injection to the hind paw, respectively (Iwasaki et al. 2006). In this case, most of the ginger compounds also promoted adrenal catecholamine secretion, which influences energy consumption (Iwasaki et al. 2006).

Ginger has been suggested to be effective against inflammation, osteoarthritis, and rheumatism (Reginster et al. 2000). However, inconsistencies in clinical studies have led to debate regarding the effectiveness and safety of ginger for treatment of arthritis (Marcus and Suarez-Almazor 2001). An earlier study showed that ginger oil (33 mg/kg), administered orally to rats for 26 days, caused a significant repression of paw and joint swelling associated with severe chronic adjuvant arthritis (Sharma, Srivastava, and Gan 1994). More recently, the effectiveness of a crude ginger extract was compared with a fraction containing only gingerols and derivatives to inhibit joint swelling in the streptococcal cell wall–induced arthritis animal model of rheumatoid arthritis (Funk et al. 2009). Results indicated that although both extracts could prevent joint inflammation, the crude dichloromethane extract, which also contained essential oils and more polar compounds, was more effective (when normalized to gingerol content) in preventing both joint inflammation and destruction (Funk et al. 2009). In humans, one study showed no difference between placebo and ginger in patients with osteoarthritis of the hip or knee (Bliddal et al. 2000). In contrast, patients suffering from osteoarthritis of the knee showed a consistently greater response to treatment with ginger extract compared with the control group (Altman and Marcussen 2001). In addition, relief from pain and swelling was reported in patients suffering from rheumatoid arthritis, osteoarthritis, or general muscular discomfort when using powdered ginger as a dietary supplement for 3 months to 2 years (Srivastava and Mustafa 1992). Besides pain relief from arthritis, results of a double-blind comparative clinical trial indicated that ginger (250-mg capsules) was as effective as the nonsteroidal anti-inflammatory drugs mefenamic acid (250 mg) and ibuprofen (400 mg) in relieving pain in women with primary dysmenorrhea (Ozgoli, Goli, and Moattar 2009). In contrast, consumption of 2 g of ginger before 30 minutes of cycling exercise (60% VO_2) had no effect on quadriceps muscle pain, rating of perceived exertion, work rate, heart rate, or oxygen uptake (Black and Oconnor 2008).

Researchers have hypothesized that the anti-inflammatory effects of ginger might be related to its ability to inhibit prostaglandin and leukotriene biosynthesis (Srivastava and Mustafa 1992). Some others have showed that gingerols actively inhibit arachidonate 5-lipoxygenase, an enzyme of leukotriene biosynthesis (Kiuchi et al. 1992). [8]-gingerol, but not [6]-gingerol, was shown to inhibit cyclooxygenase-2 (COX-2) expression, which is induced during inflammation to increase formation of prostaglandins (Tjendraputra et al. 2001). Others have also reported that ginger

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extract suppresses the activation of tumor necrosis factor α (TNF-α) and expression of COX-2 in human synoviocytes (Frondoza et al. 2004). Proinflammatory cytokines such as TNF-α, interleukin (IL)-1β, and IL-12, which are produced primarily by macrophages, play an important role in sepsis, ischemia/reperfusion injury, and transplant rejection. [6]-gingerol was reported to inhibit the production of proinflammatory cytokines from LPS-stimulated peritoneal macrophages, but to have no effect on the function of antigen presenting cells (APC) or the LPS-induced expression of proinflammatory chemokines (Tripathi et al. 2007). However, this same group later reported that a ginger extract attenuated the production of IL-12, TNF-α, and IL-1β proinflammatory cytokines and RANTES (regulated upon activation, normal T cell expressed and secreted) and monocyte chemoattractant protein 1 (MCP-1) proinflammatory chemokines in LPS-stimulated murine peritoneal macrophages (Tripathi, Bruch, and Kittur 2008). In general, ginger extract inhibited macrophage activation and APC function, and indirectly suppressed T-cell activation (Tripathi, Bruch, and Kittur 2008). Other stable [6]-gingerol metabolites or analogs were reported to suppress LPS-induced NO production in murine macrophages mainly by reducing *inos* gene and iNOS protein production (Aktan et al. 2006). Some of ginger's anti-inflammatory effects appear to be associated with decreased IκBα degradation and impaired nuclear factor κB (NF-κB) nuclear translocation of p65 (Aktan et al. 2006; Lee et al. 2009). The majority of scientific evidence does seem to suggest that ginger and its various components have anti-inflammatory effects both in vitro and ex vivo. However, the data supporting ginger as an effective anti-inflammatory agent in humans in vivo are still contradictory and incomplete.

7.6.3 Ginger as an Antinausea Agent

The most common and well-established use of ginger throughout history is probably its utilization in alleviating symptoms of nausea and vomiting. The benefits and dangers of herbal treatment of liver and gastrointestinal distress have been reviewed (Langmead and Rampton 2001), and several controlled studies have reported that ginger is generally effective as an antiemetic (Aikins Murphy 1998; Ernst and Pittler 2000; Jewell and Young 2000, 2002, 2003; Langmead and Rampton 2001; Dupuis and Nathan 2003; Boone and Shields 2005; Borrelli et al. 2005; Bryer 2005; Mahesh, Perumal, and Pandi 2005; Chaiyakunapruk et al. 2006; Thompson and Potter 2006; Quimby 2007). The effectiveness of ginger as an antiemetic has been attributed to its carminative effect, which helps to break up and expel intestinal gas. This idea was supported by the results of a randomized, double-blind trial in which healthy volunteers reported that ginger effectively accelerated gastric emptying and stimulated antral contractions (Wu et al. 2008). Previously, [6]-gingesulfonic acid, isolated from ginger root, was showed to be effective against HCl/ethanol-induced gastric lesions in rats (Yoshikawa et al. 1992). This compound showed weaker pungency but more potent antiulcer activity than [6]-gingerol or [6]-shogaol (Yoshikawa et al. 1994).

Ginger root is commonly recommended for preventing seasickness (Schmid et al. 1994) and is found to be superior to dimenhydrinate (Dramamine) or placebo against symptoms of motion sickness (Mowrey and Clayson 1982). A follow-up study also indicated that 1 g of ginger might be effective in reducing the subjective severity of seasickness in naval cadets on the high seas (Grontved et al. 1988). On the other hand, additional research studies showed no benefits of using ginger for treating motion sickness (Wood et al. 1988; Stewart et al. 1991), and at least one group reported that patients receiving ginger extract for treating osteoarthritis experienced more, although mild, gastrointestinal adverse events compared to a placebo-treated group (Altman and Marcussen 2001). The exact antiemetic mechanism of ginger is not clear, although some evidence suggests that it inhibits serotonin receptors and exerts its antiemetic effects directly on the gastrointestinal system and in the central nervous system (DerMarderosian and Beutler 2006). Although the antiemetic effects of ginger are the most well-studied effects of this condiment and have been reviewed extensively, the effectiveness and safety of ginger for treating nausea and vomiting have been questioned in the past

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because the findings reported were often contradictory (Wilkinson 2000b). At the same time, ginger continues to be recommended for alleviating nausea and vomiting associated with pregnancy, chemotherapy, and certain surgical procedures.

Nausea and vomiting during pregnancy affects most pregnant women, and over the years ginger has been used to try to alleviate the condition (Aikins Murphy 1998; Jewell and Young 2000, 2002, 2003; Fugh-Berman and Kronenberg 2003; Boone and Shields 2005; Borrelli et al. 2005; Bryer 2005; Chrubasik, Pittler, and Roufogalis 2005; White 2007). At least one survey indicated that the overall use of dietary supplements in pregnant women appears to be low, but ginger is commonly recommended and used to prevent nausea (Tsui, Dennehy, and Tsourounis 2001). Several double-blind, randomized, placebo-controlled clinical trials have indicated that ginger consumption is effective and safe in helping to prevent nausea and vomiting during pregnancy (Portnoi et al. 2003; Willetts, Ekangaki, and Eden 2003). Randomized trials suggest that although ginger might not be as potent as some treatments (Jewell and Young 2000), its consumption for treating nausea or vomiting or both in early pregnancy has very few or no adverse side effects and seems to be effective (Niebyl 1992; Jackson 2001; Vutyavanich, Kraisarin, and Ruangsri 2001; Jewell and Young 2002; Niebyl and Goodwin 2002). In fact, ginger has been reported to be as effective as dimenhydrinate (i.e., Dramamine) in treating nausea and vomiting in pregnancy with fewer side effects (Pongrojpaw, Somprasit, and Chanthasenanont 2007). Women who received ginger (250-mg capsules) appeared to experience less vomiting and nausea compared to those receiving placebo (Ozgoli, Goli, and Simbar 2009), and ginger also relieved pain from primary dysmenorrhea (Ozgoli, Goli, and Simbar 2009). The effectiveness of ginger has been compared with that of vitamin B6 (another recommended therapy) in randomized, double-blind, controlled trials. Results indicated that ginger and vitamin B6 therapy were equally effective in reducing nausea and the number of vomiting episodes during pregnancy (Sripramote and Lekhyananda 2003; Smith et al. 2004). In a later randomized, double-blind, controlled trial, pregnant women were randomly divided to receive either 650 mg of ginger or 25 mg of vitamin B6 (3xd/4 days). In this case, ginger actually appeared to be more effective than vitamin B6, with only minor side effects (Chittumma, Kaewkiattikun, and Wiriyasiriwach 2007). These results were supported in an additional trial in which pregnant women with nausea were randomized into groups to receive either 1 g of ginger/day or 40 mg of vitamin B6/day for 4 days. Results of this trial indicated that compared with a baseline, nausea and vomiting in the ginger group were significantly less than those reported by the vitamin B6 group (Ensiyeh and Sakineh 2009). A systematic review of the results of other double-blind, randomized, controlled trials, uncontrolled trials, case reports, and observational studies indicated that ginger is superior to placebo and as effective as vitamin B6 in relieving the severity of nausea and vomiting, with no reported side effects or adverse effects on pregnancy (Borrelli et al. 2005). A similar review of the literature regarding the safety and efficacy of ginger in the management of nausea and vomiting during pregnancy revealed that ginger appears to be a relatively low-risk and effective treatment for these symptoms (Boone and Shields 2005). Importantly, no differences in birth weight, gestational age, or frequencies of congenital abnormalities have been observed between ginger-treated and untreated mothers (Willetts, Ekangaki, and Eden 2003). A survey of a group of obstetricians and gynecologists revealed that most of them would recommend taking an antiemetic (71.3%), and specifically ginger (51.8%), to patients suffering from moderate to severe nausea (Power, Holzman, and Schulkin 2001).

Ginger has been recommended to combat nausea associated with chemotherapy (Sharma and Gupta 1998; Grant and Lutz 2000). Gingerol was reported to reduce cisplatin (a platinum-based chemotherapy drug)-induced emesis in a vomiting model of mink possibly by inhibiting the central or peripheral increase of 5-hydroxytryptamine, dopamine, and substance P (Qian et al. 2009). In contrast, addition of ginger root powder (1 g/day) to a standard antiemetic regimen with metoclopramide had no advantage in reducing nausea or vomiting in acute or delayed phases of cisplatin-induced emesis in gynecologic cancer patients (Manusirivithaya et al. 2004). Cisplatin can cause renal oxidative and nitrosative stress and dysfunction. However, rats that were administered

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cisplatin and [6]-gingerol exhibited lower lipid peroxidation and conservation of GSH coupled with enhanced superoxide dismutase and catalase, which resulted in a restoration of normal renal function (Kuhad et al. 2006). Complementary intervention with ginger has also been suggested to have possible benefits in preventing acute chemotherapy-induced nausea and vomiting (CINV) in children (Dupuis and Nathan 2003). However, the results of a randomized, double-blind, placebo-controlled trial indicated that ginger did not provide any additional benefit in reducing CINV when given with a 5-hydroxytryptamine 3 (HT3) receptor antagonist and/or aprepitant (a substance P antagonist; Zick et al. 2009). Notably, compared with a normal diet, high-protein meals with ginger consumed twice daily were reported to reduce the delayed nausea of chemotherapy and decrease the use of antiemetic medications (Levine et al. 2008).

Ginger was suggested to be an effective postoperative prophylactic antiemetic (Phillips, Ruggier, and Hutchinson 1993) that is not associated with effects on gastric emptying (Phillips, Ruggier, and Hutchinson 1993). However, the effectiveness of ginger in preventing postoperative nausea and vomiting has been disputed (Visalyaputra et al. 1998). One study indicated that pretreatment with ginger extracts reversed experimentally induced delay in gastric emptying in rats (Gupta and Sharma 2001), and ginger was also reported to reduce food transit time in experimental rats, an effect that might have implications in the prevention of colon cancer or constipation (Platel and Srinivasan 2001). The digestive stimulatory effects of ginger and other spices might be associated with positive effects on trypsin and pancreatic lipase (Platel and Srinivasan 2000) and ginger's ability to increase gastric motility (Micklefield et al. 1999).

Several groups have studied the effectiveness of ginger in preventing nausea associated with gynecological laparoscopy. Patients who took ginger (1 g) appeared to experience less nausea incidence, especially within 2–4 hours of the procedure, and some reported less vomiting also (Pongrojpaw and Chiamchanya 2003). These results were supported by a later study involving 60 patients who received either 3 g of ginger or placebo 1 hour before the procedure. Although nausea was less in the ginger group at 2 hours postprocedure, vomiting did not vary between the two groups (Apariman, Ratchanon, and Wiriyasirivej 2006). However, at 6 hours, patients who had received ginger reported significantly less nausea and vomiting than the placebo group (Apariman, Ratchanon, and Wiriyasirivej 2006). Results of another similar trial indicated that ginger (1 g) taken 1 hour before major gynecologic surgery decreased nausea and vomiting at 2 and 6 hours postsurgery compared to placebo, and had no adverse side effects (Nanthakomon and Pongrojpaw 2006). In contrast, at least one trial indicated that ginger was not effective in reducing the incidence of postoperative nausea and vomiting in patients undergoing gynecologic laparoscopy (Eberhart et al. 2003). Finally, a systematic review and meta-analysis of randomized, controlled trials comparing ginger with placebo in preventing postoperative nausea and vomiting revealed that a fixed dose of at least 1 g of ginger appears to be more effective than placebo (Chaiyakunapruk et al. 2006). Overall, these results suggest that ginger is probably fairly effective in alleviating nausea and vomiting associated with a variety of conditions. Although the mechanism is not clear, ginger appears to have no adverse side effects and never seems to worsen nausea and vomiting.

7.6.4 Anticarcinogenic Activities of Ginger

A great deal of interest by numerous research groups, including our own, is now being focused on the cancer-preventive and potential cancer therapeutic applications of ginger and its various components. Several aspects of the chemopreventive effects of numerous phytochemical dietary and medicinal substances, including ginger, have been reviewed previously (Surh, Lee, and Lee 1998; Surh 1999, 2002; Bode and Dong 2004; Shukla and Singh 2007; Aggarwal et al. 2008). Studies focused on the anticancer activities of various forms of ginger from a crude or partially purified extract to gingerols, especially [6]-gingerol; shogaols, especially [6]-shogaol; and zerumbone, a sesquiterpene compound derived from ginger and a number of minor components and metabolites. The effectiveness of ginger in preventing or suppressing cancer growth has been examined in a variety

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of cancer types, including lymphoma, hepatoma, colorectal cancer, breast cancer, skin cancer, liver cancer, and bladder cancer. The mechanisms proposed to explain the anticancer activities of ginger and its components include antioxidant activity and the ability to induce apoptosis, decrease proliferation, cause cell-cycle arrest, and suppress activator protein 1 (AP-1) and NF-κB/COX-2 signaling pathways (Figure 7.3).

The anticancer activities of [6]-gingerol and zerumbone have been associated with their antioxidant activities. Several ginger components were reported to have effective anticancer promoter activity based on their ability to inhibit TPA-induced Epstein–Barr virus early antigen (EBV-EA) in Raji cells (Vimala, Norhanom, and Yadav 1999; Kapadia et al. 2002). [6]-gingerol was reported to suppress the reactive oxygen species–potentiated invasive capacity of ascites hepatoma AH109A cells by reducing peroxide levels (Yagihashi, Miura, and Yagasaki 2008). In normal RL34 rat liver epithelial cells, zerumbone was found to induce glutathione S-transferase and the nuclear localization of the transcription factor Nrf2, which binds to the antioxidant response element (ARE) of phase II enzyme genes (Nakamura et al. 2004). Zerumbone potentiated the expression of several Nrf2/ARE-dependent phase II enzyme genes, including γ-glutamyl-cysteine synthetase, glutathione peroxidase, and hemeoxygenase-1 (Nakamura et al. 2004). Others have reported that zerumbone decreases TPA-induced hydrogen peroxide formation and edema corresponding to enhanced levels of various antioxidant enzymes (Murakami et al. 2004). These types of changes have been linked with lower 7,12-dimethylbenz[a]anthracene (DMBA)-initiated/TPA-promoted tumor incidence, number of tumors per mouse, and tumor volume (Murakami et al. 2004).

Zerumbone has also been reported to downregulate CXC chemokine receptor 4 (CXCR4), which is highly expressed in various tumors, including breast, ovary, prostate, gastrointestinal, head

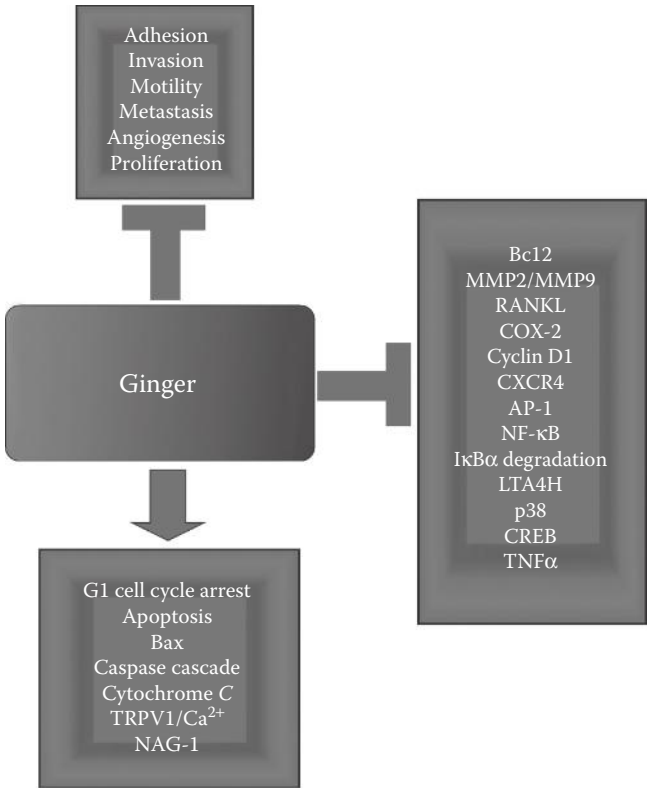


FIGURE 7.3 The anticancer activities exerted by ginger.

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and neck, bladder, brain, and melanoma tumors (Sung et al. 2008). Because the CXCR4 mediates homing of tumor cells to specific organs that express its ligand, CXCL12, zerumbone was suggested as a potential suppressor of cancer metastasis and was effective in suppressing CXCR4 in a variety of cancers, including those of the pancreas, lung, kidney, and skin (Sung et al. 2008). Furthermore, zerumbone effectively attenuated osteoclast formation induced by human breast tumor cells and by multiple myeloma and decreased osteolysis dose-dependently in MDA-MB-231 breast cancer tumor-bearing athymic nude mice, suggesting that it might be effective in preventing cancer-associated bone loss or osteoporosis (Sung et al. 2009). [6]-gingerol has also been reported to suppress adhesion, invasion, motility, matrix metalloproteinase (MMP)-2, and MMP-9 messenger ribonucleic acid (mRNA) expression and protein activities in MDA-MB-231 human breast cancer cell lines (Lee, Seo, Kang, and Kim 2008).

Ginger and its constituents have been reported to inhibit tumor promotion in mouse skin (Katiyar, Agarwal, and Mukhtar 1996). In particular, [6]-gingerol has been reported to be highly effective as an anticancer agent in skin in vivo in the two-stage initiation–promotion mouse skin model. In this model, tumors are initiated by a one time application of DMBA followed by repeated topical applications of TPA beginning a few days later. Topical application of [6]-gingerol on the shaved backs of female ICR mice decreased the incidence of DMBA-initiated/TPA-promoted skin papilloma formation and also suppressed TPA-induced epidermal ornithine decarboxylase activity and inflammation (Park et al. 1998). Results of a similar study indicated that in the DMBA/TPA skin tumor model, topical application of [6]-paradol or [6]-dehydroparadol prior to the application of TPA significantly decreased both the number of tumors per mouse and the number of mice exhibiting tumors (Chung et al. 2001).

Earlier studies suggest that gingerol is an effective inhibitor of azoxymethane-induced intestinal carcinogenesis in rats (Yoshimi et al. 1992). Ginger supplementation (50 mg/kg BW) was reported to suppress the number of tumors as well as the incidence of 1,2-dimethylhydrazine (DMH)-induced colon cancer (Manju and Nalini 2005). The effect was attributed to decreased oxidative damage associated with enhanced catalase, superoxide dismutase, glutathione peroxidase, and glutathione transferase activities as well as increased GSH (Manju and Nalini 2005). This group later reported that administration of ginger to DMH-treated rats significantly decreased the incidence and number of tumors as well as the activity of microbial enzymes, β-glucuronidase, and mucinase (Manju and Nalini 2006). Finally, Wistar rats that were fed a ginger extract (1% mixed in diet) exhibited significantly lower multiplicity of urothelial lesions (hyperplasia and neoplasia) than untreated groups (Ihlaseh et al. 2006).

Studies suggest that ginger compounds suppress proliferation of human cancer cells through the induction of apoptosis (Lee et al. 1998; Lee and Surh 1998; Thatte, Bagadey, and Dahanukar 2000). A saline extract prepared from ginger extract suppressed the proliferation of HEp-2 cells by inducing cytotoxic effects and DNA fragmentation (Vijaya Padma, Arul Diana Christie, and Ramkuma 2007). Ginger extract and especially [6]-gingerol were reported to effectively decrease proliferation of YYT colon cancer cells and the angiogenic potential of endothelial cell tubule formation in immortalized MS1 endothelial cells (Brown et al. 2009). [10]-gingerol was reported to cause a significant and prolonged increase in intracellular calcium and cytotoxicity in human colorectal cancer SW480 cells (Chen, Li, and Kuo 2009). [6]-gingerol was reported to inhibit both proliferation and invasion of ascites hepatoma AH109A cells and appeared to act by causing an S-phase arrest, elongated doubling time of hepatoma cells, and an increased rate of apoptosis (Yagihashi, Miura, and Yagasaki 2008). This compound also induced cell-cycle arrest and suppressed the growth of human pancreatic cancer cell lines, human pancreatic adenocarcinoma (HPAC) cells, which express wild-type p53 and BxPC-3 cells that express a mutant p53 protein (Park et al. 2006). Interestingly, [6]-gingerol appeared to be most effective in inducing apoptosis in p53-mutant cells and induced arrest, but not apoptosis, in p53-expressing cells (Park et al. 2006). [6]-gingerol was further reported to suppress proliferation and induce apoptosis or G1 cell-cycle arrest in several colorectal cell lines, including HCT116, SW480, HT29, LoVo, and Caco2

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cells (Lee, Cekanova, and Baek 2008). These effects were associated with a decreased abundance of cyclin D1 (a proto-oncoprotein that is overexpressed in cancer) and increased expression of a nonsteroidal anti-inflammatory drug (NSAID)-activated gene (NAG-1), a proapoptotic and antitumorigenic protein (Lee, Cekanova, and Baek 2008).

Through the comparison of promotion-sensitive (P^+) and promotion-resistant (P^-) derivatives of the mouse epidermal JB6 cell lines, AP-1 was reported to have a critical role in tumor promotion (Huang, Ma, Bowden, and Dong 1996; Huang, Ma, and Dong 1996). In addition, blocking the tumor promoter–induced activation of AP-1 inhibited neoplastic transformation (Dong et al. 1994). Epidermal growth factor (EGF) is known to induce a relatively high level of AP-1 activity and cell transformation (Huang, Ma, and Dong 1996). We previously investigated the effect of two structurally related compounds of the ginger family, [6]-gingerol and [6]-paradol, on EGF-induced cell transformation and AP-1 activation (Bode et al. 2001). Our results provided the first evidence that both compounds block EGF-induced cell transformation, but by different mechanisms. [6]-gingerol appeared to act by directly inhibiting AP-1 DNA binding activity and transactivation, whereas [6]-paradol appeared to act by inducing apoptosis (Bode et al. 2001). Others report that [6]-gingerol causes DNA fragmentation and suppresses Bcl-2 expression in promyelocytic leukemia HL-60 cells (Wang et al. 2003), and also induces growth inhibition and caspase-mediated apoptosis in human epidermoid carcinoma A431 cells (Nigam et al. 2009). [6]-paradol and other structurally related derivatives, such as [10]-paradol, [3]-dehydroparadol, [6]-dehydroparadol, and [10]-dehydroparadol, inhibited proliferation of KB oral squamous carcinoma cells in a time- and dose-dependent manner (Keum et al. 2002). [6]-dehydroparadol (75 μM) was more potent than the other compounds tested, and it induced apoptosis through a caspase-3-dependent mechanism (Keum et al. 2002).

[6]-shogaol [1-(4-hydroxy-3-methoxyphenyl)-4-decen-3-one], an alkanone from ginger, exhibited the most potent cytotoxicity against human A549, SK-OV-3, SK-MEL-2, and HCT15 tumor cells, compared to [4]-, [6]-, [8]-, and [10]-gingerols (Kim et al. 2008). This compound also inhibited proliferation of several transgenic mouse ovarian cancer cell lines, including C1 and C2 (Kim et al. 2008). Further, [6]-shogaol was reported to inhibit the growth of and induce apoptosis in COLO 205 cells (Pan et al. 2008). Treatment with [6]-shogaol, but not [6]-gingerol, induced DNA fragmentation in COLO 205 colon cancer cells. Apoptosis was mediated by activation of caspase-9, -3, and -8, resulting in the release of mitochondrial cytochrome *c*, upregulation of proapoptotic Bax, and downregulation of antiapoptotic Bcl2, and the induction of growth arrest and DNA damage (GADD)-inducible transcription factor 153 (GADD153) mRNA and protein (Pan et al. 2008). [6]-shogaol induced apoptosis of hepatoma cells mediated by activation of caspase-3 and -7 (Chen et al. 2007). The compound was also reported to reduce the viability of gastric cancer cells by directly damaging microtubules and inducing mitotic arrest (Ishiguro et al. 2007).

NF-κB is a rapidly induced stress-responsive transcription factor that functions to intensify the transcription of a variety of genes, including cytokines, growth factors, and acute response proteins (Baldwin 1996). Its activation is also linked to mitogen-activated protein (MAP) kinase signaling pathways (Schulze-Osthoff et al. 1997). The mechanism for NF-κB activation is well known. In its inactive form, NF-κB is found in the cytosol bound to an inhibitory protein called inhibitory kappa B (IκB). When stimulated, IκB is phosphorylated by an IκB kinase, which releases it from NF-κB and is subsequently degraded. Following its separation from IκB, NF-κB is translocated into the nucleus, where it activates gene transcription by binding to its specific DNA sequence found in certain genes. Importantly, NF-κB activation is associated with initiation or acceleration of tumorigenesis (Gilmore 1997), and in JB6 cells, inhibition of NF-κB also blocks tumor promoter–induced cell transformation (Li et al. 1997). [6]-gingerol might exert its effects by suppressing the NF-κB/COX-2 pathway. This idea is supported by data indicating that the reduction of UVB-induced expression and transactivation of COX-2 by [6]-gingerol was associated with the suppression of IκBα phosphorylation (Ser32) resulting in a decreased translocation of NF-κB from cytosol to nucleus in HaCaT cells (Kim et al. 2007). A ginger extract fed to rats with experimentally induced liver

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cancer resulted in decreased NF-κB and TNF-α expression (Habib et al. 2008). [6]-gingerol was reported to suppress TNF related apoptosis induced ligand (TRAIL)-induced NF-κB activation, resulting in apoptosis mediated by caspase-3 or -7 activation, which was associated with the downregulation of clAP1, a negative regulator of these caspases (Ishiguro et al. 2007).

Zerumbone has been reported to suppress NF-κB activation induced by a variety of stimuli, including tumor necrosis factor (TNF), cigarette smoke condensate, and hydrogen peroxide (Takada, Murakami, and Aggarwal 2005). It also suppressed IκBα kinase phosphorylation and degradation, resulting in a downregulation of constitutively active NF-κB and many of its regulated gene targets, such as COX-2, cyclin D1, Bcl2, and other antiapoptotic genes, thereby enhancing apoptosis induced by chemotherapeutic agents (Takada, Murakami, and Aggarwal 2005). Zerumbone was also reported to suppress receptor activator of NF-κB ligand (RANKL) activity in mouse monocytes (osteoclast precursor cells) by inhibiting IκBα kinase activity, phosphorylation, and degradation (Sung et al. 2009). Oral administration of zerumbone (100, 250, or 500 ppm) to ICR mice decreased inflammation and the multiplicity of colon adenocarcinomas induced by intraperitoneal injection of azoxymethane (AOM, 10 mg/kg BW; Kim et al. 2009). Additionally, zerumbone (250 or 500 ppm) effectively suppressed 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung adenoma formation in female A/J mice (Kim et al. 2009). This ginger derivative appeared to exert its effects by inhibition of proliferation, induction of apoptosis, and suppression of NF-κB and heme oxygenase expression in both colon and lung cancer tissues (Kim et al. 2009). In an earlier study, [6]-gingerol was reported to inhibit both the vascular endothelial growth factor (VEGF)- and basic fibroblast growth factor (bFGF)-induced proliferation of human endothelial cells and cause cell-cycle arrest in the G1 phase (Kim, Min et al. 2005). [6]-gingerol also blocked capillary-like tube formation by endothelial cells in response to vascular endothelial growth factor (VEGF), and strongly inhibited sprouting of endothelial cells in the rat aorta and formation of new blood vessels in the mouse cornea in response to VEGF (Kim, Min et al. 2005).

Investigators suggested that the effectiveness of ginger might be related to its ability to inhibit prostaglandin and leukotriene biosynthesis (Srivastava and Mustafa 1992). Some researchers showed that gingerol actively inhibits arachidonate 5-lipoxygenase, an enzyme of leukotriene biosynthesis (Kiuchi et al. 1992). The leukotriene A4 hydrolase (LTA4H) protein is regarded as a relevant target for cancer therapy, and our *in silico* prediction using a reverse-docking approach revealed that LTA4H might be a potential target for [6]-gingerol (Jeong et al. 2009). Our prediction was supported by work showing that [6]-gingerol suppresses anchorage-independent cancer cell growth by binding to LTA4H and inhibiting LTA4H activity in HCT116 colorectal cancer cells. We further found that [6]-gingerol effectively suppressed tumor growth in vivo in nude mice, an effect that was mediated by the inhibition of LTA4H activity. Collectively, these findings indicate a crucial role of LTA4H in cancer and also support the anticancer efficacy of [6]-gingerol targeting of LTA4H for the prevention of colorectal cancer (Jeong et al. 2009). Importantly, these are the first results that identify a direct target of [6]-gingerol to explain its anticancer activity.

Cyclooxygenase-2 is an important enzyme in prostaglandin biosynthesis, and is regarded as a promising molecular target for many anti-inflammatory as well as chemopreventive agents. Topical application of [6]-gingerol was reported to suppress TPA-induced COX-2 expression, p38 phosphorylation, and NF-κB DNA binding activity in mouse skin (Kim et al. 2004). These results were further expanded to show that pretreatment of mouse skin with [6]-gingerol resulted in decreased TPA-induced NF-κB DNA binding and transcriptional activity by suppressing both IκBα phosphorylation and degradation and p65 phosphorylation and nuclear translocation (Kim, Kundu et al. 2005). The interaction of phosphorylated p65 (Ser536) with CREB (cAMP response element binding) protein, a transcriptional coactivator of NF-κB, was prevented by [6]-gingerol, and the inhibitory effect of [6]-gingerol on p38 phosphorylation, an upstream mediator of COX-2 activation, was observed (Kim, Kundu et al. 2005).

Treatment of cultured ovarian cancer cells with [6]-shogaol caused a marked growth inhibition that was associated with suppression of NF-κB activation as well as the diminished secretion

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of angiogenic factors, VEGF and IL-8 (Rhode et al. 2007), suggesting a role for this compound in preventing angiogenesis in cancer. In contrast to most reports, dietary consumption of ginger (0.5% or 1.0%) did not suppress aberrant crypt foci (ACF) formation or reduce the number of crypts per ACF in DMH-treated rats compared to untreated control rats (Dias et al. 2006). Dietary ginger did not significantly change the proliferative or apoptotic indexes of the colonic crypt cells induced by DMH (Dias, 2006). In marked contrast to many studies, ginger extract was not able to inhibit the development of *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine (BBN)/*N*-methyl-*N*-nitrosourea (MNU)-induced bladder cancer in male Swiss mice. In fact, in BBN/MNU/2% ginger-treated mice, the incidence of grade 2 transitional cell carcinoma was increased (Dias et al. 2006; Bidinotto et al. 2006).

7.6.5 Cardiovascular and Other Disease-Preventive Effects of Ginger

In addition to its effects in relation to cancer, some evidence supports a protective role for ginger in cardiovascular function and a number of other disease conditions. Ginger has gained interest for its potential to treat various aspects of cardiovascular disease, and the in vitro and animal data supporting the anti-inflammatory, antioxidant, antiplatelet, hypotensive, and hypolipidemic effects of this condiment have been reviewed (Nicoll and Henein 2009). However, human trials are less convincing and more investigations are needed (Nicoll and Henein 2009). Caution when taking ginger and other herbal extracts has been suggested because of an apparent association of ginger with reported incidences of increased risk of bleeding following surgery (Chang and Whitaker 2001; Pribitkin and Boger 2001) or if taken with anticoagulant drugs such as warfarin (Heck, DeWitt, and Lukes 2000). However, the data are not conclusive (Vaes and Chyka 2000). At least one study indicates that ginger has no effect on blood pressure, heart rate, or coagulation parameters and does not interact with anticoagulant drugs such as warfarin (Weidner and Sigwart 2000). These findings were supported in a later study in which ginger was reported to have no effect on clotting status or the pharmacokinetics or pharmacodynamics of warfarin in healthy subjects (Jiang, Williams et al. 2005). An aqueous ginger extract was reported to induce a dose-dependent decrease in arterial blood pressure in a variety of animal models (Ghayur and Gilani 2005a,b).

At least one group found that administration or consumption of standardized ginger extract decreased aortic atherosclerotic lesion areas, plasma triglycerides and cholesterol, low-density lipoprotein (LDL)-associated lipid peroxides, and LDL aggregation in mice (Fuhrman et al. 2000). In rabbits that were fed a high-cholesterol diet, administration of ginger extract resulted in a significant antihyperlipidemic effect and a lower degree of atherosclerosis compared to the group that was fed cholesterol alone (Bhandari, Sharma, and Zafar 1998). Importantly, ginger powder (3 g/day in 1-g capsule 3xd) significantly lowered lipid levels in volunteer patients in a double-blind, controlled clinical trial study (Alizadeh-Navaei et al. 2008). Triglyceride and cholesterol were substantially decreased as was LDL levels compared to placebo group. Notably, the high-density lipoprotein (HDL) level of the ginger group was higher than that of the placebo group, whereas the very-low-density lipoprotein (VLDL) level of the placebo group was higher than that of the ginger group (Alizadeh-Navaei et al. 2008). Dried ginger powder (0.1 g/kg BW, per oral administration [p.o.] for 75 days) significantly lowered (50%) the development of atheroma in the aorta and coronary arteries of rabbits that were fed cholesterol (Verma et al. 2004). This effect was associated with decreased lipid peroxidation and increased fibrinolytic activity with ginger, but blood lipid levels were not different from control animals (Verma et al. 2004). Another compound isolated from ginger, (E)-8 β,17-epoxylabd-12-ene-15,16-dial, was reported to inhibit cholesterol biosynthesis (Tanabe et al. 1993), and ginger meal (1%) decreased serum cholesterol levels significantly (Dias et al. 2006). Ginger was also reported to slightly reduce retinoid-binding protein mRNA expression levels in liver and visceral fat in male rats that were fed cholesterol to induce hyperlipidemia (Matsuda et al. 2009). These results hint that ginger consumption might improve lipid metabolism (Matsuda et al. 2009).

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Antiplatelet therapy is an effective approach for preventing coronary heart disease. Ginger components are suggested as a potential new class of platelet-activation inhibitors without the potential side effects of aspirin, which is most commonly used in this approach. In a comparison of gingerols and analogs with aspirin, ginger compounds were found to be less potent compared to aspirin in inhibiting arachidonic acid–induced platelet release and aggregation and COX activity (Koo et al. 2001). However, several analogs had a significant inhibitory effect, suggesting that further development of more potent gingerol analogs might have value as an alternative to aspirin therapy in preventing ischemic heart disease (Koo et al. 2001). Consumption of ginger (5 g) inhibited platelet aggregation induced in men who consumed 100 g of butter daily for 7 days (Verma et al. 1993), and a later study showed that ginger enhanced fibrinolytic activity (Verma and Bordia 2001). An evaluation of the antiplatelet activity of 20 pungent constituents of ginger revealed that [8]-paradol was the most potent COX-1 inhibitor and antiplatelet aggregation agent (Nurtjahja-Tjendraputra et al. 2003). [8]-gingerol and [8]-shogaol were also found to be effective antiplatelet aggregation agents (Nurtjahja-Tjendraputra et al. 2003). Ginger and nifedipine (a calcium-channel blocker) were reported to have a synergistic effect on antiplatelet aggregation in normal human volunteers and hypertensive patients (Young et al. 2006). Ginger oil (24% citral) effectively lowered spontaneous or prostoglandin F2-alpha (PGF2-alpha)-2α-induced rat myometrial (uterus) contractility, and increases in external calcium concentration reversed the relaxant effects of ginger oil (Buddhakala et al. 2008). Ginger compounds have been reported to directly stimulate myocardial sarcoplasmic reticulum (SR) calcium uptake (Antipenko, Spielman, and Kirchberger 1999; Maier et al. 2000), but its therapeutic use in treating heart failure has not been advocated (Maier et al. 2000). Ginger is also used to treat asthma, diabetes, and other conditions.

Asthma is a chronic disease characterized by inflammation and hypersensitivity of airway smooth muscle cells to different substances that induce spasms, and ginger has been used for centuries in treating respiratory illnesses. Components of ginger rhizomes are reported to contain potent compounds capable of suppressing allergic reactions and might be useful for the treatment and prevention of allergic diseases (Chen et al. 2009). Ghayur, Gilani, and Janssen (2008) reported that a ginger extract inhibits airway contraction and associated calcium signaling, possibly by blocking plasma membrane calcium channels. In a mouse model of Th2-mediated pulmonary inflammation, an intraperitoneal injection of a ginger extract mainly comprised of gingerols markedly decreased the recruitment of eosinophils to the lungs in ovalbumin-sensitized mice and also suppressed the Th2 cell-driven response to allergen (Ahui et al. 2008).

Ginger has been suggested to have antidiabetic effects. In the streptozotocin-induced diabetic rat model, rats that were fed ginger exhibited better glucose tolerance and higher serum insulin levels than untreated rats, suggesting that it can help control blood sugar levels (Islam and Choi 2008). Treatment with a ginger extract produced a significant reduction in fructose-induced elevation in lipid levels, body weight, hyperglycemia, and hyperinsulinemia associated with insulin resistance (Kadnur and Goyal 2005). An aqueous extract of raw ginger (administered daily, 500 mg/kg intraperitoneally) to streptozotocin-induced diabetic rats lowered serum glucose, cholesterol, and triacylglycerol levels; decreased urine protein levels, water intake, and urine output; and prevented the weight loss associated with diabetes in this model (Al-Amin et al. 2006). [6]-gingerol has also been found to enhance differentiation of 3T3-L1 preadipocytes and to enhance insulin-sensitive glucose uptake (Sekiya, Ohtani, and Kusano 2004). A later study showed that [6]-shogaol or [6]-gingerol significantly inhibited TNF-α-mediated downregulation of adiponectin expression in 3T3-L1 adipocytes (Isa et al. 2008). [6]-shogaol appeared to function as a peroxisome proliferator-activated receptor (PPAR)γ agonist, whereas [6]-gingerol acted by suppressing TNF-α-induced JNKs signaling (Isa et al. 2008). These results give some suggestion that ginger might be valuable in managing the effects of diabetes in humans.

Dried ginger may have beneficial effects in treating dementia, including Alzheimer's disease (Ghayur, Gilani, Ahmed, Khalid, Nawaz, Agbedahunsi, Choudhary, and Houghton 2008). Ulcerative colitis is a chronically recurrent inflammatory bowel disease of unknown origin, and

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in rats, ginger extract alleviated the symptoms of acetic acid–induced ulcerative colitis (El-Abhar, Hammad, and Gawad 2008).

7.7 SAFETY, EFFICACY, AND CONTRAINDICATIONS

Ginger is recognized by the U.S. Food and Drug Administration (FDA) as a food additive that is "generally recognized as safe." However, and notably, in 1930, thousands of Americans were poisoned and paralyzed by an illicit extract of Jamaican ginger (jake) that was used to circumvent Prohibition laws. The extract had been adulterated with a neurotoxic organophosphate compound, triorthocresyl phosphate (TOCP; Crandall 1931; Morgan and Penovich 1978). The extract was banned in 1931.

Early studies suggest that ginger extract increased the mutagenesis ability of 2(2-furyl)-3(5-nitro-2-fury)acryl amide (AF2) or *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine (NTG), and [6]-gingerol was determined to be an active mutagen (Nakamura and Yamamoto 1982). A later study suggests that [6]-shogaol is much less mutagenic than [6]-gingerol and that the active part of [6]-gingerol is the aliphatic chain moiety containing a hydroxyl group (Nakamura and Yamamoto 1983). To our knowledge, these studies have not been confirmed nor repeated, and no recent evidence suggests that ginger or its components are mutagenic.

Oral administration of a ginger extract (1000 mg/kg) was reported to be tolerated well by pregnant rats, and it exerted no adverse effects on the mothers or in the development of fetuses (Weidner and Sigwart 2001). This result is somewhat in contrast to an earlier study, in which administration of ginger tea to pregnant rats resulted in twice the loss of embryos but heavier surviving fetuses compared to untreated controls (Wilkinson 2000a). Ginger rhizome extract (0.5–10.0 g/kg) administered intraperitoneally to mice was reported to have no clastogenic effects compared to ginger oil, which produced some chromosomal irregularities (Mukhopadhyay and Mukherjee 2000).

Most recently, male and female rats that were fed ginger powder (500, 1000, or 2000 mg/kg BW) by gavage for 35 days did not exhibit any overall mortalities or abnormalities in behavior, growth, or food and water consumption (Rong et al. 2009). No overt organ abnormalities were observed and hematological and blood biochemical parameters in treated and untreated control animals were similar. The only real difference observed was a slightly decreased absolute and relative weight of the testes only at the highest dose (2000 mg/kg; Rong et al. 2009). Observational studies in humans suggest no evidence of teratogenicity from treatments for early pregnancy nausea that included ginger (Jewell and Young 2003). These results were confirmed in a similar trial showing that administration of ginger beginning at the first trimester of pregnancy did not appear to increase the rates of major malformations above the baseline rate of 1–3% (Portnoi et al. 2003). Overall, these data indicate that ginger consumption appears to be very safe with very limited side effects.

7.8 SUMMARY AND CONCLUSIONS

Ginger is not only an extremely popular dietary condiment used for flavoring food but also an herb that has been used for thousands of years as a medicinal herb to treat a variety of ailments. Chemical and metabolic analyses have revealed that ginger comprises hundreds of compounds and metabolites. The most extensively studied bioactive components include gingerols and shogaols, especially [6]-gingerol and [6]-shogaol, respectively. The content of each component is clearly dependent on the source and preparation of the ginger rhizome. Research interest in determining the role of natural compounds in preventing disease has increased markedly over the last few years. In spite of the abundance of research studies, many of the results are phenomenon based and provide data that are descriptive and observational rather than mechanistic. More studies are needed in animals and humans on the kinetics of ginger and its constituents and on the effects of consumption over a long

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period of time. Specific molecular targets and mechanisms of action need to be identified. Ginger clearly has a vast number of components and metabolites, many of which have not been studied in detail. The lack of standardization of ginger supplements is disconcerting, and whether consumption of high levels of isolated components (e.g., [6]-gingerol) is advisable is uncertain. [6]-gingerol or other ginger components might require inter-reactivity or dependency on other components in the whole food source to exert their positive effects.

Research data indicate that ginger and its constituents accumulate in the gastrointestinal tract, which supports the many observations of ginger's effectiveness as an antinausea agent and as a possible colon cancer–preventing compound. Ginger acts as a potent antioxidant in vitro and ex vivo, but the data are not obvious for in vivo application and specific targets and mechanisms are lacking. Ginger appears to exert anti-inflammatory effects by suppressing COX-2 with subsequent inhibition of prostaglandin and leukotriene biosynthesis. On the other hand, the data supporting the effectiveness of ginger in alleviating pain and swelling associated with arthritis are somewhat conflicting. The most common use of ginger is to alleviate the vomiting and nausea associated with pregnancy, chemotherapy, and some types of surgery. The clinical data undoubtedly indicate that ginger is at least as effective, and may be better, than vitamin B6 in treating these symptoms. Again, mechanisms are lacking, but no reports indicate that ginger has any adverse side effects or that it can worsen illness in pregnant women or patients. Interest in ginger as an anticancer agent has markedly increased over the last few years and a direct protein target has been identified in colon cancer. Ginger also appears to reduce cholesterol and improve lipid metabolism, thereby helping to decrease the risk of cardiovascular disease and diabetes.

In summary, ginger has been reported to possess diverse pharmacological properties, although its specific biological targets are largely unknown and remain to be determined. However, in spite of the lack of specific mechanistic information, use of ginger appears to be safe and its effects are mighty and amazing in its many applications.

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8 Biological Activities of Ginseng and Its Application to Human Health

Jae Joon Wee, Kyeong Mee Park, and An-Sik Chung

CONTENTS

8.1 Introduction 157
8.2 Structural Properties of Ginseng 158
8.2.1 Structural Conversion of Ginsenosides 158
8.2.2 Other Constituents in Ginseng 160
8.3 Immunomodulatory Function of Ginseng 160
8.3.1 Immunomodulating Effects of Aqueous Extracts of Ginseng 160
8.3.2 Immunomodulating Effects of Saponin Fraction 161
8.3.3 Immunomodulating Effects of Polysaccharide Fractions 161
8.4 Anticarcinogenic Function of Ginseng 162
8.4.1 Effect on Tumor Cell Cytotoxicity and Differentiation 163
8.4.2 Antitumor Promotion Related to Inflammation 163
8.4.3 Antimetastatic Effect and Inhibition of Angiogenesis 164
8.4.4 Anticarcinogenic Activities and Synergistic Effect in Combination with Chemical Therapeutic Agents 164
8.4.5 Reducing Multidrug Resistance 165
8.5 Reduction of Blood Glucose Level and Improvement of Diabetes Treatment 165
8.5.1 Modulation of Insulin Secretion 166
8.5.2 Control of Blood Glucose Level and Glucose Transport 166
8.5.3 Regulation of Adipogenic Transcriptional Factor PPAR-γ and AMPK 167
8.6 Effects of Ginseng on Central Nervous System Functions and Degenerative Diseases 167
8.6.1 Learning and Memory 167
8.6.2 Neurodegenerative Diseases 168
8.7 Summary and Research Needs 169
References 170

8.1 INTRODUCTION

Ginseng is a medicinal plant widely used for the treatment of various conditions. The pharmacological effects of ginseng have been demonstrated in cancer, diabetes, cardiovascular diseases and have been used for promoting immune function, central nervous system (CNS) function, relieving stress, and for its antioxidant activities (Jung and Jin 1996). The root of *Panax ginseng* C. A. Meyer, which is known as Korean or Asian ginseng, is a valuable and an important folk medicine in East Asian countries, including China, Korea, and Japan, for more than 2000 years. *Panax* is derived from the word "panacea," which means a cure for all diseases and a source of longevity as well as physical strength and resistance. As the use of traditional Chinese herbs for medicinal and dietary purposes

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becomes increasingly popular in Western countries, sales of *P. ginseng* are increasing in North America and Europe as well as in other parts of the world.

The major bioactive components of *P. ginseng* are the ginsenosides, a group of saponins with dammarane triterpenoid structure (Huang 1999). Almost 50 ginsenosides have been isolated from *P. ginseng* root (white and red ginsengs), and novel structures continue to be identified, particularly from *Panax quinquefolius* (American ginseng) and *Panax japonica* (Japanese ginseng) as well as their berries (Gillis 1997; Yoshikawa et al. 1998; Attele et al. 2002; Christensen 2009). In this chapter, we review the structural and pharmacological properties of ginseng, and its active constituents, including ginsenosides, polysaccharides, and polyacetylenic alcohols. The pharmacological and clinical usages of ginseng, particularly ginsenosides, are discussed in relation to its anticancer, antidiabetes, immunomodulatory functions, and improving CNS functions including learning, memory, and neurodegenerative diseases.

8.2 STRUCTURAL PROPERTIES OF GINSENG

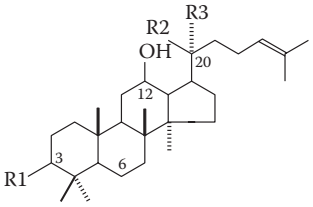
Ginsenosides, known as ginseng saponins, are the major components of ginseng and are classified into two major groups by the type of their aglycones, namely protopanaxadiol (PPD) and protopanaxatriol (PPT). PPD and PPT have dammarane triterpenoidal skeletons with sugar moieties binding at C-3, C-6, and C-20 positions (Huang 1999). The genuine structures of the PPD and PPT ginseng sapogenins are dammar-24-ene-3β,12β,20(S)-triol(PPD) and dammar-24-ene-3β,6α,12β,20(S)-tetrol(PPT), respectively (Shibata et al. 1995). Ginsenosides, which are named as ginsenoside Rx (x = o, a1, a2, b1, b2, b3, c, d, e, f, g1, g2, h1, and h2), differ from one another by the type of aglycone, sugar moieties, number of sugars, and their site of attachment (Figure 8.1). Exceptionally, ginsenoside Ro is an oleanane-type saponin, which is common in plants (Figure 8.1c). Another oleanane-type of ginsenoside is polyacetylene ginsenoside Ro, which contains a polyacetylenyl ester at the C-6′ position of glucosyl moiety (Zhang et al. 2002). These ginsenosides are usually extracted through water/n-butanol partitioning, following the extraction of ginseng root with aqueous alcohol, resulting in n-butanol extract as a saponin fraction.

The structural diversity of ginsenosides may contribute to the multiple pharmacological effects of ginseng on cancer, diabetes, inflammation, stress, immune, cardiovascular system, and CNS. Furthermore, it is of interest that the coexistence of PPD- and PPT-type ginsenosides in ginseng may be associated with its dual effects that can both stimulate and sedate the CNS. Ginsenoside Rb1 has been observed to exhibit depressant activity on the CNS, whereas ginsenoside Rg1 showed stimulant activity (Takagi, Saito, and Nabata 1972). Microinjection of Rb1 into the hypothalamic ventromedial nucleus decreased food intake, indicating CNS-suppressive action (Etou et al. 1988). In contrast, the sustained central administration of ginsenoside Rg1 attenuated anorexia, increased water intake, and decreased ambulation produced by an increase in environmental temperature (Fujimoto et al. 1989).

8.2.1 Structural Conversion of Ginsenosides

Ginsenosides undergo structural conversion under high temperature conditions, such as the decoction and steaming of ginseng, and in the acidic conditions in the stomach or due to metabolism by the intestinal bacteria. Some partially deglycosylated saponins, such as ginsenoside Rh1, Rg2, and Rg3, are obtained as byproducts produced during steaming of red ginseng (Figure 8.1a, b). Ginsenoside Rh1 is generated from the ginsenoside Rg1 via deglycosylation at the C-20 position, whereas ginsenoside Rg2 is generated from ginsenoside Re. Ginsenoside Rg3 is further converted to ginsenoside Rh2 via deglycosylation of one terminal glucose at the C-3 position. Elimination of the sugar moiety and subsequent epimerization of the hydroxyl group at the C-20 position yields 20(R)-ginsenosides Rh1, 20(R)-ginsenosides Rg2, and 20(R)-ginsenosides Rg3 as epimers (Figure 8.1a, b).

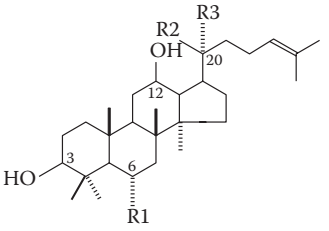
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	R1	R2	R3
20(S)-protopanaxadiol	OH	OH	CH_3
Ginsenoside Rb1	O-glc(2→1)glc	O-glc(6→1)glc	CH_3
Ginsenoside Rb2	O-glc(2→1)glc	O-glc(6→1)arap	CH_3
Ginsenoside Rc	O-glc(2→1)glc	O-glc(6→1)araf	CH_3
Ginsenoside Rd	O-glc(2→1)glc	O-glc	CH_3
20(S)-ginsenoside Rg3	O-glc(2→1)glc	OH	CH_3
20(R)-ginsenoside Rg3	O-glc(2→1)glc	CH_3	OH
Ginsenoside Rs1	O-glc(2→1)glc(6)Ac	O-glc(6→1)arap	CH_3
Compound K	OH	O-glc	CH_3
Ginsenoside Rh2	O-glc	OH	CH_3
Malonyl ginsenoside Rb1	O-glc(2→1)glc(6)Ma	O-glc(6→1)glc	CH_3

Glc : β-D-glucopyranosyl arap : α-L-arabinopyranosyl
Araf : α-L-arabinofuranosyl Ac : acetyl Ma : malonyl

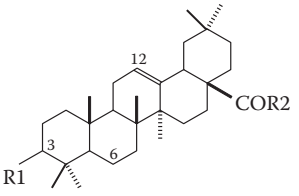
(a)



	R1	R2	R3
20(S)-protopanaxatriol	OH	OH	CH_3
Ginsenoside Re	O-glc(2→1)rha	O-glc	CH_3
Ginsenoside Rf	O-glc(2→1)glc	OH	CH_3
Ginsenoside Rg1	O-glc	O-glc	CH_3
20(S)-ginsenoside Rg2	O-glc(2→1)rha	OH	CH_3
20(R)-ginsenoside Rg2	O-glc(2→1)rha	CH_3	OH
20(R)-ginsenoside Rh1	O-glc	CH_3	OH

Glc : β-D-glucopyranosyl rha : α-L-rhamnopyranosyl

(b)



	R1	R2
Ginsenoside Ro	O-glcUA(2→1)rha	O-glc

GlcUA : β-D-glucuronic acid rha : α-L-rhamnopyranosyl

(c)

FIGURE 8.1 (a) Protopanaxadiol-type ginsenoside. (b) Protopanaxatriol-type ginsenoside. (c) Oleanane-type ginsenoside.

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Stereoisomers often differ considerably in pharmacological activity, potency, and pharmacokinetic profile, exerting different effects in biological systems. For example, 20(S)-Rg3 enhanced glucose-stimulated insulin secretion, whereas 20(R)-Rg3 did not show any effect (Park, Ha, and Chung 2008). Malonyl groups at the 6″-position of glucosyl moiety of ginsenoside Rb1, Rb2, Rc, and Rd are released during steaming, resulting in their corresponding ginsenosides (Kitagawa et al. 1983; Figure 8.1a). In red ginseng, the acetyl group remains at the 6″-position of glucosyl moiety of some saponins such as ginsenosides Rs1 and Rs2; thus, it appears that steaming inactivates the deacetylating enzyme (Kasai et al. 1983; Figure 8.1a).

The chemical structure of the side chain at the C-20 position can be modified by hydration or dehydration during steaming or decoction of the ginseng root. Recently, new dammarane glycosides with modified side chains, which are named ginsenoside Rh4, Rg5, Rg6, 20(E)-ginsenoside F4, and Rf2, have been isolated from Korean red ginseng (KRG; Park, Rhee, and Lee 2005). Further information may be found in a review by Christensen (2009). The structural conversion of ginsenosides also takes place in the gastrointestinal tract by gastric juice and digestive and bacterial enzymes after ingestion. The sugars attached to the C-3 or C-20 hydroxyl group of the aglycone are cleaved off separately from the end by intestinal flora (Hasegawa et al. 1996). PPD-type saponins are metabolized to compound K (C-K), whereas PPT-type saponins are hydrolyzed to 20(S)-PPT (Figure 8.1a, b). Pharmacological activities of C-K such as anticancer, antidiabetes, and anti-inflammation effects will be discussed in Sections 8.3 and 8.4.

8.2.2 Other Constituents in Ginseng

Ginseng contains several valuable nonsaponin components, including essential oils, antioxidants, polyacetylenic alcohols, peptides, amino acids, polysaccharides, and vitamins. Ginseng polysaccharides have also been a target of chemical and biological research, because plant polysaccharides generally show antitumor effects through modulation of innate immunity. Two acidic polysaccharides, which are named ginsenan S-IA and ginsenan S-IIA, were isolated from *P. ginseng* (Tomoda et al. 1993). Ginsenan S-IIA was shown to increase phagocytosis. Many immunological studies have been performed with crude polysaccharide fractions, which are usually prepared by ethanol precipitation after the extracting ginseng root with hot water. Their immunological activities will be described in Section 8.3.

Enormous progress has been made in understanding the chemistry of ginsenosides in transformed or metabolized forms as well as intact ones, contributing to the understanding of ginseng pharmacological properties. However, further studies on nonsaponin constituents, especially immunomodulating polysaccharides, and on the interaction and/or harmonization of constituents still remain to be explored.

8.3 IMMUNOMODULATORY FUNCTION OF GINSENG

There have been many reports describing the immunomodulating effects of ginseng, although results are somewhat controversial and inconsistent, since the chemical composition of purified fractions of ginseng employed in studies is different. In Sections 8.3.1 through 8.3.3, we describe the immunomodulating effects of aqueous extracts, saponin fractions, and polysaccharide fractions of ginseng.

8.3.1 Immunomodulating Effects of Aqueous Extracts of Ginseng

Aqueous extracts of ginseng contain amino acids, minerals, saponins, and various water-soluble low- and high-molecular weight compounds. It was reported that a ginseng extract modulated the cytokine production in a mouse model with *Pseudomonas aeruginosa* lung infection. The lung cells from the ginseng extract-treated group produced more interferon γ (IFN-γ) and tumor necrosis factor α (TNF-α), but less interleukin 4 (IL-4), with a higher ratio of IFN-γ/IL-4. Results indicated that

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a ginseng extract treatment induced a Th1-like immune response (cellular immune response) in the mice with *Panax aeruginosa* lung infection (Song et al. 2003).

Long-term oral administration of the ginseng extract appears to potentiate humoral immune response but suppresses spleen cell functions in male BALB/c mice. Mice treated with ginseng extract and immunized with ovalbumin (OVA) resulted in an eightfold increase in titers of anti-OVA immunoglobulin G (IgG) in serum, but IgG production was not affected in spleen cells (Liou, Huang, and Tseng 2005). Intranasal coadministration with inactivated influenza virus A (PR8) and ginseng extract increased the levels of influenza virus-specific antibodies and neutralizing activities and provided protective immunity compared to immunization with PR8 alone. Ginseng extract coadministration also significantly induced high levels of IL-4 and IL-5 cytokines, producing cells after PR8 infection, implying that ginseng extract plays a role as a mucosal adjuvant against influenza virus as well as an immunomodulator during influenza virus infection (Quan et al. 2007).

8.3.2 Immunomodulating Effects of Saponin Fraction

Dendritic cells (DCs) play a pivotal role in the initiation of T-cell-mediated immune responses, making them an attractive cellular adjuvant for use in cancer vaccines. Researchers investigated whether M4, end products of steroidal ginseng saponins metabolized in digestive tracts, can drive DCs maturation from human monocytes in vitro. Results showed that mature DCs differentiated with M4 induced the differentiation of naive T cells toward a helper T-cell type 1 (Th1) response and augmented cytotoxicity toward tumor cells. Takei et al. (2004) suggested that M4 might be used on DC-based vaccines for cancer immunotherapy.

In the case of ginsenoside Rg1, it was reported that Rg1 enhanced CD4(+) T-cell activities and modulated Th1/Th2 differentiation in murine splenocytes. Rg1 had no mitogenic effects on unstimulated CD4(+) T cells but augmented CD4(+) T-cell proliferation on activation with anti-CD3/anti-CD28 antibodies in a dose-dependent manner. Rg1 also enhanced the expression of cell surface protein CD69 on CD4(+) T cells. In Th0 condition, Rg1 increased the expression of IL-2 mRNA and enhanced the expression of IL-4 mRNA on CD4(+) T cells, suggesting that Rg1 prefers to induce Th2 lineage development (Lee et al. 2004). In addition, ginsenoside Rg1 induced Th1 type differentiation of CD4(+) T cells and helped mice resist disseminated candidiasis. Antimouse IFN-γ antibody treatment of Rg1-treated mice abolished the protection against disseminated candidiasis (Lee and Han 2006).

PPD saponins (Rg3, Rd, Rc, Rb1, and Rb2) and PPT saponins (Rg1, Re, and Rg2) were evaluated for their adjuvant effects on the immune responses to OVA in BALB/c mice. OVA-specific antibody responses were significantly higher in mice immunized with OVA coadministered with Rg1, Re, Rg2, Rg3, and Rb1, but not with Rd, Rc, and Rb2. Therefore, it is suggested that Rg1, Re, Rg2, Rg3, and Rb1 have more potent adjuvant effects than the others (Sun, Hu, and Song 2007). Recently, it has been reported that ginsenoside-based nanoparticles (ginsomes) played a role as a novel adjuvant and upregulated Th1 and Th2 immune response in imprinting control region (ICR) mice. The ginsomes were spherical with diameters ranging from 70 to 107 nm and contained ginsenosides Rb2, Rc, Rb1, and Rd. The ginsomes promoted significantly higher IgG responses, increased the levels of specific IgG1, IgG2a, IgG2b, and IgG3, as well as T and B lymphocyte proliferation in response to concanavalin A, LPS, and OVA. The enhanced IgG titer and subclass levels paralleled the increased production of IFN-γ (Th1 cytokine) and IL-5 (Th2 cytokine). Therefore, ginsomes as an adjuvant are assumed to upregulate both Th1 and Th2 immune responses (Song, Zang, and Hu 2009).

8.3.3 Immunomodulating Effects of Polysaccharide Fractions

Polysaccharide fractions of ginseng are high-molecular weight compounds obtained from the water-soluble and ethanol-insoluble fractions of ginseng. The in vitro immunostimulating activities of polysaccharides from ginseng were investigated. Four polysaccharides, which were found to be

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homogeneous by gel-filtration chromatography, were prepared and designated PF3111, PF3112, PBGA11, and PBGA12. Component sugar analysis revealed that they were heteroglycans with molecular weights ranging from 37 to 760 kD, composed of glucose, galactose, arabinose, mannose, and xylose in different molar ratios. Fraction PBGA12 had the most anticomplementary activity, which is mediated through both alternative and classical pathways. All the polysaccharides except PBGA11 induced the production of IFN-γ in the presence of concanavalin A. They induced the production of significant amount of TNF-α in cell cultures (Gao et al. 1996).

Incubation of murine macrophages (RAW 264.7 cells) with increasing amounts of polysaccharide fraction of ginseng showed a dose-dependent stimulation of inducible nitric oxide (NO) synthesis. This was associated with an incline in inducible nitric oxide synthase (NOS) mRNA levels as determined by semiquantitative polymerase chain reaction, and electromobility shift assay studies indicated enhanced nuclear factor κB (NF-κB) DNA binding activity. Friedl et al. (2001) suggested that polysaccharide treatment could modulate several aspects of host defense mechanisms due to stimulation of the inducible NOS. It was also reported that a polysaccharide fraction of ginseng stimulated murine normal splenocytes by inducing the mRNA expressions of Th1- and Th2-type cytokines and also restored the mRNA expression of IFN-γ, Th1 cytokine, after its inhibition by whole-body γ irradiation. Therefore, the polysaccharide fraction of ginseng was found to restore the T lymphocytes function that had been suppressed by γ irradiation in allogeneic mixed lymphocyte reactions (Han et al. 2005).

More recently, reports on the acidic polysaccharide of *P. ginseng* (APG) were described. Acidic polysaccharide fractions altered the phenotype of bone marrow cells (BMCs) and increased the viability and alloreactivity of BMCs after γ irradiation both in vitro and in vivo. A pretreatment with APG significantly increased the viability of BMCs against γ irradiation. APG-treated BMCs had a significantly higher amount of IL-12, which is a major cytokine for immune responses, compared with the medium-treated BMCs. The expression of major histocompatibility complex (MHC) class II molecules of APG-treated BMCs was also increased, and APG-treated BMCs showed significantly higher levels of allogeneic CD4(+) T lymphocyte proliferation. Furthermore, APG-treated mice had a larger number of BMCs after γ irradiation than the control mice, and the BMCs of APG-treated mice were successfully cultured into DCs, which are the representative antigen-presenting cells (Kim, Kim et al. 2007).

Various aspects of immunomodulatory effects of ginseng have been investigated for their tonic effects. Modulation of cytokine production, potentiation of humoral immune response, enhancement of CD4(+) T-cell activities, upregulation of adjuvant effects, restoration of T lymphocytes function, and BMCs viability after suppression by γ irradiation were especially remarkable.

8.4 ANTICARCINOGENIC FUNCTION OF GINSENG

The main weapons in the war against cancer have been early detection and surgical removal of the tumor, radiotherapy, and chemotherapy. There are also attempts to develop gene therapy. However, the results have been less than ideal, and strategy is now changing from therapeutic approaches to prevention of cancer by identifying effective natural products as chemopreventive agents. One of the promising candidates for cancer prevention is ginseng. People who consume ginseng preparations are at lower risk of cancers in the stomach, lung, liver, pancreas, ovaries, colon, and oral cavity (Yun 2003). *P. ginseng*, *P. quinquefolius*, and other related plants including *Panax japonicus* are frequently used for medicinal purposes. Although a complex mixture of compounds is present in these plants, the ginsenosides are mostly responsible for the pharmacological effects of these ginsengs, and Rg3 and Rh2 are recognized as major active anticancer saponins (Helms 2004). In Sections 8.4.1–8.4.5 describe the anticarcinogenic effects of ginseng based on its diverse mechanisms, including cell cytotoxicity and differentiation, antitumor promotion related to inflammation, antimetastasis and inhibition of angiogenesis, synergistic effect on chemical therapeutic agents, and decreasing multidrug resistance (MDR).

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8.4.1 Effect on Tumor Cell Cytotoxicity and Differentiation

Saponin and nonsaponin compounds have been reported to show cytotoxic activities against various kinds of cancer cell lines in culture. The major active components are ginsenoside Rh2, a peculiar component of KRG, polyacetylenes, panaxydol, panaxynol, and panaxytriol. Jia et al. (2004) demonstrated that ginsenoside Rh2 inhibited proliferation, induced apoptosis in cancer cell lines, and sensitized drug-resistance breast cancer cells to paclitaxel. Recently, it was shown that KRG extract induced apoptosis and decreased telomerase activity in human leukemia cells (Park et al. 2009). The main active ingredients in KRG are four representative ginsenosides, Rg1, Rg3, Rh2, and Rk1. Ginsenosides Rg3 and Rh2 inhibited proliferation of prostate cancer cells by detachment of the cells and by modulating mitogen-activated protein (MAP) kinases (Kim, Lee et al. 2004). Ginsenoside Rh2 treatment significantly inhibited the viability of MCF-7 and MDA-MB-231 breast cancer cells with G_0/G_1 phase cell-cycle arrest, which was caused by $p15^{Ink4B}$ and $p27^{Kip1}$-dependent inhibition of cyclin-dependent kinases (Choi, Kim, and Singh 2009). In addition, Rh2 markedly increased albumin secretion and alkaline phosphatase activity, whereas it strikingly decreased α-fetoprotein secretion and γ-glutamyl transpeptidase in SMMC-7721 hepatocarcinoma (Zeng and Tu 2003). Furthermore, Rh2 almost completely inhibited telomerase activity with the parallel induction of the cell differentiation. After steam or heat treatment of American ginseng and notoginseng, the content of Rg3 was found to increase remarkably, with increased antiproliferation of colorectal cancer cells (Wang and Yuan in press). In addition, acetylpanaxydol and panaxydolchlorohydrin, showing cytotoxicity against lymphoid leukemia L1210, have been isolated from Korean ginseng root (Ahn, Kim, and Lee 1989). Panaxydol, a polyacetylene compound isolated from *Panax notoginseng*, and *P. ginseng* inhibited proliferation and induced differentiation of the human hepatocarcinoma cell line HepG2 by increasing the expression of p21 and pRb, while reducing that of inhibitor of differentiation 1 and 2 (Guo et al. 2009). These studies suggest that ginseng compounds, such as ginsenoside Rh2 and panaxydol, block cancer cell proliferation and induce cell differentiation toward more mature forms of normal cells.

8.4.2 Antitumor Promotion Related to Inflammation

Considerable effort has been made to develop chemopreventive agents that could inhibit, retard, or reverse multistage carcinogenesis (Weinstein 1991). Tumor promotion is closely related to inflammation (DiGiovanni 1992), and compounds with strong anti-inflammatory activity possess antitumor promoter activity. Treatment with KRG extract of human leukemia cells decreased the expression levels of cyclo-oxygenase-2 (COX-2) and inducible NOS (Park et al. 2009), which are indicators of inflammation related to tumor promotion. In addition, treatment with KRG extract induced apoptosis of leukemia cells mediated by an inhibition of Bcl-2 and Bcl-X_L, and it progressively downregulated the expression of human telomerase reverse transcriptase by inhibiting the expression of c-Myc. Ginsenosides Rb1, Rc, Re, Rg1, and Rg3 from *P. ginseng* were tested for anti-inflammatory activity (Surh et al. 2002). Rg3 was found to be the most effective in terms of inhibiting 12-0-tetradecanoylphorbol-13-acetate (TPA)-induced ear edema, COX-2 expression, and NF-κB activation. One ginsenoside metabolite, 20-0-β-D-glucopyranosyl-20(S)-PPD, which is known as C-K, given to ICR mice suppressed COX-2 expression and ornithine decarboxylase activity induced by TPA (Lee et al. 2005).

The eukaryotic transcriptional factor NF-κB is involved in intracellular signaling pathways associated with inflammation and carcinogenesis. C-K pretreatment inhibited TPA-induced epidermal NF-κB activity in mouse skin. Antitumor promotional effects of C-K were shown by markedly decreased numbers of papillomas in mouse skin induced by 12-dimethylbenz[a]anthracene (DMBA). These findings suggest that C-K exerts anti-inflammatory effects by inhibiting TPA-induced COX-2 expression, which may contribute to its antitumor-promoting effects on mouse skin carcinogenesis. Ginsenoside Rb1 inhibited histamine release and IL-4 production induced by

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substance P, an allergic enhancer, via the extracellular receptor kinase (ERK) pathway (Liao et al. 2006). It was also shown that C-K as a functional ligand of the glucocorticoid receptor regulated distinct Toll-like receptor 4-mediated inflammatory responses, which suggests a novel therapy for gram-negative septic shock (Yang et al. 2008).

8.4.3 Antimetastatic Effect and Inhibition of Angiogenesis

Ginsenoside Rg3 inhibited tumor invasion and metastasis of F16 melanoma cells without impairing cell growth and proliferation of tumor cells (Mochizuki, Yoo, and Matsuzawa 1995). Rg3 inhibited the metastasis of ovarian cancer; the inhibitory effect is partially due to inhibition of tumor-induced angiogenesis and the decreased invasive ability and MMP-9 expression of SKOV-3 cells (Xu et al. 2008). Ginsenoside Rg3 significantly inhibited growth and angiogenesis of ovarian cancer when used alone or combined with cyclophosphamide (CTX; Xu et al. 2007). Another study found that low-dose CTX combined with Rg3 produced significant antiangiogenic effects, without overt toxicity, because Rg3 is capable of specific blockade of activated endothelial cell survival mechanisms (Zhang, Kang, and Zhoa 2006). These studies indicated that a ginsenoside Rg3 and CTX combination reinforced the antitumor effect on each other and improved the living quality and survival time of mice with tumors. As an antiangiogenic method, this regimen has the advantage of a lowered susceptibility to drug-resistance mechanisms and improved animal survival. Another ginsenoside, Rb1, suppressed the formation of endothelial tube-like structures through modulation of pigment epithelium-derived factors through estrogen β receptors (Leung et al. 2007). These findings demonstrated several novel mechanisms of these ginsenosides that may have value in anticancer and antiangiogenesis therapy. Ginsenoside 20(S)-PPD inhibited the proliferation and invasion of human fibrosarcoma HT1080 cells due to downregulation of the expression MMP-2 (Li et al. 2006). A ginseng saponin metabolite (C-K) suppressed phorbol ester-induced MMP-9 expression through inhibition of AP-1 and MAP kinase signaling pathways in human astroglioma cells (Jung et al. 2006). Ginsenoside Rp1, a semisynthesized ginseng saponin, strongly inhibited metastatic lung transfer of B16-melanoma cells by downregulation of β1-intergrin activation and further directly blocked the viability of cancer cells (Park, Park et al. 2008).

8.4.4 Anticarcinogenic Activities and Synergistic Effect in Combination with Chemical Therapeutic Agents

Several studies have been conducted to evaluate the inhibitory effect of ginseng on carcinogenesis induced by various chemical carcinogens. Earlier studies showed that long-term oral administration of KRG extract inhibited the incidence and the proliferation of tumors induced by 7,12-DMBA, urethane, and aflatoxin B1 (Yun, Yun, and Han 1983). The chemopreventive potential of ginseng was evaluated using DMBA-induced skin tumorigenesis (Kumar 1993). There was a marked reduction not only in tumor incidence but also in cumulative tumor frequency at the initiation phase of tumorigenesis. Ginsenosides Rg3 and Rg5 showed statistically significant reduction of lung cancer, and Rh2 tended to decrease the incidence (Yun et al. 2001). Panwar et al. (2005) showed that *P. ginseng* extract inhibited lung adenoma induced by benzo[a]pyrene and decreased the frequencies of chromosomal aberrations and micronuclei. Another study showed that Rh2 had an antiproliferative effect on human lung adenocarcinoma A549 cells with G1 arrest by downregulation of cyclin proteins and kinases and further apoptosis mediated by caspase-8 (Cheng et al. 2005). Dietary administration of KRG suppressed colon carcinogenesis induced by 1, 2-dimethylhydrazine with inhibition of cell proliferation, acting on aberrant crypt foci in the colon mucosa (Fukushima, Wanibuchi, and Li 2001). In addition, an anticarcinogenic effect of KRG on the development of liver cancer induced by diethylnitrosamine in rats was identified in preventive and curative events (Wu, Zhu, and Li 2001). Ginsenoside Rh2 was shown to inhibit cell growth at low concentrations, to induce apoptosis at high concentrations, and, interestingly, to act either additively or synergistically

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with chemotherapeutic drugs on cancer cells, especially breast cancer cells to paclitaxel (Jia et al. 2004). Panaxadiol (PD) enhanced the anticancer effects of 5-fluorouracil (5-FU) in human colorectal cancer cells by inducing apoptosis (Li et al. 2009). The enhancement of S-phase arrest and the increased susceptibility to apoptosis are synergistic effects of PD on 5-FU.

8.4.5 Reducing Multidrug Resistance

One of the major obstacles to the effective treatment of human malignancy is the acquisition of broad anticancer drug resistance by tumor cells. This phenomenon is called *multidrug resistance*. MDR is a major problem in cancer chemotherapy, and it is correlated with the overexpression of P-glycoprotein (Pgp) in the plasma membrane of resistant cells (Gotteeman and Pastan 1993). Ginsenoside Rg1, Re, Rc, and Rd were found to have a moderate inhibitory effect on the drug efflux pump in MDR mouse lymphoma and to increase intracellular drug accumulation (Molnar et al. 2000). Ginsenoside Rg3, among several ginseng components, was shown to have the most potent inhibitory activity on MDR human fibroblast carcinoma KBV20C (Park et al. 1996). Rg3 treatment of drug-resistant KBV20C cells specifically inhibited Pgp-mediated drug accumulation and further increased life span in mice implanted with adriamycin-resistant murine leukemia P388 cells in vivo (Kim et al. 2003). Subsequent studies demonstrated that Rg3 was cytotoxic against a multidrug-resistant human fibrocarcinoma KBV20C cells but not against normal WI cells in vitro, and Rg3 also promoted the accumulation of rhodamine 123 in adriamycin-resistant murine leukemia P388 cells in vivo by mediating decreased membrane fluidity, thereby blocking drug efflux (Kwon et al. 2008). Another study showed that ginsenoside metabolites Rh2, PPD, and PPT significantly enhanced the cytotoxicity of mitoxantrone (MX) to human breast carcinoma and may be potential inhibitors of breast cancer resistance protein (BCRP) in MCF-7/MX cells, which overexpress BCRP (Jin et al. 2006).

The protective influence and the complementary therapeutic potential of ginseng for cancer treatment have been shown by extensive laboratory, preclinical, and epidemiological studies. Two Korean cohort studies have suggested that ginseng consumers are associated with a 60–70% reduction in the risk of gastric cancer. However, ginseng consumers in the Shanghai women's cohort study showed no beneficial effects on gastric cancer risk (Kamangar et al. 2007). Further careful evaluation in Asian cohort studies may help clarify ginseng's effect on gastric carcinogenesis and other cancers. Additional clinical studies are needed to evaluate the potential beneficial effects of ginseng on chemoprevention and complementary therapy of cancers.

8.5 REDUCTION OF BLOOD GLUCOSE LEVEL AND IMPROVEMENT OF DIABETES TREATMENT

Diabetes, affecting almost 3.5% of the world's population, is one of the major global health problems. The root of *P. ginseng* has been used to treat diabetes and has been given as a tonic for chronic use without adverse effects. More than 90% of patients with diabetes have type 2 diabetes, which is related to aging, low physical activity, diet, and lifestyle. In this section, we focus the effects of ginseng on type 2 diabetes rather than type 1 diabetes.

Animal studies support that the roots of *P. ginseng* and other ginseng species, including American ginseng, possess antihyperglycemic activity (Kimura et al. 1981; Chung and Choi 2001; Dey et al. 2003). It has been documented that ginseng therapy decreased fasting glucose, lowered body weight (Sotaniemi, Haapakoski, and Rautio 1995), and increased glucose utilization and insulin regulation in diabetic patients (Vulksan et al. 2008). Furthermore, American ginseng has the ability to attenuate postprandial glycemia in healthy individuals (Vulksan et al. 2001). Recently, it was observed that oral administration of *P. ginseng* root had the ability to improve insulin resistance in rats receiving a fructose-rich diet (Liu, Liu, and Cheng 2005). These observations suggest that ginseng is beneficial for patients with type 2 diabetes and for nondiabetic subjects to prevent development of diabetes.

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8.5.1 Modulation of Insulin Secretion

Ginseng might mediate its antidiabetic action through a variety of mechanisms, including actions on the insulin-secreting pancreatic β-cells and the target tissues that take up glucose (Xie, Mehendale, and Yuan 2005). Korean white ginseng (KWG) and KRG, one of the heat-processed Korean ginsengs, have a long history as herbal remedies with antidiabetic effects. KWG has been reported to stimulate glucose-induced insulin release from pancreatic islets as a potentiator (Kimura et al. 1981; Su, Cheng, and Li 2007). The mode of the insulinotropic action of KRG was to act as an initiator for insulin release, not in a glucose-dependent manner. In general, the heat-processed KRG has been reported to have more potent pharmacological activities than nonprocessed KWG (Kim et al. 2000). KRG significantly evoked a stimulation of insulin release in normal pancreatic rat islets and may act by inhibiting the K_{ATP} channel, thereby depolarizing the β-cells and stimulating Ca^{2+} influx (Kim and Kim 2008). These findings suggest that *P. ginseng* has beneficial effects in the treatment of diabetes at least in part via the stimulation of insulin release.

Antihyperglycemic and antiobese effects of *P. ginseng* berry extract have been observed; its major constituent is ginsenoside Re (Attele et al. 2002). Ginsenoside Rg3 enhanced glucose-stimulated insulin secretion (Park, Ha, and Chung 2008) and was further metabolized to ginsenoside Rh2 by human intestinal bacteria, which seems to be more effective (Bae et al. 2002). Intravenous injection of ginsenoside Rh2 into rats decreased plasma glucose and increased plasma insulin by activation of muscarinic M3 receptors in pancreatic β-cells via acetylcholine (ACH) release from cholinergic terminals (Lee, Kao et al. 2006). PPD ginsenoside potentiated an insulin secretion stimulated by a low concentration of glucose, and C-K, a final metabolite of PPD ginsenoside, showed the most potent insulin secretion in pancreatic β-cells through action on the K_{ATP}-channel-dependent pathway. These observations were confirmed in an oral glucose tolerance test in ICR mice (Han et al. 2007). In *db/db* mice, multiple administration of C-K showed hypoglycemic effects and improved glucose tolerance with β-cell preservation. Both Rh2 and C-K appear to have some therapeutic value for the treatment of diabetes and might be useful candidates for the development of new antidiabetic drugs.

8.5.2 Control of Blood Glucose Level and Glucose Transport

There are numerous reports of ginseng root improving diabetic conditions in both human and animal studies. In animal studies, orally administrated ginseng root was able to counteract the effect of high fructose-induced insulin resistance in rats after 4 weeks, decreasing glucose concentration and inhibiting insulin resistance (Liu, Liu, and Cheng 2005). Ethanol extract of wild ginseng root prevented weight gain and elevated fasting blood glucose, triglycerides, and high free fatty acid levels in a high fat-induced hyperglycemia mouse model (Yun et al. 2004). Ginsenoside Re decreased blood glucose, cholesterol, and triglyceride levels as well as decreased oxidative stress in the eye and kidney of diabetic rats (Cho et al. 2006). It is suggested that ginseng is useful for the prevention of diabetes in healthy people and for improved glycemic control in type 2 diabetes patients (Luo and Luo 2008). Clinical studies have reported that American ginseng lowers blood glucose in diabetic patients (Vulksan et al. 2000; Luo, Yano, and Luo 2003). In these studies, both type 2 diabetic patients and nondiabetic subjects were shown to benefit from intake of American ginseng in terms of stabilizing postprandial glycemia. More studies are required to confirm that ginseng administration decreases the dietary glycemic index, an indicator of carbohydrate's ability to raise blood glucose level.

P. ginseng has been shown to increase glucose transport-2 protein in the liver of normal and hyperglycemic mice (Lee 1992). Recently, Shang et al. (2008) showed that ginsenoside Rb1 stimulated basal and insulin-mediated glucose uptake in a time- and dose-dependent manner in 3T3-L1 adipocytes and C2C12 myotubes. In adipocytes, Rb1 promoted GLUT1 and GLUT4 translocation to the cell membrane and further increased the phosphorylation of insulin receptor substrate-1, protein

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kinase B, and stimulated phosphatidylinositol 3(P13)-kinase activity in the absence of the activation of the insulin receptor. Ginsenoside Rg3 enhanced glucose-stimulated insulin secretion and AMP-activated protein kinase (AMPK) in HIT-T15 cells, and further lowered the plasma glucose level by stimulating insulin secretion in ICR mice associated with ATP-sensitive K^+ channels (Park, Ha, and Chung 2008). AMPK is considered a master switch, regulating glucose and lipid metabolism, and an enzyme that works as a fuel gauge that is activated in conditions of high-energy phosphate depletion. Collectively, the findings provide insight into the hypoglycemic and antidiabetic properties of ginseng and ginsenosides and their potential to provide beneficial treatment for diabetes.

8.5.3 Regulation of Adipogenic Transcriptional Factor PPAR-γ and AMPK

Obesity is a major obstacle to human health because it predisposes individuals to various diseases, such as type 2 diabetes, cardiovascular diseases, and cancer. Two major proteins regulate adipocyte differentiation: AMPK and the peroxisome proliferator-activated receptor (PPAR; Yin, Mu, and Birnbaum 2003; Zhang, Lavan, and Greggore 2004). Both AMPK and PPAR-γ are major regulatory proteins involved in both obesity and diabetes. PPAR-γ is activated under conditions of adipocyte differentiation (Nedergaard et al. 2005; Lehrke and Lazar 2005). AMPK plays a role in intracellular energy homeostasis. The AMPK signaling pathway is induced by genistein, epigallocatechin gallate, and capsaicin and by decreasing 3T3-L1 adipocyte differentiation (Hwang et al. 2005). Ginsenoside Rh2 effectively inhibited adipocyte differentiation via PPAR-γ inhibition and activated AMPK in 3T3 L1 adipocytes (Hwang et al. 2007). Another study showed that ginsenoside Rb1 and Rg1 suppress triglycerides accumulation in 3T3-L1 adipocytes by activating PKA with increased intracellular cAMP (Park, Ahn et al. 2008). However, the insulin-stimulated glucose uptake was enhanced by Rb1 and Rg1 via activation of P13-kinase, and these ginsenosides promoted glucose-stimulated insulin secretion and cell viability in Min6 cells through PKA, which was associated with insulin response substrate 2 expression to insulin and insulin-like growth factor 1 signaling.

Some ginsenosides in ginseng improve insulin resistance by decreasing intracellular triglycerides accumulation. Ginsenoside Rb1 reduces rat liver triglycerides (Park et al. 2002) and Rh2 decreases triglyceride accumulation through AMPK activation in 3T3-L1 adipocytes (Hwang et al. 2007). Ginsenoside Rg3 was effective in inhibiting 3T3-L1 adipocyte differentiation through PPAR-γ induction by rosiglitazone and also was effective in activating AMPK (Hwang et al. 2009). The antiobesity effects of ginseng and ginsenosides Rg3, Rh2, and Rb1 may involve the AMPK and PPAR-γ signaling pathways. Further studies on the connection between the AMPK and PPAR-γ signaling pathways may be desirable to understand the antiobesity qualities of ginseng and its use in antidiabetic treatment.

8.6 EFFECTS OF GINSENG ON CENTRAL NERVOUS SYSTEM FUNCTIONS AND DEGENERATIVE DISEASES

Learning is the acquisition and storage of information as a consequence of experience, and memory is the relatively permanent storage form of the learned information, although it is not a single, unitary phenomenon. Alzheimer's disease is the predominant age-related neurodegenerative disorder, and it is known mainly for its progressive memory loss and consequent dementia in the elderly. Ginsenoside, the active principle in *P. ginseng* root, has been demonstrated to show both neurotrophic effects in memory and learning and neuroprotective actions for the prevention of neuron degeneration.

8.6.1 Learning and Memory

Various memory-impairment models have been used to evaluate the effects of ginseng and its active ingredients on learning and memory. In passive avoidance test, ginsenoside Rg1 improved learning

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and memory acquisition, consolidation, and retrieval, indicating that Rg1 can improve all stages of memory (Zhang et al. 1990). To study the effect of ginsenoside Rg1 on learning and memory loss induced by β-amyloid, passive avoidance and performance in the Morris water maze were assayed after the final treatment. Ginsenoside Rg1 significantly decreased latency and swimming distance, improved corresponding changes in search strategies in the Morris water maze, and increased step-through latency (Wang and Zhang 2001). In another study, Rg1 significantly improved memory deficits in aged rats, ovariectomized rats, and cerebral ischemia-reperfusion rats (Qiu et al. 1995; Chen, Gong, and Zhang 2001). Results showed that ginseng extract and ginsenosides Rg1 and Rb1 facilitated acquisition and retrieval of memory. Moreover, these ginsenosides also antagonized memory loss and cognitive deficit under various pathological conditions, such as cerebral ischemia and dementia (Qiu et al. 1995).

Among the mechanisms underlying the positive impact on brain aging related to impairment of cognitive function and memory, ginsenosides might potentiate the cholinergic system in CNS. ACH is a very important neurotransmitter in the brain, and its scarcity often leads to learning and memory impairment. Ginsenosides Rg1 and Rb1 were found to enhance the functions of the cholinergic system by increasing the density of central M-cholinergic receptors and increasing the level of ACH in the CNS (Zhang et al. 1988). Glutamate, another neurotransmitter, is also important for learning, memory, and cognitive function. Ginsenosides Rb1 and Rg1 facilitate the release of glutamate evoked by 4-aminopyridine, a potassium channel blocker that depolarizes nerve terminals in vitro (Chang et al. 2008), in a manner corresponding to in vivo depolarization (Tibbs et al. 1989). Ginsenosides Rb1 and Rg1 mediated facilitations of glutamate release are associated with an enhancement of vesicular exocytosis, an increase in Ca^{2+} influx through presynaptic N- and P/Q-type voltage-dependent Ca^{2+} channels and protein kinase A, which subsequently enhances Ca^{2+} entry to cause an increase in evoked glutamate release from rat cortical synaptosomes (Chang et al. 2008). Further study of this group showed that ginsenosides Rb1 and Rg1 enhanced glutamate exocytosis from rat cortical nerve terminals by affecting vesicle mobilization through the activation of protein kinase C (Chang and Wang 2008).

8.6.2 Neurodegenerative Diseases

Apoptosis is a process by which a cell actively commits suicide under tightly controlled circumstances, and it plays a fundamental role in the development of multicellular organisms, maintenance of homeostasis, and numerous pathophysiological processes. However, defective control of apoptosis might play a role in the etiology of cancer, autoimmune diseases, and neurodegenerative disorders. It was first reported that ginsenoside Rg1 inhibited apoptosis induced by withdrawing serum from the culture system of primary cortical neurons (Li, Zhang, and Zhang 1997). An antiapoptotic effect of Rg1 was shown in aged rats in vivo. Further studies demonstrated that mechanisms of Rg1 on apoptosis involved decreasing NO content and NOS activity, reducing intracellular calcium concentration and enhancing superoxide dismutase activity. Li et al. (1997) found that both NOS expression and the activity of NOS were elevated significantly in aged rats, which leads to increased NO concentration in rat cortex. NO played a role in the acceleration of senescence, and the inhibitory effect of Rg1 on NOS activity may be related to its antiaging function. Other studies on the antiapoptotic effect of Rg1 on neurons suggest that the effect of Rg1 may contribute to enhancing the ratio of Bcl-2 to Bax protein and inhibiting activation of caspase-3 (Chen et al. 2002).

Among 11 ginsenosides (Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, Rg2, Rg3, Rh1, and Rh2), Rg3 was the most effective ginsenoside in terms of inhibitory activity of N-methyl-D-aspartic acid (NMDA) on hippocampal neurons (Kim, Kim et al. 2004). Selective blockers of the active glycine site on NMDA receptors are considered to be promising therapeutics that may decrease the devastating effects of excitotoxicity (Lee, Zipfel, and Choi 1999). It was demonstrated that ginsenoside Rg3 significantly protects neurons from NMDA-induced neurotoxicity by blocking the glycine-binding site. Homocysteine could exert its excitotoxicity through NMDA receptor activation. It was shown

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that ginsenoside Rg3 significantly and dose-dependently inhibits homocysteine-induced hippocampal cell death. Ginsenoside Rg3 not only significantly lowered homocysteine-induced DNA damage, but also in vitro attenuated caspase-3 activity in a dose-dependent manner (Kim, Cho et al. 2007). Furthermore, it was also demonstrated that Rg3 dose-dependently inhibits homocysteine-induced increase of intracellular Ca^{2+} levels. In addition, ginsenoside Rg3 dose-dependently inhibited homocysteine-induced currents in Xenopus oocytes expressing NMDA receptors (Kim, Cho et al. 2007). These results collectively suggest that ginsenoside Rg3 protects from homocysteine-induced neurotoxicity in rat hippocampus; this effect is likely to be due to inhibition of homocysteine-mediated activation of NMDA receptors.

Ginsenoside Rh2 was identified as an active ingredient of ginseng that can act at the hippocampal NMDA receptors (Lee, Kim et al. 2006), but its neuroprotective activity came from an in vivo experiment showing that at 100 mg/kg p.o., ginsenoside Rh2 protects brain from ischemia-reperfusion injury (Park et al. 2004). These results indicate that ginsenoside Rg3 protects neurons in vitro from NMDA-induced neurotoxicity and in vivo ginsenoside Rh2 protects from ischemia-reperfusion brain injury. The neuroprotective activity of these ginsenosides can be attributed to the specific inhibition of NMDA-induced receptor activation.

Alzheimer's disease, characterized microscopically by the deposition of amyloid plaques and formation of neurofibrillary tangles in the brain, has become the most common cause of senile dementia. Loss of cholinergic neurons along with muscarinic ACH receptors in the cerebral cortex and hippocampus is closely associated with Alzheimer's disease. It was reported that Rg3 effectively decreased inflammatory cytokine expression in Abeta42-treated murine BV-2 microglial cells, inhibited the binding of NF-κB p65 to its DNA consensus sequences, and significantly decreased expression of TNF-α in activated microglia. Results suggest that inhibition of the inflammatory repertoire of microglia, neuroprotection, and increased macrophage scavenger receptor type A expression induced by Rg3 may at least partly explain its therapeutic effects in chronic neurodegenerative diseases (Joo et al. 2008). In addition, the effect of ginsenoside Rg3 on the metabolism of Abeta40 and Abeta42 was investigated in SK-N-SH cells transfected with Swedish mutant β-amyloid precursor protein (Yang et al. 2009). The results of enzyme-linked immunosorbent assay (ELISA) and Western blot analysis showed that Rg3 significantly lowered levels of Abeta40 and Abeta42, leading to the suggestion that Rg3 would be useful for treating patients suffering from Alzheimer's disease (Yang et al. 2009).

Pituitary adenylate cyclase-activating polypeptide (PACAP) is introduced as a neurotrophic factor to promote cell survival. Ginsenoside Rh2 stimulated PACAP gene expression and cell proliferation in type 1 rat brain astrocytes (RBA1) cells and ameliorated the RBA1 growth inhibition of Abeta. These results suggested that Rh2 can induce an increase in PACAP to activate PAC1 and thereby lead to attenuating Abeta-induced toxicity (Shieh et al. 2008). Thus, it is suggested that ginseng is useful in the prevention of age-related neurodegenerative diseases such as dementia.

To summarize this section, ginsenosides Rg1, Rb1, Rg3, and Rh2 were shown to be effective in potentiating learning and memory acquisition, enhancing releases of ACH and glutamate, inhibiting apoptosis, and protecting neurons from neurotoxic insults. Ginsenosides Rg3 and Rh2 were particularly effective in protecting CNS, preventing neurodegenerative diseases, and might also be useful in the treatment of Alzheimer's disease.

8.7 SUMMARY AND RESEARCH NEEDS

Ginseng has been widely used as a folk medicine in East Asian countries for thousands of years, mainly as a general tonic and adaptogen to maintain the body's resistance to adverse factors and homeostasis, including improving physical and sexual function, general vitality, and antiaging. Ginseng and ginsenosides seem to be beneficial for immunity, cancer, diabetes, CNS functions, and other conditions. Although a single ginsenoside is demonstrated to be beneficial regarding some effects or conditions, it remains to be determined whether a single component or mixtures

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of components derived from ginseng can maximize benefit across several diseases and conditions. Therefore, more research works concerning the structure–activity relationship between ginseng constituents, acting individually or synergistically in a mixture, are required for predicting and ensuring physiological and pharmacological efficacy. In addition, as many steps must be taken to standardize the usage of ginseng root through isolating specific ginsenosides, the formulated standardization of ginseng extract and ginsenoside isolation is clearly required to have constant results and desirable efficacy in animal and human experiments. Finally, large-scale, controlled clinical studies are needed to validate the results in terms of their applicability to humans to extend those reported experiments that have been performed using animal models.

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9 *Ganoderma lucidum* (Lingzhi or Reishi) *A Medicinal Mushroom*

Sissi Wachtel-Galor, John Yuen, John A. Buswell, and Iris F. F. Benzie

CONTENTS

9.1 Introduction 175
9.2 History: Lingzhi as a Medicinal Mushroom 176
9.3 Taxonomy 177
9.4 Cultivation, Global Use, and Manufacture of Products 178
9.5 Major Bioactive Components 179
9.5.1 Polysaccharides and Peptidoglycans 180
9.5.2 Triterpenes 181
9.5.3 Other Components 182
9.6 Therapeutic Applications 183
9.6.1 Cancer 183
9.6.1.1 Introduction 183
9.6.1.2 In Vitro Anticancer Activities 183
9.6.1.3 Animal Studies 185
9.6.1.4 Human Studies 186
9.6.2 Immunomodulation 186
9.6.3 Lingzhi as an Antioxidant 187
9.6.4 Viral and Bacterial Infections 188
9.6.5 Diabetes Mellitus 189
9.6.6 Liver and Gastric Injury 190
9.7 Concluding Remarks 191
Acknowledgments 192
References 192

9.1 INTRODUCTION

Ganoderma lucidum, an oriental fungus (Figure 9.1), has a long history of use for promoting health and longevity in China, Japan, and other Asian countries. It is a large, dark mushroom with a glossy exterior and a woody texture. The Latin word *lucidus* means "shiny" or "brilliant" and refers to the varnished appearance of the surface of the mushroom. In China, *G. lucidum* is called lingzhi, whereas in Japan the name for the Ganodermataceae family is reishi or mannentake.

In Chinese, the name lingzhi represents a combination of spiritual potency and essence of immortality, and is regarded as the "herb of spiritual potency," symbolizing success, well-being, divine power, and longevity. Among cultivated mushrooms, *G. lucidum* is unique in that its pharmaceutical rather than nutritional value is paramount. A variety of commercial *G. lucidum* products

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FIGURE 9.1 (See color insert.) The lingzhi mushroom (*Ganoderma lucidum*). (Courtesy of North American Reishi/Nammex. With permission.)

are available in various forms, such as powders, dietary supplements, and tea. These are produced from different parts of the mushroom, including mycelia, spores, and fruit body. The specific applications and attributed health benefits of lingzhi include control of blood glucose levels, modulation of the immune system, hepatoprotection, bacteriostasis, and more. The various beliefs regarding the health benefits of *G. lucidum* (Figure 9.2) are based largely on anecdotal evidence, traditional use, and cultural mores. However, recent reports provide scientific support to some of the ancient claims of the health benefits of lingzhi.

9.2 HISTORY: LINGZHI AS A MEDICINAL MUSHROOM

Lingzhi has been recognized as a medicinal mushroom for over 2000 years, and its powerful effects have been documented in ancient scripts (Wasser 2005). The proliferation of *G. lucidum* images in art began in 1400 AD, and they are associated with Taoism (McMeekin 2005). However, *G. lucidum* images extended beyond religion and appeared in paintings, carvings, furniture, and even women's accessories (Wasser 2005). The first book wholly devoted to the description of herbs and their medicinal value was *Shen Nong Ben Cao Jing*, written in the Eastern Han dynasty of China (25–220 AD). This book is also known as "Classic of the Materia Medica" or "Shen-nong's Herbal Classics." It describes botanical, zoological, and mineral substances, and was composed in the second century under the pseudonym of Shen-nong ("the holy farmer"; Zhu, 1998). The book, which has been continually updated and extended, describes the beneficial effects of several mushrooms with a reference to the medicinal mushroom *G. lucidum* (Zhu, 1998; Upton 2000; Sanodiya et al. 2009). In the *Supplement to Classic of Materia Medica* (502–536 AD) and the *Ben Cao Gang Mu* by Li Shin-Zhen, which is considered to be the first pharmacopoeia in China (1590 AD; Ming dynasty), the mushroom was attributed with therapeutic properties, such as tonifying effects, enhancing vital energy, strengthening cardiac function, increasing memory, and antiaging effects. According to the *State Pharmacopoeia of the People's Republic of China* (2000), *G. lucidum* acts to replenish Qi, ease the mind, and relieve cough and asthma, and it is recommended for dizziness, insomnia, palpitation, and shortness of breath.

Wild lingzhi is rare, and in the years before it was cultivated, only the nobility could afford it. It was believed that the sacred fungus grew in the home of the immortals on the "three aisles of the

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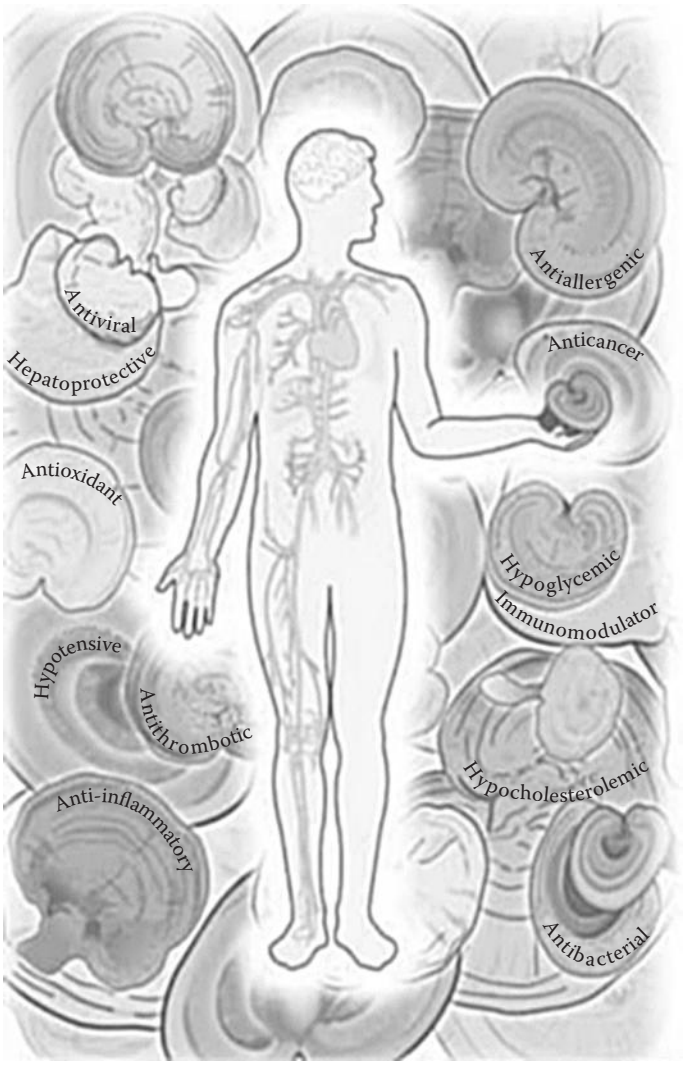


FIGURE 9.2 Postulated health benefits of lingzhi (*Ganoderma lucidum*).

blest" off the coast of China (McMeekin 2005). However, its reputation as a panacea may have been earned more by virtue of its irregular distribution, rarity, and use by the rich and privileged members of Chinese society than by its actual effects. Nevertheless, the *Ganoderma* species continue to be a popular traditional medicine in Asia and their use is growing throughout the world (Wachtel-Galor, Buswell et al. 2004; Lindequist, Niedermeyer, and Jülich 2005).

9.3 TAXONOMY

The family Ganodermataceae describes polypore basidiomycetous fungi having a double-walled basidiospore (Donk 1964). In all, 219 species within the family have been assigned to the genus *Ganoderma*, of which *G. lucidum* (W. Curt.: Fr.) P. Karsten is the species type (Moncalvo 2000). Basidiocarps of this genus have a laccate (shiny) surface that is associated with the presence of thick-walled pilocystidia embedded in an extracellular melanin matrix (Moncalvo 2000). *Ganoderma* species are found all over the world, and different characteristics, such as shape and color (red, black,

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blue/green, white, yellow, and purple) of the fruit body, host specificity, and geographical origin, are used to identify individual members of the species (Zhao and Zhang 1994; Woo et al. 1999; Upton 2000). Unfortunately, the morphological characteristics are subject to variation resulting from, for example, differences in cultivation in different geographical locations under different climatic conditions and the natural genetic development (e.g., mutation, recombination) of individual species. Consequently, the use of macroscopic characteristics has resulted in a large number of synonyms and a confused, overlapping, and unclear taxonomy for this mushroom. Some taxonomists also consider macromorphological features to be of limited value in the identification of *Ganoderma* species due to its high phenotypic plasticity (Ryvarden 1994; Zhao and Zhang 1994). More reliable morphological characteristics for *Ganoderma* species are thought to include spore shape and size, context color and consistency, and the microanatomy of the pilear crust. Chlamydospore production and shape, enzymatic studies and, to a lesser extent, the range and optima of growth temperatures have also been used for differentiating morphologically similar species (Gottlieb, Saidman, and Wright 1998; Moncalvo 2000; Saltarelli et al. 2009). Biochemical, genetic, and molecular approaches have also been used in *Ganoderma* species taxonomy. Molecular-based methodologies adopted for identifying *Ganoderma* species include recombinant (rDNA) sequencing (Moncalvo et al. 1995; Gottlieb, Ferref, and Wright 2000), random amplified polymorphic DNA-PCR (RAPD; PCR stands for polymerase chain reaction), internal transcribed spacer (ITS) sequences (Hseu et al. 1996), sequence-related amplified polymorphism (SRAP; Sun et al. 2006), enterobacterial repetitive intergenic consensus (ERIC) elements, and amplified fragment length polymorphism (AFLP; Zheng et al. 2009). Other approaches to the problem of *G. lucidum* taxonomy include nondestructive near-infrared (NIR) methods combined with chemometrics (Chen et al. 2008), nuclear magnetic resonance (NMR)-based metabolomics (Wen et al. 2010), and high-performance liquid chromatography (HPLC) for generating chemical fingerprints (Su et al. 2001; Chen et al. 2008; Shi, Zhang et al. 2008; Chen et al. 2010).

9.4 CULTIVATION, GLOBAL USE, AND MANUFACTURE OF PRODUCTS

Owing to its irregular distribution in the wild and to an increasing demand for *G. lucidum* as a medicinal herb, attempts were made to cultivate the mushroom (Chang and Buswell 2008). Different members of the *Ganoderma* genus need different conditions for growth and cultivation (Mayzumi, Okamoto, and Mizuno 1997). Moreover, different types are favored in different geographical regions. For example, in South China, black *G. lucidum* is popular, whereas red *G. lucidum* is preferred in Japan. *G. lucidum* thrives under hot and humid conditions, and many wild varieties are found in the subtropical regions of the Orient. Since the early 1970s, cultivation of *G. lucidum* has become a major source of the mushroom. Artificial cultivation of *G. lucidum* has been achieved using substrates such as grain, sawdust, wood logs (Chang and Buswell 1999; Wasser 2005; Boh et al. 2007), and cork residues (Riu, Roig, and Sancho 1997).

Since it takes several months to culture the fruiting body of *G. lucidum*, mycelia-based and culture broth–based products have assumed greater importance due to demands for increased quality control and year-round production (Sanodiya et al. 2009). The processes and different growth parameters (e.g., temperature, pH) involved in submerged mycelial culture can easily be standardized under controlled conditions, and purification and other downstream processing of active components such as polysaccharides released into the culture medium usually involve relatively simple procedures. Different culture conditions and medium compositions have also been reported to strongly influence mycelial growth and the production of biopolymers (e.g., polysaccharides) that are extruded from the cell (exopolysaccharides [EPSs]; Mayzumi, Okamoto, and Mizuno 1997; Chang and Buswell 1999; Habijanic and Berovic 2000; Fang and Zhong 2002; Boh et al. 2007; Sanodiya et al. 2009). For example, Yang and Liau (1998) reported that polysaccharide production by fermenter-grown mycelia of *G. lucidum* was optimum at 30°C–35°C and a pH of 4–4.5, and the addition of supplements such as fatty acids was found to accelerate mycelial growth and the

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production of bioactive components. In a submerged culture of *G. lucidum*, the optimum pH for cell growth has been shown to be lower than that for EPS formation. A two-stage pH-control strategy, developed to maximize mycelial biomass and EPS production, revealed that culture pH had a significant effect on EPS yield, chemical composition and molecular weight, and mycelial morphology (Kim, Park, and Yun 2006). The productive mycelial morphological form for EPS production was a dispersed pellet (controlled pH shift from 3.0 to 6.0) rather than a compact pellet with a dense core (pH maintained at 4.5) or a featherlike pellet (controlled pH shift from 6.0 to 3.0). Three different polysaccharides were obtained under each pH condition, and their molecular weights and chemical compositions were significantly different (Kim, Park, and Yun 2006). More recently, a novel three-stage light irradiation strategy has been developed in submerged cultures of *G. lucidum* for the efficient production of polysaccharides and one of the triterpene components, ganoderic acid (Zhang and Tang 2008).

A decade ago, more than 90 brands of *G. lucidum* products were registered and marketed internationally (Lin 2000). Worldwide consumption is now estimated at several thousand tonnes, and the market is growing rapidly. Although there are no recently published data relating to the total world market value of ganoderma products, in 1995, the total estimated annual market value given by different commercial sources was US$1628 million (Chang and Buswell 1999). Numerous *G. lucidum* products, prepared from different parts of the mushroom, are currently available on the market (Chang and Buswell 2008). In manufacturing terms, the simplest type consists of intact fruiting bodies ground to powder and then processed to capsule or tablet form. Other "nonextracted" products are prepared from the following three sources: (1) dried and powdered mycelia harvested from submerged liquid cultures grown in fermentation tanks; (2) dried and powdered combinations of substrate, mycelia, and mushroom primordia, following inoculation and incubation of a semisolid medium with fungal mycelia; and (3) intact fungal spores or spores that have been broken by mechanical means or have had the spore walls removed. Although spore preparations have been researched and promoted vigorously in recent years, any added medicinal effects attributable to the removal or breakage of spore walls, which represents an additional and often costly step in the production process, are still controversial. Other products are prepared with materials (e.g., polysaccharides, triterpenes) extracted, usually with hot water or ethanol, from fruiting bodies or mycelia harvested from submerged liquid cultures and then evaporated to dryness and tabulated/encapsulated either separately or integrated together in designated proportions. The adoption of supercritical fluid CO_2 extraction technologies has enlarged the spectrum of extracted substances due to the low temperature required during processing. Several other products have been prepared as binary, ternary or more complex mixtures of powdered ganoderma and other mushrooms (e.g., *Lentinula edodes*, *Agaricus brasiliensis*, *Grifola frondosa*, *Pleurotus* spp., and *Flammulina velutipes*) and even with other medicinal herbs (e.g., spirulina powder or flower pollen grains).

9.5 MAJOR BIOACTIVE COMPONENTS

Most mushrooms are composed of around 90% water by weight. The remaining 10% consists of 10–40% protein, 2–8% fat, 3–28% carbohydrate, 3–32% fiber, 8–10% ash, and some vitamins and minerals, with potassium, calcium, phosphorus, magnesium, selenium, iron, zinc, and copper accounting for most of the mineral content (Borchers et al. 1999). In a study of the nonvolatile components of *G. lucidum*, it was found that the mushroom contains 1.8% ash, 26–28% carbohydrate, 3–5% crude fat, 59% crude fiber, and 7–8% crude protein (Mau, Lin, and Chen 2001).

In addition to these, mushrooms contain a wide variety of bioactive molecules, such as terpenoids, steroids, phenols, nucleotides and their derivatives, glycoproteins, and polysaccharides. Mushroom proteins contain all the essential amino acids and are especially rich in lysine and leucine. The low total fat content and high proportion of polyunsaturated fatty acids relative to the total fatty acids of mushrooms are considered significant contributors to the health value of mushrooms (Chang and Buswell 1996; Borchers et al. 1999; Sanodiya et al. 2009).

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Polysaccharides, peptidoglycans, and triterpenes are three major physiologically active constituents in *G. lucidum* (Boh et al. 2007; Zhou et al. 2007). However, the amount and percentage of each component can be very diverse in natural and commercial products, as exemplified by the data shown in Table 9.1. When 11 randomly selected samples of commercial lingzhi products purchased in Hong Kong shops were evaluated for the two major active components, triterpenes and polysaccharides, it was found that the triterpene content ranged from undetectable to 7.8% and the polysaccharide content varied from 1.1–5.8% (Chang and Buswell 2008). Such variations can occur for several reasons, including differences in the species or strains of mushroom used and differences in production methods.

9.5.1 Polysaccharides and Peptidoglycans

Fungi are remarkable for the variety of high-molecular-weight polysaccharide structures that they produce, and bioactive polyglycans are found in all parts of the mushroom. Polysaccharides represent structurally diverse biological macromolecules with wide-ranging physiochemical properties (Zhou et al. 2007). Various polysaccharides have been extracted from the fruit body, spores, and mycelia of lingzhi; they are produced by fungal mycelia cultured in fermenters and can differ in their sugar and peptide compositions and molecular weight (e.g., ganoderans A, B, and C). *G. lucidum* polysaccharides (GL-PSs) are reported to exhibit a broad range of bioactivities, including anti-inflammatory, hypoglycemic, antiulcer, antitumorigenic, and immunostimulating effects (Miyazaki and Nishijima 1981; Hikino et al. 1985; Tomoda et al. 1986; Bao et al. 2001; Wachtel-Galor, Buswell et al. 2004). Polysaccharides are normally obtained from the mushroom by extraction with hot water followed by precipitation with ethanol or methanol, but they can also be extracted with water and alkali. Structural analyses of GL-PSs indicate that glucose is their major sugar component (Bao et al. 2001; Wang et al. 2002). However, GL-PSs are heteropolymers and can also contain xylose, mannose, galactose, and fucose in different conformations, including 1–3, 1–4, and 1–6-linked β and α-D (or L)-substitutions (Lee, Lee, and Lee 1999; Bao et al. 2002). Branching conformation and solubility characteristics are said to affect the antitumorigenic properties of these polysaccharides (Bao et al. 2001; Zhang, Zhang,

TABLE 9.1
Comparison of Triterpene and Polysaccharide Contents of 11 Commercial Lingzhi (*G. lucidum*) Products Currently Available on the Market

Nature of Product	Triterpenes (%)	Polysaccharide (%)
A (fruit body extract)	1.36	4.48
B (fruit body extract)	2.36	5.32
C (fruit body extract)	1.88	15.70
D (fruit body extract)	1.06	10.97
E (fruit body extract)	0.44	7.51
F (fruit body extract)	1.78	6.18
G (fruit body extract)	1.44	13.30
H (fruit body extract)	0.50	15.80
I (fruit body extract)	7.82	7.66
J (fruit body powder)	0.46	1.10
K (mycelium powder)	Undetectable	12.78

Source: Reproduced from Chang, S. T., and J. A. Buswell. 2008. Safety, quality control and regulational aspects relating to mushroom nutriceuticals. *Proc. 6th Intl.* Conf. *Mushroom Biology and Mushroom Products*, 188–95. Krefeld, Germany: GAMU Gmbh. With permission.

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and Chen 2001). The mushroom also consists of a matrix of the polysaccharide chitin, which is largely indigestible by the human body and is partly responsible for the physical hardness of the mushroom (Upton 2000). Numerous refined polysaccharide preparations extracted from *G. lucidum* are now marketed as over-the-counter treatment for chronic diseases, including cancer and liver disease (Gao et al. 2005).

Various bioactive peptidoglycans have also been isolated from *G. lucidum*, including *G. lucidum* proteoglycan (GLPG; with antiviral activity; Li, Liu and Zhao 2005), *G. lucidum* immunomodulating substance (GLIS; Ji et al. 2007), PGY (a water-soluble glycopeptide fractionated and purified from aqueous extracts of *G. lucidum* fruit bodies; Wu and Wang 2009), GL-PS peptide (GL-PP; Ho et al. 2007), and F3 (a fucose-containing glycoprotein fraction; Chien et al. 2004).

9.5.2 Triterpenes

Terpenes are a class of naturally occurring compounds whose carbon skeletons are composed of one or more isoprene C_5 units. Examples of terpenes are menthol (monoterpene) and β-carotene (tetraterpene). Many are alkenes, although some contain other functional groups, and many are cyclic. These compounds are widely distributed throughout the plant world and are found in prokaryotes as well as eukaryotes. Terpenes have also been found to have anti-inflammatory, antitumorigenic, and hypolipidemic activity. Terpenes in *Ginkgo biloba*, rosemary (*Rosemarinus officinalis*), and ginseng (*Panax ginseng*) are reported to contribute to the health-promoting effects of these herbs (Mahato and Sen 1997; Mashour, Lin, and Frishman 1998; Haralampidis, Trojanowska, and Osbourn 2002).

Triterpenes are a subclass of terpenes and have a basic skeleton of C_{30}. In general, triterpenoids have molecular weights ranging from 400 to 600 kDa and their chemical structure is complex and highly oxidized (Mahato and Sen 1997; Zhou et al. 2007). Many plant species synthesize triterpenes as part of their normal program of growth and development. Some plants contain large quantities of triterpenes in their latex and resins, and these are believed to contribute to disease resistance. Although hundreds of triterpenes have been isolated from various plants and terpenes as a class have been shown to have many potentially beneficial effects, there is only limited application of triterpenes as successful therapeutic agents to date. In general, very little is known about the enzymes and biochemical pathways involved in their biosynthesis.

In *G. lucidum*, the chemical structure of the triterpenes is based on lanostane, which is a metabolite of lanosterol, the biosynthesis of which is based on cyclization of squalene (Haralampidis, Trojanowska, and Osbourn 2002). Extraction of triterpenes is usually done by means of methanol, ethanol, acetone, chloroform, ether, or a mixture of these solvents. The extracts can be further purified by various separation methods, including normal and reverse-phase HPLC (Chen et al. 1999; Su et al. 2001). The first triterpenes isolated from *G. lucidum* are the ganoderic acids A and B, which were identified by Kubota et al. (1982). Since then, more than 100 triterpenes with known chemical compositions and molecular configurations have been reported to occur in *G. lucidum*. Among them, more than 50 were found to be new and unique to this fungus. The vast majority are ganoderic and lucidenic acids, but other triterpenes such as ganoderals, ganoderiols, and ganodermic acids have also been identified (Nishitoba et al. 1984; Sato et al. 1986; Budavari 1989; Gonzalez et al. 1999; Ma et al. 2002; Akihisa et al. 2007; Zhou et al. 2007; Jiang et al. 2008; Chen et al. 2010). Examples of triterpenes are shown in Figure 9.3.

G. lucidum is clearly rich in triterpenes, and it is this class of compounds that gives the herb its bitter taste and, it is believed, confers on it various health benefits, such as lipid-lowering and antioxidant effects. However, the triterpene content is different in different parts and growing stages of the mushroom. The profile of the different triterpenes in *G. lucidum* can be used to distinguish this medicinal fungus from other taxonomically related species, and can serve as supporting evidence for classification. The triterpene content can also be used as a measure of quality of different ganoderma samples (Chen et al. 1999; Su et al. 2001)

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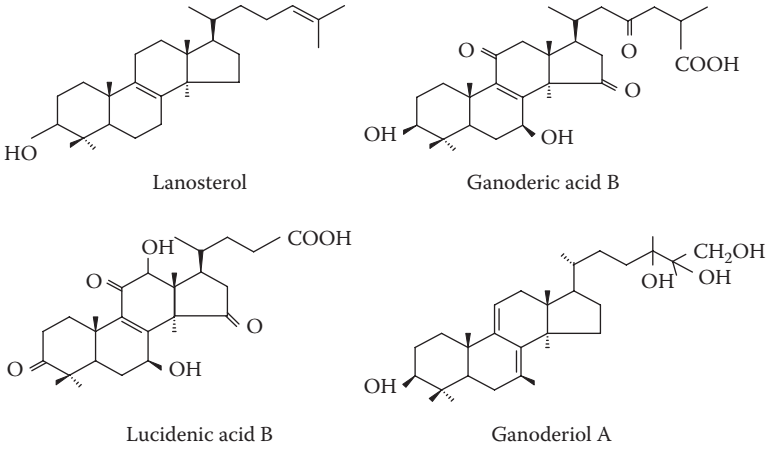


FIGURE 9.3 Chemical structure of lanosterol and three of the many triterpenes isolated from *Ganoderma lucidum*. (From Kubota, T., Y. Asaka, I. Miura, and H. Mori. 1982. *Helv Chim Acta* 65:611–9; Nishitoba, T., H. Sato, T. Kasai, H. Kawagishi, and S. Sakamura. 1984. *Agric Biol Chem* 48:2905–7; Sato, H., T. Nishitoba, S. Shirasu, K. Oda, and S. Sakamura. 1986. *Agric Biol Chem* 50:2887–90; Budavari, S. 1989. *The Merck Index*. 11 ed, 845. New Jersey: Merck & Co., INC. With permission.)

9.5.3 Other Components

Elemental analysis of log-cultivated fruit bodies of *G. lucidum* revealed phosphorus, silica, sulfur, potassium, calcium, and magnesium to be their main mineral components. Iron, sodium, zinc, copper, manganese, and strontium were also detected in lower amounts, as were the heavy metals lead, cadmium, and mercury (Chen et al. 1998). Freeze-dried fruit bodies of unidentified *Ganoderma* spp. collected from the wild were reported to have a mineral content of 10.2%, with potassium, calcium, and magnesium as the major components (Chiu et al. 2000). Significantly, no cadmium or mercury was detected in these samples. *G. lucidum* can also contain up to 72 μg/g dry weight of selenium (Se; Falandysz 2008) and can biotransform 20–30% of inorganic selenium present in the growth substrate into selenium-containing proteins (Du et al. 2008).

Some attention has been given to the germanium content of *Ganoderma* spp. Germanium was fifth highest in terms of concentration (489 μg/g) among the minerals detected in *G. lucidum* fruit bodies collected from the wild (Chiu et al. 2000). This mineral is also present in the order of parts per billion in many plant-based foods, including ginseng, aloe, and garlic (Mino et al. 1980). Although germanium is not an essential element, at low doses, it has been credited with immunopotentiating, antitumor, antioxidant, and antimutagenic activities (Kolesnikova, Tuzova, and Kozlov 1997). However, although the germanium content of *G. lucidum* has been used to promote *G. lucidum*-based products, there is no firm evidence linking this element with the specific health benefits associated with the mushroom.

G. lucidum contains some other compounds that may contribute to its reported medicinal effect, such as proteins and lectins. The protein content of dried *G. lucidum* was found to be around 7–8%, which is lower than that of many other mushrooms (Chang and Buswell 1996; Mau, Lin, and Chen 2001). Bioactive proteins are reported to contribute to the medicinal properties of *G. lucidum*, including LZ-8, an immunosuppressive protein purified from the mycelia (Van Der Hem et al. 1995); a peptide preparation (GLP) exhibiting hepatoprotective and antioxidant activities (Sun, He, and Xie 2004; Shi, Sun et al. 2008); and a 15-kDa antifungal protein, ganodermin, which is isolated from *G. lucidum* fruiting bodies (Wang and Ng. 2006).

The carbohydrate and crude fiber content of the dried mushroom was examined and found to be 26–28% and 59%, respectively (Mau, Lin, and Chen 2001). Lectins were also isolated from the fruit body and mycelium of the mushroom (Kawagishi et al. 1997), including a novel 114-kDa

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hexameric lectin, which was revealed to be a glycoprotein having 9.3% neutral sugar and showing hemagglutinating activity on pronase-treated human erythrocytes (Thakur et al. 2007). Lectins (from the Latin word *legere*, which means to pick up, choose) are nonenzymatic proteins or glycoproteins that bind carbohydrates. Many species of animals, plants, and microorganisms produce lectins, and they exhibit a wide range of functions. In animals, for example, lectins are involved in a variety of cellular processes and the functioning of the immune system (Wang, Ng, and Ooi 1998).

Other compounds that have been isolated from *G. lucidum* include enzymes such as metalloprotease, which delays clotting time; ergosterol (provitamin D_2); nucleosides; and nucleotides (adenosine and guanosine; Wasser 2005; Paterson 2006). Kim and Nho (2004) also described the isolation and physicochemical properties of a highly specific and effective reversible inhibitor of α-glucosidase, SKG-3, from *G. lucidum* fruit bodies. Furthermore, *G. lucidum* spores were reported to contain a mixture of several long-chain fatty acids that may contribute to the antitumor activity of the mushroom (Fukuzawa et al. 2008).

9.6 THERAPEUTIC APPLICATIONS

The combination of benefit without toxicity represents the desired end result in the development of effective therapeutic interventions. *G. lucidum* has been used for hundreds of years as a health promotion and treatment strategy; there are now many published studies that are based on animal and cell-culture models and on in vitro assessment of the health effects of *G. lucidum*, and there are also some reports of human trials in the field. However, there is no cohesive body of research, and the objective evaluation of this traditional therapy in terms of human health remains to be clearly established. In Sections 9.6.1 through 9.6.6, studies on the properties of *G. lucidum* in relation to cancer (which has attracted the most interest), viral and bacterial infection, diabetes, and liver injury are discussed.

9.6.1 Cancer

G. lucidum is a popular supplement taken by healthy individual to boost the immune system and by cancer patients along with conventional therapies. In this section, the scientific studies of *G. lucidum* on its anticancer properties are summerized.

9.6.1.1 Introduction

Cancer is a worldwide leading cause of death, and despite comprehensive advances in the early diagnosis of the disease and chemotherapy, it remains a major clinical challenge (WHO 2008). As part of searching for new chemopreventive and chemotherapeutic agents, hundreds of plant species, including mushrooms, have been evaluated. This has resulted in the isolation of thousands of bioactive molecules that were shown to have antitumor activity from numerous mushroom species, including *Ganoderma* species (Wasser and Weis 1999; Borchers et al. 2008). In *G. lucidum*, a large number of chemical compounds can be extracted from the fruiting body, mycelia, or spores. Many polysaccharides and triterpenes, the two major groups of components in the mushroom, exhibit chemopreventive and/or tumoricidal effects, as proved by numerous studies from in vitro experiments and animal and human in vivo studies (Yuen and Gohel 2005; Zaidman et al. 2005). Tumor-implanted animal models have shown inhibitory effects on angiogenesis and metastasis. However, evidence from well-designed human trials is still scarce.

9.6.1.2 In Vitro Anticancer Activities

Tomasi et al. (2004) tested 58 basidiomycetes mushrooms, of which *G. lucidum* was shown to be the most effective in killing cancer cells. *G. lucidum* induced cell-cycle arrest and apoptosis in various human and rodent tumor cells, including murine lymphocytic leukemia L1210 and Lewis lung carcinoma (LLC; Min et al. 2000; Tomasi et al. 2004), mouse reticulocyte sarcoma L-II (Liu et al. 2002), murine sarcoma Meth-A (Min et al. 2000; Gao, Min et al. 2002) and S180

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(Gao, Min et al. 2002; Liu et al. 2002), human leukemia HL-60 (Muller et al. 2006; Kim et al. 2007; Fukuzawa et al. 2008; Liu et al. 2009) and U937, K562, Blin-1, Nalm-6, RPMI8226 (Muller et al. 2006; Shang et al. 2009), human hepatoma PLC/PRF/5, KB (Lin et al. 2003), HepG2 (Liu et al. 2009; Weng et al. 2009), Hep3B (Chung et al. 2001), Huh-7 (Lin et al. 2003; Li, Chan et al. 2005), human liver tumor SMMC7721 (Tang et al. 2006), human breast cancer MDA-MB-123 (Jiang et al. 2008; Liu et al. 2009; Zhao et al. 2010), MCF-7 (Jiang, Slivova, and Sliva 2006; Liu et al. 2009; Shang et al. 2009), T-47D (Gao, Min et al. 2002) and MT-1 (Wu et al. 2006; Xie et al. 2009), human prostate cancer PC-3 (Jiang et al. 2004; Evans et al. 2009), human cervix uteri tumor Hela (Liu et al. 2002; Tang et al. 2006; Shang et al. 2009), human ovarian cancer SKOV4 (Shang et al. 2009), human colonic cancer HT-29 (Hong et al. 2004) and SW480 (Xie et al. 2006), human lung carcinoma PG (Cao and Lin 2006; Cao, Lin, and Wang 2007) and 95-D (Tang et al. 2006), human small-cell lung carcinoma NCI-H69 and multidrug-resistant strain VPA (Sadava et al. 2009), low-grade bladder cancer MTC-11 (Lu et al. 2004), and human uroepithelial HUC-PC (Yuen, Gohel, and Au 2008) cells.

Through the regulation of expression of different signals, tumor cells were arrested by *G. lucidum* at different points of cell cycle, for example, breast at G0/G1 phase; lung at G1 phase; liver at G1/G2 phase; and bladder, prostate, and leukemia at G2 phase. A selenium-enriched extract of *G. lucidum* mycelia was shown to induce G1/S phase arrest in human erythroid chronic myeloid leukemia K562 cells (Shang et al. 2009). Another extract induced G0/G1 phase arrest in estrogen-dependent breast MCF-7 cells through the downregulation of estrogen-α receptor and serine/threonine-specific protein kinase Akt/nuclear factor κB (NF-κB) signaling (Jiang, Slivova, and Sliva 2006). In various human cancer cell lines, extracts of *G. lucidum* were shown to suppress the progression of the G1 phase in cell cycle, and apoptosis was confirmed by using terminal deoxynucleotidyl transferase dUTP nick and labeling (TUNEL) assay (Liu et al. 2009). Many of these activities were accompanied by apoptosis. Cao and Lin (2006) demonstrated that a fraction of GL-PP decreased the anti-apoptotic protein Bcl-2 expression and increased the proapoptotic protein Bax expression in human umbilical cord vascular endothelial cells (HUVECs). A triterpene-rich extract from *G. lucidum* induced progressive apoptosis in the premalignant HUC-PC cell line by increasing the early apoptosis marker annexin-V within 3 hours. Half the cells stained positive for 7-amino-actinomycin D (indicating late apoptosis) after 8 hours. All cells were dead at 24 hours, and this was associated with the downregulation of telomerase (Yuen, Gohel, and Au 2008). Similar apoptotic activities were also demonstrated in other human cancer cells (Fukuzawa et al. 2008). An ethanol extract of *G. lucidum* decreased cyclooxygenase 2 (COX)-2 enzyme expression and increased nitric oxide synthesis in colon HT-29 cells (Hong et al. 2004). In lung 95-D tumor cells, the pure compound ganoderic acid T caused mitochondrial dysfunction, which resulted from the upregulation of pro-apoptotic p53 and Bax expression (Tang et al. 2006). Moreover, the use of a combination of *G. lucidum* and *Duschesnea* extracts upregulated cytochrome *c* and Bax translocation to trigger caspase-3 apoptosis in leukemia HL-60 cells (Kim et al. 2007). Activation of caspases-7 and -9 by *G. lucidum* has been demonstrated in breast MCF-7 and lung H69-SCLC cancer cells, respectively (Hu et al. 2002; Sadava et al. 2009). In hepatoma HepG2 cells, a lucidenic acid–rich *G. lucidum* extract was shown to suppress phosphorylation of ERK1/2 and Akt signaling, which downregulated their downstream NF-κB and proto-oncoproteins (c-Jun and c-Fos) activities, favoring apoptosis (Weng et al. 2009).

A tumor mass requires a continuous nutrient supply via new blood vessels formed by the process of angiogenesis. Invasive cancer cells spread to distant sites through blood and lymphoid vessels. Therefore, agents that inhibit angiogenesis inhibit tumor growth and spread. The potential antiangiogenic activities of *G. lucidum* have been demonstrated in ex vivo chick embryo chorioallantoic membrane (CAM) assay (Cao and Lin 2004; Song et al. 2004). Polysaccharide peptide and ethanol extract from *G. lucidum* has been proved to decrease microvessels around a microfiber filter disc containing an embryo with intact yolks. Using a prostate cancer cell line, two angiogenic factors, known as vascular endothelial growth factor (VEGF) and transforming growth factor (TGF)-β1,

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were suppressed by *G. lucidum* through inhibition of the ras/extracellular signal–regulated kinase (Erk1/2) and Akt signaling pathways (Johnston 2005; Stanley et al. 2005). Similar effects were also observed in a human lung cancer cell lines under hypoxic conditions after exposure to a high dose of GL-PP (Cao and Lin 2006).

Cell adhesion, invasion, and migration are the key factors in determining the aggressiveness of cancer; hence, control of cell motility is effective in avoiding cancer metastasis. A polysaccharide extract of *G. lucidum* mycelia inhibited the formation of oncogenic ras-induced transformed foci in an R6 embryo fibroblast cell line (Hsiao et al. 2004). Spores and the fruiting body of *G. lucidum* were shown to inhibit the regulatory proteins phosphatidylinositol and NF-κB in highly invasive breast and prostate cancer cells (Sliva et al. 2002). Cell adhesion, invasion, and colony formation of breast cancer cells were significantly inhibited on exposure to *G. lucidum* extracts (Sliva 2004). In addition, Lu et al. (2004) demonstrated that water and ethanol extracts of *G. lucidum* modulated the F/G-actin ratio, which, in turn, reduced the formation of stress fiber and focal adhesion complexes of bladder cancer cells, suggesting the actin remodeling was associated with the inhibition of carcinogen-induced cell migration. Inhibition of mitogen-induced invasion of HepG2 cells was demonstrated in a study by using Matrigel-coated filter inserts assay (Weng et al. 2009).

9.6.1.3 Animal Studies

Rodent studies of possible antitumorigenic effects of *G. lucidum* can be traced back to the early 1980s. Ten days of intraperitoneal (i.p.) injections of a polysaccharide fraction (GL-1) from the fruit body was reported to inhibit (by 95–98%) the growth of transplanted sarcoma 180 tumor cells in mice (Miyazaki and Nishijima 1981). A complex of polysaccharides and protein from the mushroom was also found to show significant antitumor activity in a similar study conducted by Kim et al. (1980). An inhibition rate of 88% was reported, and there was complete regression of tumor in a third of the test animals. In a study conducted by Hyun, Choi, and Kim (1990), which used a similar protocol but used various extracted polysaccharides, inhibition rates of 52–81% were found. A hot water extract (2 mg/mouse) given i.p. for 3 days resulted in an average 74% inhibition of tumor growth in mice, with 3 out of 10 animals showing complete regression, and an oral administration (daily for 5 weeks) showed 45–63% inhibition (Ohno et al. 1998). Similar inhibitory effects were shown with implanted sarcoma 180 cells after polysaccharide was given orally to mice (Zhang and Lin 1999). A pure β-(1→3) glucan was tested in parallel with crude *G. lucidum* extracts, which resulted in 90% inhibition of tumor growth (Ohno et al. 1998). A dry powder preparation of the antlered form of *G. lucidum* (known as deerhorn lingzhi due to its appearance) was shown to inhibit tumor growth and elongate the life span in both allogeneic sarcoma-180-bearing ddY mice and synergenic MM-46 mammary tumor–bearing C3H/He mice (Nonaka et al. 2006).

G. lucidum is a major component of many traditional botanical formulations, such as TBS-101, which was demonstrated to inhibit tumor growth and invasion in PC-3-implanted mice (Evans et al. 2009). Yun (1999) reported that 9 weeks of oral administration of mycelial extract significantly inhibited lung adenoma formation in mice. Oral administration of triterpenoid fractions for 18 consecutive days inhibited Martigel-induced angiogenesis, which significantly reduced tumor weight and the number of tumor cell colonies that had metastasized to the liver in female C57BL/6J strain mice with intrasplenic implantation of Lewis lung cancer cells (Kimura, Taniguchi, and Baba 2002; Wang et al. 2007). In male ICR-nu/nu nude mice injected with hepatoma HepG2 cells, daily oral administration of lucidenic acid–rich extract for 68 days (800 mg/kg dosage) decreased both the number and size of tumors by up to 99%, and also the number of metastatic tumors occurring in liver and lung (Weng et al. 2009). An aqueous extract (administered i.p. at 10, 20, and 40 mg/mouse) of the fruit body significantly increased the life span of mice implanted with Lewis lung carcinoma cells. However, no dose-response effect was seen (Furusawa et al. 1992). An additive effect was seen when *G. lucidum* was given in combination with cytotoxic antineoplastic drugs, and there was a suggestion of a possible synergistic effect with cisplatin (Furusawa et al. 1992). In another study, *G. lucidum* was found to also prolong the life span of tumor-transplanted mice by inhibiting metastasis to the lung (Lee et al.

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1995). When given 1 week prior to the administration of a carcinogenic agent, a hot water extract of the mycelium/growth medium complex decreased the development of aberrant crypt foci (ACF) and precancerous lesions in the colon (Lu et al. 2001; Lu et al. 2003). No toxicity or side effects were seen in the rats when the extract was administered for 3 months. When tested with mouse colon tumor–implanted chambers, a polysaccharide mixture containing isoflavone aglycons from cultured *G. lucidum* mycelia was found to inhibit angiogenesis in vivo (Miura et al. 2002).

The chemopreventive activities of the mushroom on prostate cancer were demonstrated by a triterpenoid-rich extract of *G. lucidum* that suppressed the ventral prostate growth induced by testosterone (Liu et al. 2007a). Ganoderol B was identified as the active principle that was able to bind to an androgen receptor and inhibit 5α-reductase, suppressing androgen-induced LNCaP cell growth and downregulating the prostate-specific antigen (Liu et al. 2007b).

9.6.1.4 Human Studies

In humans, whether the antitumor effect of lingzhi is a direct one or is mediated via effects on the immune system is a key question to address. *G. lucidum* is one of the eight components of an herbal mixture called "prostate cancer-hope" (known as PC-SEPS), which has been used as an alternative in the treatment of androgen-dependent and -independent prostate cancer (Gao and Zhou 2009). However, only a few clinical trials have used *G. lucidum* as a single agent on cancer patients (Gao, Zhou et al. 2002; Gao, Zhou et al. 2003; Gao, Sai et al. 2003). Two randomized, controlled trials have been conducted using a GL-PS-rich extract (a patented over-the-counter product, Ganopoly; Gao et al. 2003; Gao and Sai et al. 2003). Gao, Zhou et al. (2003) recruited 134 patients with advanced cancers of different sites and supplemented them with *G. lucidum* capsules at a dosage of 1800 mg/day for 12 weeks. Cellular immunity in 80% of these patients was significantly enhanced in terms of elevated plasma interleukin (IL)-2, IL-6, and interferon γ (IFN-γ) levels and natural killer (NK) cell activity. In another study, the same protocol was followed with 68 lung cancer patients (Gao, Sai et al. 2003) in whom immune parameters including total T cells, NK cells, and CD4/CD8 ratio were significantly enhanced in the *G. lucidum*–treated group. In addition, quality of life in terms of Karnofsky score was improved in about 65% of these patients (Gao, Sai et al. 2003). Ganopoly was also demonstrated to enhance mitogenic activity and NK cells in patients with advanced cancer in a before-and-after comparison study (Gao, Min et al. 2002). These results provide some evidence that the antitumor effects of *G. lucidum* are mediated via effects on the immune system. However, it must be noted that all studies were conducted by the same research group and that other direct antitumor effects of *G. lucidum* have not yet been studied on humans in vivo.

9.6.2 Immunomodulation

Agents that enhance the functioning of the host immune system could be expected to enhance health in terms of improved resistance and, thus, removal of malignant or premalignant cells. Many *G. lucidum* products on the market are labeled or promoted as immunomodulating agents.

There is considerable evidence to support the immunostimulating activities of *G. lucidum* via induction of cytokines and enhancement of immunological effector (Wang et al. 1997; Zhu and Lin 2006). Different components from *G. lucidum* were proved to enhance the proliferation and maturation of T and B lymphocytes, splenic mononuclear cells, NK cells, and dendritic cells in culture in vitro and in animal studies in vivo (Bao et al. 2001; Cao and Lin 2002; Zhu, Chen, and Lin 2007; Ma et al. 2008). In normal BALB/c mice, a polysaccharide-rich extract of *G. lucidum* promoted the proliferation of splenocytes and enhanced the activities of macrophages and NK cells, which resulted in the increase of IL-6 and IFN-γ (Chang et al. 2009). Although a commercial *G. lucidum* extract did not stimulate proliferation of lymphocytes, it activated the gene expression of IL-1β, IL-6, IL-10, and tumor necrosis factor (TNF)-α (Mao et al. 1999). A polysaccharide fraction (F3) was shown to enhance both adaptive and innate immunities by triggering the production of cytokines IL-1, IL-6, IL-12, IFN-γ, TNF-α, and colony stimulating factors (CSFs) from mouse

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splenocytes (Chen et al. 2004). It was reported also that TNF-α and IL-6 production were stimulated in human and murine macrophages by *G. lucidum* mycelia (Kuo et al. 2006). This effect might be due to increased synthesis of nitric oxide (NO) induced by β-D-glucan (Ohno et al. 1998). These polysaccharides were also found to be highly suppressive to tumor cell proliferation in vivo while enhancing the host's immune response (Ooi and Liu 2000).

Wang et al. (1997) found that a polysaccharide-enriched fraction from *G. lucidum* activated cultured macrophages and T lymphocytes in vitro, which led to an increase of IL-1β, TNF-α, and IL-6 in the culture medium. In another study (Zhang and Lin 1999), incubation of macrophages and T lymphocytes with a polysaccharide resulted in an increase in TNF-α and INF-γ levels in the culture medium. This "conditioned" culture medium was found to inhibit cell growth and induce apoptosis in sarcoma 180 and HL-60 cells (Zhang and Lin 1999). Furthermore, serum-incorporated treatment with a polysaccharide peptide fraction from *G. lucidum* markedly inhibited the proliferation of human lung carcinoma (PG) cells, whereas the pure fraction by itself did not induce similar effects (Cao and Lin 2004). In addition to polysaccharides, a lanostane triterpenoid, ganoderic acid Me, inhibited tumor growth and metastasis of Lewis lung carcinoma in "T helper 1 responder" C57BL/6 mice by enhancing immune function in terms of IL-2 and IFN-γ expression and NK cell activity (Wang et al. 2007). Zhu and Lin (2006) used cytokine-induced killer (CIK) cells to investigate the interaction between GL-PSs and cytokines, which mediated cell proliferation and antitumor activity. The cytotoxicity of CIK cells was correlated well with the expression of perforin and granzyme B induced by IL-2 and anti-CD3. Results indicated that GL-PSs enhance IL-2 and TNF-α production as well as protein and messenger ribonucleic acid (mRNA) expression of granzyme B and perforin in CIK cells culture, and thus decrease the doses of IL-2 and anti-CD3 without affecting the killing effects on NK-resistant mouse P815 mastocytoma cells and NK-sensitive mouse YAC-1 lymphoma cells (Zhu and Lin 2006).

9.6.3 Lingzhi as an Antioxidant

Consumption of antioxidant-rich plants may help prevent cancer and other chronic diseases (Collins 2005; Benzie and Wachtel-Galor 2009). Antioxidants protect cellular components from oxidative damage, which is likely to decrease risk of mutations and carcinogenesis and also protect immune cells, allowing them to maintain immune surveillance and response. Various components of *G. lucidum*, in particular polysaccharides and triterpenoids, show antioxidant activity in vitro (Lee et al. 2001; Mau, Lin, and Chen 2002; Shi et al. 2002; Wachtel-Galor, Choi, and Benzie 2005; Yuen and Gohel 2008; Saltarelli et al. 2009; Wu and Wang 2009). As shown in Figure 9.4, antioxidants

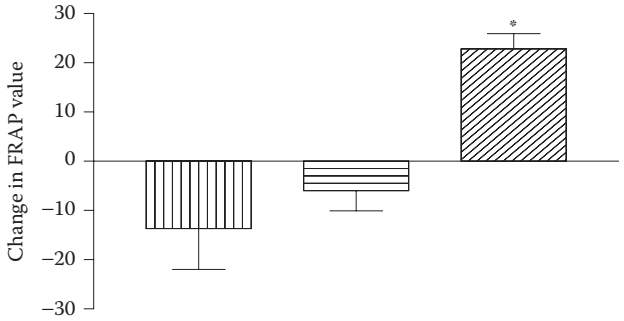


FIGURE 9.4 Mean +SEM (standard errors of the mean) change in plasma total antioxidant power (as the ferric reducing ability of plasma [FRAP] value) at 90 minutes postingestion of placebo (vertical lines), 1.1 g of *G. lucidum* extract (horizontal lines), and 3.3 g of *G. lucidum* extract (diagonal lines) in a human intervention trial ($n = 10$). A significant ($^*p < .05$) increase in plasma FRAP was seen after *G. lucidum* administration compared with placebo intake, indicating an absorption of antioxidant compounds into plasma. (From Wachtel-Galor, S., Y. T. Szeto, B. Tomlinson, and I. F. Benzie. F. 2004. *Int J Food Sci Nutr* 1:75–83. With permission.)

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from lingzhi were found to be absorbed quickly after ingestion, resulting in an increase in the plasma total antioxidant activity of human subjects (Figure 9.4; Wachtel-Galor, Szeto et al. 2004).

Ooi and Liu (2000) reported that protein-bound polysaccharide (PBP) and polysaccharide peptide were able to mimic the endogenous antioxidant superoxide dismutase (SOD) in cancer-bearing animals in vivo. These polysaccharides were also reported to protect the immune cells from oxidative damage (Ooi and Lui 2000). The protective effects of *G. lucidum* on DNA strand scission induced by a metal-catalyzed Fenton reaction, ultraviolet irradiation, and hydroxyl radical attack were shown in agarose gel electrophoresis in vitro (Lee et al. 2001). Hot water extracts of *G. lucidum* significantly protected Raji cells from hydrogen peroxide (H_2O_2)-induced DNA damage (Shi et al. 2002). Hot water extracts protected human lymphocyte DNA only at low (<.001% w/v) concentrations, and caused H_2O_2-mediated damage at higher concentrations (>.01% w/v) (Wachtel-Galor, Choi, and Benzie 2005). Two antioxidant-enriched extracts from *G. lucidum* acted oppositely in premalignant HUC-PC cells under carcinogenic attack (Yuen and Gohel 2008). The aqueous extract protected cellular DNA from oxidative damage, whereas the ethanolic extract damaged cellular DNA, with increased H_2O_2 production and significant cell-killing effects observed. The results suggested that different effects of *G. lucidum* could be exhibited by different extractable components in bladder chemoprevention. Methanol extracts of *G. lucidum* were reported to prevent kidney damage (induced by the anticancer drug cisplatin) through restoration of the renal antioxidant defense system (Sheena, Ajith, and Janardhanan 2003). In contrast, a fraction of ganoderma triterpenes (GTS) was found to enhance the intracellular reactive oxygen species (ROS)-producing effect of doxorubicin (DOX) in Hela cells, leading to more DNA damage and apoptosis, whereas such synergism was inhibited by a ROS scavenger (Yue et al. 2008). In an animal study (diabetic rats), nonenzymic and enzymic antioxidants levels increased and lipid peroxidation levels decreased with *G. lucidum* treatment (Jia et al. 2009). However, a direct link has not been established between the antioxidant properties of *G. lucidum* and its immunomodulatory and anticancer effects, and whether lingzhi acts as an antioxidant or pro-oxidant may depend on concentration and environment.

9.6.4 Viral and Bacterial Infections

The goal of research in the treatment of viral and bacterial infections is the discovery of agents that specifically inhibit viral and bacterial multiplication without affecting normal cells. The undesired side effects of antibiotics and antivirals and the appearance of resistant and mutant strains make the development of new agents an urgent requirement. This has led researchers to investigate the antibacterial and antiviral activity of medicinal plants and fungi (Wasser and Weis 1999; Zhong and Xiao 2009). Isolation of various water- and methanol-soluble, high-molecular-weight PBPs from *G. lucidum* showed inhibitory effects on herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), and vesicular stomatitis virus (VSV) New Jersey strain in a tissue culture system. Using the plaque reduction method, a significant inhibitory effect was seen at doses that showed no cytotoxicity (Eo et al. 1999; Oh et al. 2000). In addition, there was a marked synergistic effect when PBP from *G. lucidum* was used in tissue culture in conjunction with antiherpetic agents, acyclovir or vidarabine, and with IFN-α (Kim et al. 2000; Oh et al. 2000). Similar results were shown in HSV-1 and HSV-2 with a GLPG isolated from the mycelia of *G. lucidum* (Liu et al. 2004; Li, Liu, and Zhao 2005). The cells were treated before, during, and after infection, and viral titer in the supernatant of cell culture 48 hours postinfection was determined. The antiviral effects of the GLPG were more remarkable before viral treatment than after treatment. Although the mechanism was not defined, the authors concluded that GLPG inhibits viral replication by interfering with early events of viral adsorption (Li, Liu, and Zhao 2005).

Some triterpenes from *G. lucidum* have also been reported to have an inhibitory effect against human immunodeficiency virus (HIV)-1 protease activity, with IC_{50} values ranging from 20 to more than 1000 μM; however, not all of the examined triterpenes showed anti-HIV activity (El-Mekkawy et al. 1998; Min et al. 1998). In another study, a ganoderic acid isolated from *G. lucidum* showed

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inhibitory effects on the replication of hepatitis B virus (HBV) in HepG2215 cells (HepG2-HBV-producing cell line) over 8 days. Production of HBV surface antigen (HBsAg) and HBV e antigen (HBeAg) were, respectively, 20% and 44% of controls without ganoderic acid treatment (Li and Wang 2006).

Some small studies in human patients have also reported beneficial effects of lingzhi intake. A dried hot water extract of *G. lucidum* taken orally (equivalent to 36 or 72 g of dried mushroom per day) was used as the sole treatment for postherpetic (varicella zoster virus) neuralgia in 4 elderly patients. This treatment was reported to dramatically decrease pain and promote the healing of lesions, without any toxicity even at very high doses (Hijikata and Yamada 1998). In another study, a mixture of *G. lucidum* with other herbs improved recovery time in patients with herpes genitalis ($n = 15$) and herpes labiallis ($n = 13$; Hijikata, Yamada, and Yasuhara 2007).

For evaluating the antibacterial effects of the mushroom, several in vitro and in vivo animal studies using *G. lucidum* were performed. Mice injected with *G. lucidum* extract (2 mg/mouse) 1 day prior to injection with *Escherichia coli* showed markedly improved survival rates (>80% compared to 33% in controls; Ohno et al. 1998). In an in vitro study that used the disk assay (Keypour et al. 2008), a chloroform extract of *G. lucidum* was investigated for its antibacterial effect on gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*) and gram-negative bacteria (*E. coli*, *Pseudomonas aeruginosa*). Results showed that the extract had growth-inhibitory effects on two of the gram-positive bacteria with a minimal inhibitory concentration (MIC) of 8 mg/mL for *S. aureus* and *B. subtilis*. In another in vitro study, the direct antimicrobial effect of a *G. lucidum* water extract was examined against 15 species of bacteria alone and in combination with 4 kinds of antibiotics (Yoon et al. 1994). *G. lucidum* was found to be more effective than antibiotics against *E. coli, Micrococcus luteus*, *S. aureus*, *B. cereus*, *Proteus vulgaris*, and *Salmonella typhi,* but less effective against other species tested. The antimicrobial combination of *G. lucidum* with four commonly used antibiotics (Yoon et al. 1994) resulted in an additive or synergistic effect in most, but not all, instances, with apparent antagonism against cefazolin and ampicillin effects on *P. vulgaris.*

To date, the antimicrobial components of the tested crude extracts have not been identified, although antimicrobial polysaccharides have been identified in other fungi and plant terpenes have been reported to have antimicrobial activity (Wasser and Weis 1999; Zhong and Xiao 2009). In addition, the bioavailability of putative antimicrobial components of *G. lucidum* has not been established. Nonetheless, *G. lucidum* offers a potentially effective therapy. There is also the implication that combination therapy may be more safe and cost effective, as lower amounts of cytotoxic antiviral and antibacterial drugs could be used with a concomitant decrease in the risk of side effects. However, this needs further investigation in terms of in vitro studies and well-designed clinical trials.

9.6.5 Diabetes Mellitus

Components of *G. lucidum* have been proved to have a hypoglycemic effect in animals. The administration of ganoderans A and B (dose of 100 mg/kg), two polysaccharides isolated from fruitbody water extracts, by i.p. injection to normal and alloxan-induced diabetic mice significantly decreased (by up to 50%) the plasma glucose concentrations, and the hypoglycemic effect was still evident after 24 hours (Hikino et al. 1985). Using a mouse model, ganoderan B was also reported to increase plasma insulin, decrease hepatic glycogen content, and modulate the activity of glucose-metabolizing enzymes in the liver (Hikino et al. 1989). The same group reported that a third polysaccharide (ganoderan C) isolated from *G. lucidum* also showed significant hypoglycemic effects in mice, and that ganoderan B increased plasma insulin levels in both normal and glucose-loaded mice (Hikino et al. 1989; Tomoda et al. 1986).

In a more recent study, oral administration of *G. lucidum* hot water extract (0.03 and 0.3 g/kg BW) for 4 weeks was found to lower the serum glucose levels in obese/diabetic (+db/+db) mice, with effects seen after the first week of treatment (Seto et al. 2009). However, the glucose levels were still higher in these animals than in the control animals, and insulin levels were not altered.

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The extract markedly reduced levels of phosphoenol-pyruvate carboxykinase (PEPCK), which are usually high in obese/diabetic mice. The suggested mechanism, according to the authors, is that of lowering the serum glucose levels through suppression of the hepatic PEPCK gene expression. In another study (Jia et al. 2009), a polysaccharides-rich extract showed beneficial effects in streptozotocin-induced diabetic rats. The diabetic rats were treated with *G. lucidum* for 30 days. Following the treatment, serum insulin levels increased (compared with the non-treated diabetic group) and glucose levels decreased in a dose-dependent way. Treatment with streptozotocin also elevated levels of lipid peroxidation markers (thiobarbituric acid reactive substances [TBARS]), lipid hydroperoxides, and conjugated dienes); decreased levels of nonenzymic antioxidants (vitamin C, reduced glutathione [GSH] vitamin E); and decreased activities of the antioxidant enzymes, SOD, catalase, and glutathione peroxidase (Gpx). Following treatment with GL-PSs, levels of nonenzymic and enzymic antioxidants increased and lipid peroxidation levels decreased. Therefore, in addition to its glycemic modulation, treatment with *G. lucidum* helped to decrease oxidative stress (Jia et al. 2009).

In one study reported in the literature, 71 adult patients with confirmed type 2 diabetes mellitus (DM) were supplemented with Ganopoly (polysaccharide fractions extracted from *G. lucidum*). The patients received either Ganopoly or placebo orally at 1800 mg, three times daily for 12 weeks. Glycosylated hemoglobin ($HbA1_C$) and plasma glucose decreased significantly after 12 weeks, indicating a hypoglycemic effect of the extract (Gao, Lan et al. 2004). Overall, the data from different studies suggest that *G. lucidum* intake helps in modulating blood glucose levels. However, the studies were performed mostly in animals. More support from well-planned human clinical studies is needed with and without combination with conventional medicines.

9.6.6 Liver and Gastric Injury

Hot water and water–ether extracts of the fruit body of *G. lucidum* were found to have a potent hepatoprotective effect on liver injury induced by carbon tetrachloride (CCl_4) given orally and intraperitoneally to rats (Lin et al. 1995; Kim et al. 1999). The measured markers for liver injury included aspartate and alanine transaminases (AST and ALT) and lactate dehydrogenase (LDH). One active compound of the extract was separated and identified as ganoderenic acid A. This was found to have a potent inhibitory effect on β-glucuronidase, and the authors suggest that this inhibitory effect may have mediated the hepatoprotection seen when this isolated compound was given (Kim et al. 1999). Protection was also reported in a study in which a hot water extract of *G. lucidum* was given orally to mice 30 minutes before administration of ethanol. The extract was found to have an inhibitory effect against the formation of malondialdehyde (MDA), a degradation product of lipid peroxides, in mouse liver and renal homogenate, with evidence of a dose response seen (Shieh et al. 2001). The MDA effect was also reported by Shi et al. (2008) when the extract was given orally to mice (at 60, 120, and 180 mg/kg/day) for 2 weeks prior to treatment with D-galactosamine, which induced hepatic injury. In addition, pretreatment with *G. lucidum* maintained normal values of AST, ALT, SOD, and GSH (Shi et al. 2008). Alcohol and CCl_4 toxicity is associated with increased oxidative stress and free-radical-associated injury. Therefore, hepatoprotection may also be mediated by the radical-scavenging properties of *G. lucidum*. Lin et al. (1995) reported that hot water extracts of *G. lucidum* showed significant radical-scavenging activity against both superoxide and hydroxyl radicals.

Further, *G. lucidum* methanolic extract was reported to show hepatic protection. The extract was given orally to rats (500 mg/kg/day) for 30 days before hepatic damage was caused by benzo(a) pyrene (Lakshmi et al. 2006). The extract prevented the increase of serum AST, ALT, and alkaline phosphatase (ALP) activities that result from benzo(a)pyrene challenge, and enhanced the levels of GSH, SOD, GpX, CAT, and glutathione S-transferase (GST). Protection of liver injury induced by CCl_4 was also observed in mice treated with ganoderic acid (from *G. lucidum*) at 10 mg and 30 mg/kg/day given by intravenous injection for 7 days (Li and Wang 2006). The medium in which

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G. lucidum was grown was also proved to have liver-protective effects in an animal study of CCl_4-induced liver damage (Liu et al. 1998). Oral administration of the medium in which *G. lucidum* mycelia were grown (but not the mycelia alone) had marked beneficial effects, as assessed by lower serum AST and ALT activities at 96 hours postinjury. No decrease was seen in the actual damage caused, as transaminase activities at 24 hours were not different from levels in control animals, implying that the mycelium medium may have promoted recovery in some way. The release of a hepatoprotective component from *G. lucidum* mycelium was also reported by Song et al. (1998). In this study, an extracellular peptidoglycan (a polysaccharide/amino acid complex named WK-003) produced during mycelium fermentation was administered orally to rats for 4 days prior to CCl_4 intoxication. The increase in serum ALT levels was significantly decreased (by 70%; $P < .01$) at 24 hours postinjury compared with untreated, intoxicated rats. The AST levels decreased by 27%; however, this was not statistically significant. These studies of a possible mycelial product with hepatoprotective activity being extruded into the culture medium are of interest because the mycelia of *G. lucidum* are much easier and less costly to cultivate than the fruit body.

Polysaccharides extracted from *G. lucidum* and given orally to rats for 28 days were found to ameliorate cirrhosis induced by biliary ligation (Park et al. 1997). In addition, collagen (measured by hydroxyproline) content in the rat liver was lowered and improved liver morphology was found in comparison with control animals. The treatment significantly decreased ligation-induced increases in serum biochemical markers of liver damage (AST, ALT, ALP, and total bilirubin). Similar results were noticed in a study conducted by Wu, Fang, and Lin (2010) in which a decrease in hepatic hydroxyproline content and an improved liver histology were found in mice. In this study, liver fibrosis was induced by the administration of thioacetamide (TAA) for 12 weeks, which was followed by 4 weeks of treatment with *G. lucidum* extract (0.5 and 1.0 g/kg/day, per oral administration). The RT-QPCR analysis showed the extract treatment decreased mRNA expression of collagen (α1), smooth muscle α actin, and the enzymes metalloproteinase-1 and metalloproteinase-13. In addition, the TAA-induced decrease in total collagenase activity was reversed by the extract treatment, indicating that *G. lucidum* protection against injury may be related to the enhancement of collagenase activity.

Apart from its effects on chemical-induced liver injury, the effects of lingzhi on gastric injury have also been investigated. Gastric ulcers were induced in rats by acetic acid (Gao, Tang et al. 2004), and treatment with GL-PS fractions of 0.5 and 1.0 g/kg for 14 days significantly accelerated the ulcer healing by 40% and 56%, respectively. Treatment with 1.0 g/kg extract significantly restored mucus and prostaglandin levels compared with the control group.

9.7 CONCLUDING REMARKS

G. lucidum is a well-known Asian herbal remedy with a long and impressive range of applications. Global consumption of *G. lucidum* is high, and a large, increasing series of patented and commercially available products that incorporate *G. lucidum* as an active ingredient are available as food supplements. These include extracts and isolated constituents in various formulations, which are marketed all over the world in the form of capsules, creams, hair tonics, and syrups.

With its growing popularity, many studies on *G. lucidum* composition, cultivation, and reputed effects are being carried out, and there are data that support its positive health benefits, including anticancer effects; blood glucose regulation; antioxidant, antibacterial, and antiviral effects; and protection against liver and gastric injury. However, most studies have been performed on animals or in cell-culture models. Human experimental studies have often been small, and the results are not always supportive of the in vitro findings. Now, the great wealth of chemical data and anecdotal evidence on the effects of *G. lucidum* needs to be complemented by reliable experimental and clinical data from well-designed human trials in order to clearly establish if the reported health-related effects are valid and significant. Many challenges are encountered due to a range of factors from dosage to production quality. Strategies for enhancing quality control procedures to define and

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standardize *G. lucidum* preparations are needed to determine mechanisms of action and to help characterize the active component(s) of this putative medicinal mushroom.

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10 Pomegranate Ellagitannins

David Heber

CONTENTS

10.1 Introduction 201
10.2 Bioactivity of Pomegranate Polyphenols and Metabolites 201
10.3 Cancer Preventive Potential of Pomegranate Polyphenols 203
10.4 Mechanistic Insights from Cell Culture and Animal Studies 204
10.5 Evidence of Bioactivity from Human Clinical Studies 206
10.6 Detailed Studies of Bioavailability and Metabolism 206
10.7 Conclusions 207
References 207

10.1 INTRODUCTION

Eating a diet rich in fruits and vegetables may lead to a reduction in the risk of common forms of cancer and may be useful in cancer prevention. For pomegranate (*Punica granatum* L.), both basic and clinical evidence of the benefits of particular classes of bioactive substances have been developed specifically for the juice and juice extracts. Since ancient times, pomegranate has been used for medicinal purposes. Extensive research on bioactive substances in the pomegranate extract has shown potential applications in the chemoprevention of common forms of cancer. This work has progressed in cell culture, human studies, and in some clinical research demonstrating the preventive potential of pomegranate.

Pomegranates have been shown to contain 124 different phytochemicals, and some of them act in concert to exert antioxidant and anti-inflammatory effects on cancer cells. Ellagitannins are bioactive polyphenols present in pomegranate. Pomegranate juice obtained by squeezing the whole fruit has the highest concentration of ellagitannins than any commonly consumed juice and contains the unique ellagitannin, punicalagin. Punicalagin is the known largest molecular weight polyphenol. Pomegranate ellagitannins are not absorbed intact into the blood stream but are hydrolyzed to ellagic acid over several hours in the intestine. Ellagitannins are also metabolized into urolithins by gut flora, which are conjugated in the liver and excreted in the urine. These urolithins are also bioactive and inhibit prostate cancer cell growth. Research on basic mechanisms of action in cell culture, animal model systems, and limited clinical research in prostate cancer patients has been carried out with pomegranate juice. This chapter discusses the evidence for the bioactivity of pomegranate ellagitannins, along with the progress in defining the pharmacokinetics and metabolism of ellagitannins in pomegranate juice in humans.

10.2 BIOACTIVITY OF POMEGRANATE POLYPHENOLS AND METABOLITES

Ellagitannins are a family of bioactive polyphenols in fruits and nuts such as pomegranates, black raspberries, raspberries, strawberries, walnuts, and almonds (Amakura et al. 2000; Clifford and Scalbert 2000). Squeezing whole pomegranate fruit (*P. granatum* L.) yields the richest source of ellagitannins among other fruit juices. This juice has been used for centuries in ancient cultures for

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medicinal purposes (Longtin 2003). Commercial pomegranate juice, which has recently become popular in the United States, has more potent antioxidant properties than other common fruit juices, and this is attributed to its high content of polyphenols. Emerging science has demonstrated anticancer effects, with the most impressive data so far in prostate cancer. However, the inhibition of subcellular pathways of inflammation triggered by nuclear factor κB (NF-κB), angiogenesis under hypoxic conditions triggered by the hypoxia-inducible factor 1α (HIF-1α), and cellular proliferation along with the stimulation of apoptosis suggests that ellagitannins can act through multiple pathways and may be used as a dietary agent for preventing and treating many common forms of cancer.

The most abundant type of polyphenols in pomegranate juice are ellagitannins that are hydrolyzable tannins releasing ellagic acid on hydrolysis (Gil et al. 2000) and form urolithins such as urolithin A following metabolism by gut flora (Figure 10.1). Punicalagin is unique to pomegranate and is part of a family of ellagitannins, which also include minor tannins such as punicalin and gallagic acid (structures not shown here). All these ellagitannins have in common the ability to be hydrolyzed to ellagic acid, resulting in a prolonged release of ellagic acid into the blood following the ingestion of pomegranate juice.

Among the pomegranate ellagitannins, punicalagin, which is the largest polyphenol, having a molecular weight of greater than 1000, is reported to be responsible for more than half the potent antioxidant activity of the juice (Gil et al. 2000). Punicalagin is most abundant in the fruit husk as opposed to the juicy seeds (arils) found within the fruit. By pressing the whole fruit during the squeezing process, ellagitannins are extracted into pomegranate juice in significant quantities, reaching levels of >2 g/L juice (Gil et al. 2000). Pomegranate juice also contains other polyphenols, such as anthocyanins (cyanidin, delphinidin, and pelargonidin glycosides) and flavonols (quercetin,

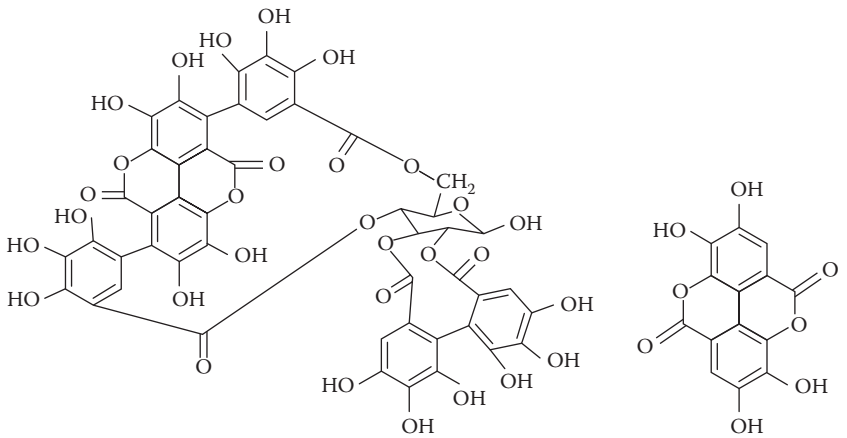


FIGURE 10.1 Chemical structures of punicalagin isomers, the major ellagitannins present in pomegranate juice and its metabolites, dimethylellagic acid glucuronide, ellagic acid, and urolithins A and B.

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kaempferol, and luteolin glycosides; Gil et al. 2000). In all, about 124 phytochemicals have been identified in the pomegranate (Seeram, Schulman, and Heber 2006).

Following metabolism by gut flora, urolithins A and B are formed and conjugated in the liver prior to excretion in the urine over 12–56 hours after a single administration of 8 oz (250 mL) of pomegranate juice. These urolithins circulate in the blood and can reach many of the target organs where the effects of pomegranate ellagitannins are noted.

10.3 CANCER PREVENTIVE POTENTIAL OF POMEGRANATE POLYPHENOLS

Results from studies in cells, animals, and humans clearly point to the importance of following ellagitannin metabolites as markers of pomegranate juice intake and studying them in detail to explain the effects of pomegranate juice on the inhibition of prostate cancer cell growth in vitro and in severe combined immunodeficient (SCID) mice with orthotopically transplanted human prostate cancer cells. Our group and others have also shown that pomegranate fruit extract and its purified ellagitannins inhibit the proliferation of human cancer cells and modulate the inflammatory subcellular signaling pathways and apoptosis (Afaq et al. 2005; Seeram et al. 2005; Adams et al. 2006).

Based on the observation that a pomegranate fruit extract inhibited prostate cancer growth in athymic nude mice, some authors have proposed that anthocyanins are the major phytochemical group responsible for the observed anticancer effects (Malik et al. 2005). Although anthocyanins do contribute to the observed antioxidant activity of pomegranate juice, it is unlikely that anthocyanins account for the complete profile of activities observed with pomegranate juice and extracts. In fact, pomegranate extracts containing ellagitannins without anthocyanins have previously been shown to exhibit in vitro and in vivo anticarcinogenic properties, including induction of cell-cycle arrest and apoptosis, inhibition of tumor formation, and xenograft tumor growth (Castonguay et al. 1997). The bioavailability of ellagitannins from pomegranate extract is equivalent to that observed after the administration of pomegranate juice, so that this point is highly relevant to the potential utility of pomegranate extracts as dietary supplements (Seeram, Zhang et al. 2008).

Inflammation is a hallmark of prostate cancer and is observed in prostate tissue at the time of prostatectomy. Inflammation has also been implicated in colon cancer, breast cancer, and other common forms of cancer. Indeed, the proliferative inflammatory atrophy may be a precursor to Prostatic Intraepithelial Neoplasia (PIN) and prostate cancer (De Marzo et al. 2003). Multiple molecular targets are related to the inflammatory pathway in prostate and other types of cancer cells (Figure 10.2). Inflammatory cells are found in prostate tissue at the time of prostatectomy and

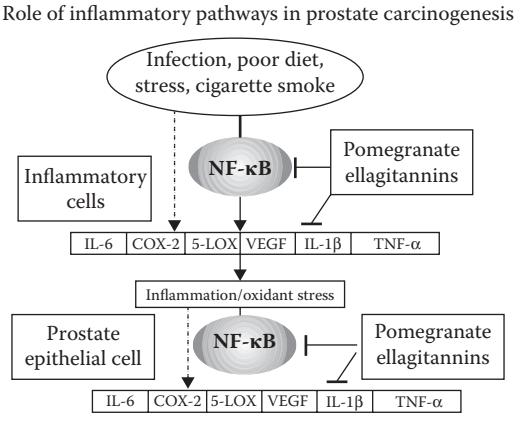


FIGURE 10.2 NF-κB activation in inflammatory cells can lead to oxidant stress and inflammation in prostate epithelial cells. Constitutive activation of NF-κB is then found in prostate cancer cells and may be the result of the interaction of inflammatory stromal cells and epithelial cells.

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the NF-κB expression is increased in more advanced lesions. In fact, nuclear localization of NF-κB is a risk factor for prostate cancer recurrence following the prostatectomy (Fradet et al. 2004). In other cancers also, this transcription factor is found to be of central importance. It is fair to say that without this transcription factor, there is no inflammation. It is unknown whether this is the only pathway mediating the effects of ellagitannins, and it is entirely possible that other interacting pathways reviewed in this section may well be involved.

NF-κB activation leads to immune activation, inflammation, and cell proliferation (Biswas et al. 2000; Kim, Sovak, and Zanieski 2000). NF-κB can also upregulate the transcription of genes that produce collagenases, cell adhesion molecules, and inflammatory cytokines, including tumor necrosis factor α (TNF-α) and interleukins (IL) 1, 2, 6, and 8 (Conner and Grisham 1996; Allison 1997; Winyard and Blake 1997). NF-κB regulates genes involved in the immune and inflammatory responses, as well as the cell-cycle control and cell death in response to proinflammatory cytokines such as IL-1 and TNF-α (Rayet and Gelinas 1999; Mayo and Baldwin 2000). NF-κB is also associated with the transcription of genes involved in cell survival, such as Bcl_x and inhibitors of apoptosis. Constitutive activation of NF-κB is identified in prostate cancers (Domingo-Domenech et al. 2005). Interestingly, the genes coding for the NF-κB proteins p52 and p65 have been mapped to sites of frequent rearrangement and amplification, which give rise to many cancers. PC3 and DU145 prostate cancer cell lines and prostate carcinoma xenografts have demonstrated constitutive NF-κB activity through constitutive activation of IκB kinase α (IKKα) protein complex (Palayoor et al. 1999; Gasparian et al. 2002; Suh et al. 2002).

Activation of NF-κB also regulates a number of downstream genes, including cyclooxygenase-2 (COX-2). COX-2 is the key enzyme regulating the production of prostaglandins, the central mediators of inflammation. The COX-2 expression is induced by several extracellular signals, including proinflammatory and growth-promoting stimuli. COX-2 mRNA expression is regulated by several transcription factors, including the cyclic-AMP response element-binding protein (CREB), NF-κB, and the CCAAT-enhancer-binding protein (C/EBP). COX-2 is also affected posttranscriptionally at the level of mRNA stability. Inflammatory cells such as macrophages and mast cells release angiogenic factors and cytokines, such as TNF-α, IL-1, and vascular endothelial growth factor (VEGF; O'Byrne and Dalgleish 2001), which signal cell growth and proliferation.

Ellagitannins and their hydrolysis product, ellagic acid, inhibit prostate cancer cell growth through cell-cycle arrest and stimulation of apoptosis (Castonguay et al. 1997; Albrecht et al. 2004; Lansky et al. 2005). In addition, they inhibit the activation of inflammatory pathways including, but not limited to, the NF-κB pathway. Inhibition of angiogenesis has also been demonstrated both in vitro and in vivo for prostate cancer. The universal nature of these mechanisms in common forms of cancer suggests that pomegranate ellagitannins, which have been tested in both prostate and colon cancer cells by our group, may also be useful dietary agents for the prevention and treatment of other forms of cancer, such as breast cancer.

10.4 MECHANISTIC INSIGHTS FROM CELL CULTURE AND ANIMAL STUDIES

In cell culture, combinations of ellagitannins are more potent than any single compound (Narayanan et al. 1999; Losso et al. 2004; Seeram et al. 2005; Sartippour et al. 2008). They have activity in combination against both prostate and colon cancer cells. While punicalagin is the most active of ellagitannins, it is possible to design experiments that demonstrate the additional effects of other phytochemicals found in pomegranate juice.

Overall, the significance of these in vitro findings is in doubt because only ellagic acid (along with the urolithins) is found in the blood circulation after the ingestion of pomegranate juice. Urolithins are formed by the action of gut bacteria on ellagitannins and are recirculated through the liver prior to excretion in the urine. Urolithins inhibit the growth of both androgen-dependent and

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androgen-independent prostate cancer cell lines, with IC_{50} values lower than that of ellagic acid. Future studies to evaluate the mechanistic basis for the antiproliferative effects of urolithins are required.

Angiogenesis is critical to tumor growth and is stimulated by tissue hypoxia due to poor oxygen delivery. Cellular hypoxia leads to angiogenesis via the induction of HIF-1α and VEGF at a cellular level. Pomegranate juice and extracts, which are rich sources of ellagitannins, have been shown to have chemopreventive potential against prostate cancer, but there have been no studies to date on the effects of an ellagitannin-rich pomegranate extract on angiogenesis directly. However, in the study of human prostate cancer cells (LNCaP) and human umbilical vein endothelial cells (HUVEC) incubated in vitro with a pomegranate extract standardized to ellagitannin content (POMx), the proliferation of LNCaP and HUVEC cells was significantly inhibited under both normoxic and hypoxic conditions, and HIF-1α and VEGF protein levels were decreased by POMx under hypoxic conditions (Sartippour et al. 2008).

Recently, there have been a number of reports on antiproliferative, proapoptotic, and antiangiogenic activity by pomegranate polyphenols, as well as on the inhibition of NF-κβ activity and xenograft growth (Albrecht et al. 2004; Afaq et al. 2005; Lansky et al. 2005). In a recent study by Sartippour et al. (2008), human prostate cancer cells (LAPC4) were injected subcutaneously into SCID mice, and the effects of the oral administration of POMx on tumor growth, microvessel density, and HIF-1α and VEGF expressions were determined after 4 weeks of treatment. POMx decreased prostate cancer xenograft size, tumor vessel density, VEGF peptide levels, and HIF-1α expression (Sartippour et al. 2008). These results demonstrate that an ellagitannin-rich pomegranate extract can inhibit tumor-associated angiogenesis as one of the several potential mechanisms for slowing the growth of prostate cancer in chemopreventive applications. Further studies in humans are needed to confirm that angiogenesis can be inhibited by an ellagitannin-rich pomegranate extract administered orally as a dietary supplement.

Constitutive NF-κB activation is observed in androgen-independent prostate cancer and represents a predictor for biochemical recurrence after the radical prostatectomy. Pomegranate extract inhibited NF-κ B activation and cell viability of prostate cancer cell lines in a dose-dependent fashion in vitro. Importantly, maximal pomegranate extract-induced apoptosis was dependent on pomegranate extract-mediated NF-κB blockade. In the LAPC4 xenograft model, pomegranate extract also delayed the emergence of LAPC4 androgen-independent xenografts in castrated mice through an inhibition of proliferation and induction of apoptosis. Moreover, the observed increase in NF-κB activity during the transition from androgen dependence to androgen independence in the LAPC4 xenograft model was abrogated by pomegranate extract. These observations suggest that pomegranate extract may be active in the prevention of the emergence of androgen independence that is driven in part by heightened NF-κB activity (Rettig et al. 2008).

The androgen independence of prostate cancer is characterized by intracellular androgen synthesis. In men treated with antiandrogen therapy, blood levels of testosterone are at castrate levels, whereas intracellular testosterone levels are about 50% the normal levels. Pomegranate extract was shown to inhibit the expression of the genes coding for the major androgen-synthesizing enzymes in vitro. This may be another mechanism through which pomegranate ellagitannins inhibit androgen-independent prostate cancer cell proliferation (Hong, Seeram, and Heber 2008).

The aromatase enzyme found in fat tissue and some breast tumors converts adrenal androgens into estrogen and represents the primary source of estrogens in postmenopausal women. Recent studies have demonstrated that in cell culture, urolithins, the metabolites of pomegranate ellagitannins, can inhibit the proliferation of MCF-7 breast cancer cells with transgenic overexpression of aromatase (MCF7aro cells). In addition, the isolated enzyme is inhibited by urolithin A in vitro. Our laboratory is currently studying the relevance of these observations in animal and human studies relevant to the prevention of breast cancer and the potential interaction of diet and exercise for breast cancer prevention (Adams et al. 2010).

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Finally, insulin-like growth factor 1 is known to stimulate prostate cancer cell proliferation, whereas insulin-like growth factor binding protein 3 (IGFBP-3) induces apoptosis of prostate cancer cells. Recently, pomegranate extract was shown to enhance the proapoptotic effects of IGFBP-3 (Koyama et al. 2010).

10.5 EVIDENCE OF BIOACTIVITY FROM HUMAN CLINICAL STUDIES

Plasma prostate-specific antigen (PSA) is a biomarker of prostate cancer progression. A clinical study in men with increasing levels of PSA after surgery or radiotherapy was begun in January 2003. Eligible patients had a PSA level > .2 ng/mL and < 5 ng/mL, and a Gleason score of 7 or less. The Gleason score is a pathological grading of tumors that predicts to some extent the future clinical course and guides therapy. The score is a composite of the predominant histology. The transition between the aggressive biology and the more commonly encountered biology is a score of 7. About 30% of men with a Gleason score of 7 have biochemical recurrence after primary therapy by surgery or radiation. Patients were provided with 8 oz (250 mL) of pomegranate juice (wonderful variety) to consume daily. Each 8 oz serving contained 570 mg of total polyphenols, quantitated as gallic acid equivalents. Interim results were published by Pantuck et al. (2006) and showed a significant increase in mean PSA doubling time following the treatment from 15 months at baseline to 54 months post-treatment ($p < .001$). In this study, 85% of patients had a decrease in the rate of PSA rise, which is being secreted solely under these conditions by prostate cancer cells that have proliferated after the removal of the primary tumor and all normal prostate tissue.

The PSA doubling time is a predictor of survival in prostate cancer patients with recurrent disease. The study was amended to allow patients to continue the pomegranate juice treatment and to undergo evaluation at 3-month intervals until clinical disease progression, defined as the decision by their urologist to use androgen blockade. In an ex vivo mitogenic bioassay, serum obtained from these pomegranate juice-treated patients, who were given 8 oz (250 mL) of pomegranate juice daily for 2 years, inhibited proliferation and stimulated apoptosis of LNCaP prostate cancer cells in vitro when the patient's serum was substituted for fetal calf serum in the cell-culture medium (Aronson et al. 2010). Further studies have been designed to determine whether ellagic acid, urolithins, and related metabolites in patient sera are responsible for these antiproliferative and proapoptotic effects.

10.6 DETAILED STUDIES OF BIOAVAILABILITY AND METABOLISM

Because the bioavailability of phytochemicals is critical to their bioactivity, it was studied in 18 volunteers to quantitate the plasma appearance and disappearance rates of ellagic acid hydrolyzed from ellagitannins in administered pomegranate juice (Seeram, Lee, and Heber 2004). In addition, it was demonstrated that the absorbed ellagic acid from the hydrolysis of pomegranate juice punicalagin is converted to dimethylellagic acid glucuronide (DMEAG) in plasma and urine on the day of administration of pomegranate juice (Seeram, Lee, and Heber 2004; Seeram, Henning et al. 2006). Urolithins derived from ellagic acid appeared in human urine after the disappearance of DMEAG about 12 hours after the administration of pomegranate juice.

Additional bioavailability data on DMEAG and urolithins were obtained in mice in support of our planned studies on the effects of pomegranate juice on orthotopically transplanted LNCaP cells in SCID mice (Seeram et al. 2007). Studies in rats and humans have shown that ellagitannins are hydrolyzed to ellagic acid in the gut, and this is metabolized by the colon microflora to form urolithins A and B. Urolithins can be absorbed into the enterohepatic circulation and can be excreted in urine and feces (Cerda et al. 2003; Cerda et al. 2004; Espin et al. 2007). Ellagic acid and urolithins can accumulate in the intestine and prostate (Larrosa, Tomás-Barberán, and Espin 2006; Seeram

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et al. 2007). Ellagitannins, ellagic acid, and urolithin A exhibit cancer chemopreventive activities in various cell and animal models. Oral administration of the pomegranate extract to wild-type mice led to increased plasma levels of ellagic acid, but ellagic acid was not detected in the prostate gland. On the other hand, intraperitoneal administration of pomegranate extract led to tenfold higher ellagic acid levels in the plasma and detectable and higher ellagic acid levels in the prostate, intestine, and colon relative to other organ systems. The detectable ellagic acid levels in prostate tissue following intraperitoneal, but not oral, administration were likely due to higher plasma levels attained after the intraperitoneal administration.

Intraperitoneal and oral administration of synthesized urolithin A led to uptake of urolithin A and its conjugates in prostate tissue, and levels were higher in prostate, colon, and intestinal tissues relative to other organs. It is unclear why pomegranate ellagitannins metabolites localize at higher levels in the prostate, colon, and intestinal tissues relative to the other organs studied. Importantly, the predilection of bioactive pomegranate ellagitannins metabolites to localize in prostate tissue, combined with clinical data demonstrating the anticancer effects of pomegranate juice, suggests the potential for pomegranate products to play a role in prostate cancer chemoprevention. Whether urolithins in human prostate tissue can be used as a biomarker following the long-term administration of pomegranate juice or pomegranate extract remains to be determined.

10.7 CONCLUSIONS

The ellagitannins found in pomegranate fruit are very potent antioxidants, and pomegranate juice exceeds the in vitro antioxidant potency of other common commercial fruit juices (Seeram, Aviram et al. 2008). There are limited treatment options for prostate cancer patients who have undergone primary therapy such as radical prostatectomy with curative intent but who have progressive elevations in their PSA. Pomegranate juice given daily for 2 years to 40 prostate cancer patients with increasing PSA levels provides evidence for the possible utilization of a nontoxic option for prevention or delay of prostate carcinogenesis. It is remarkable that 85% of patients responded to pomegranate juice in this study. Both in vitro and in vivo investigations in prostate cancer models of the molecular mechanisms that may account for these pomegranate juice effects have been explored.

Although researchers focus on the idea of a single pathway, it is evident that pomegranate ellagitannins, like other phytochemicals, work through multiple targeted pathways. Nonetheless, evidence that NF-κB activation is associated with heightened proliferation, increased neoangiogenesis, and resistance to apoptosis suggests that the antitumor action of pomegranate juice polyphenols are mainly mediated through their NF-κB inhibitory effects. This hypothesis is given added support by the recent implication of NF-κB as an independent risk factor for PSA rise (i.e., the sign of biochemical recurrence of prostate cancer) after the prostatectomy. Studies of the effects of pomegranate juice and dietary supplements made from pomegranate extract in patients prior to prostatectomy and after biochemical recurrence with increasing PSA are ongoing and should provide further information on the prostate cancer prevention and treatment potential of the juice of this ancient fruit. Further studies on other forms of cancer, including colon cancer and breast cancer, may reveal additional potentials for pomegranate juice in cancer chemoprevention.

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11 Medical Attributes of St. John's Wort (*Hypericum perforatum*)

Kenneth M. Klemow, Andrew Bartlow, Justin Crawford, Neil Kocher, Jay Shah, and Michael Ritsick

CONTENTS

11.1 Introduction 211
11.2 Taxonomy and Description 212
11.3 Traditional Origins 212
11.4 Current Usage and Preparations 213
11.5 Active Ingredients 214
11.5.1 Naphthodianthrones 215
11.5.2 Flavonoids 215
11.5.3 Lipophilic Compounds 215
11.5.4 Additional Compounds 215
11.6 Health Effects: The Scientific Evidence 216
11.6.1 St. John's Wort and Depression 216
11.6.1.1 Active Principals: In Vitro Studies 216
11.6.1.2 Clinical Studies 217
11.6.2 Antibacterial and Antiviral Properties 219
11.6.3 Anticancer Properties 220
11.6.4 Antioxidant and Neuroprotective Properties 221
11.6.5 Anti-Inflammatory Activity 222
11.6.6 Effects as a Wound-Healing Agent 222
11.6.7 Effects on Reducing Opium Dependence 223
11.7 Adverse Effects and Interactions with Other Drugs 223
11.7.1 Adverse Effects 223
11.7.2 Drug Interactions: Overview 223
11.7.2.1 P-glycoprotein 224
11.7.2.2 Cytochrome P450 224
11.7.2.3 Specific Drug Interactions 226
11.8 Future Research Needs 228
11.9 Conclusions 228
References 229

11.1 INTRODUCTION

St. John's wort (SJW), known botanically as *Hypericum perforatum*, is a sprawling, leafy herb that grows in open, disturbed areas throughout much of the world's temperate regions. The use of this species as an herbal remedy to treat a variety of internal and external ailments dates back to the time of the ancient Greeks. Since then, it has remained a popular treatment for anxiety, depression, cuts, and burns. Recent research suggests the effectiveness of this herb in treating other ailments,

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including cancer, inflammation-related disorders, and bacterial and viral diseases, and as an antioxidant and neuroprotective agent. Pharmaceutical companies, particularly in Europe, prepare standard formulations of this herb that are taken by millions of people. Worldwide annual sales of products made from SJW presently exceed several billion dollars. Further, SJW produces dozens of biologically active substances, although two—hypericin (a naphthodianthrone) and hyperforin (a lipophilic phloroglucinol)—have the greatest medical activity. Other compounds, including the flavonoids rutin, quercetin, and kaempferol, also appear to have medical activity. *H. perforatum* has been intensively studied on isolated tissue samples, using animal models and through human clinical trials. The effectiveness of SJW as an antidepression agent is particularly well studied, and the underlying mechanisms are well understood. SJW preparations have relatively few adverse effects when taken alone at the recommended dosages. However, numerous interactions with other drugs have been reported. Recent research shows these interactions result from the ability of SJW constituents to induce intestinal or hepatic enzymes that either remove drugs from the body or metabolize them to inactive forms. This chapter examines the constituents, modes of action, and adverse interactions of SJW, providing an up-to-date synthesis of a large body of literature that has developed over the past 30 years regarding this widely taken herbal remedy. Some recommendations regarding future research needs are also presented.

11.2 TAXONOMY AND DESCRIPTION

Commonly called SJW, klamath weed, tipton weed, goat weed, and enola weed (Muenscher 1946), *H. perforatum* is a perennial flowering herb belonging to the Clusiaceae (Mangosteen family; alternatively, Hypericaceae and Guttiferae). The genus *Hypericum* consists of approximately 400 species of herbs and shrubs having yellow or coppery flowers with four to five petals, numerous stamens, and a single pistil (Gleason and Cronquist 1991).

H. perforatum consist of freely branching shrubby herbs that typically range from 40 to 80 cm in height (Muenscher 1946; Gleason and Cronquist 1991). The stems and branches are densely covered by oblong, smooth-margined leaves that range from 1 to 3 cm long and 0.3–1.0 cm wide (Figure 11.1). The leaves are interrupted by minute translucent spots that are evident when held up to the light. The upper portions of mature plants can produce several dozen five-petaled yellow flowers that are typically 1.0–2.0 cm wide. The edges of the petals are usually covered with black dots. Crushed flowers produce a blood-red pigment. By late summer, the flowers produce capsules that contain dozens of tiny, dark-brown seeds. The species is a native of Europe, but has spread to temperate locations in Asia, Africa, Australia, and North and South America (Gleason and Cronquist 1991; Foster 2000). It thrives in poor soils, and is commonly found in meadows, fields, waste areas, roadsides, and abandoned mines and quarries (Muenscher 1946; Klemow and Raynal 1983; Gleason and Cronquist 1991). Due to concerns over phototoxicity to livestock, *H. perforatum* is listed as a noxious weed in seven western states in the United States. Programs promoting its eradication are underway in Canada, California, and Australia.

11.3 TRADITIONAL ORIGINS

The SJW has been considered a medicinally valuable plant for over 2000 years. The Greek physicians of the first century, Galen, Dioscorides, Pliny, and Hippocrates, recommended SJW as a diuretic, wound-healing herb, treatment for menstrual disorders, and cure for intestinal worms and snakebites (Foster 2000; Castleman 2001; Redvers et al. 2001). Dried flowering tops placed in olive oil caused the oil to turn red after 3 weeks. The ancients believed the plant had mystical qualities, and plants were collected for protection from demons and to drive away evil spirits. In fact, the generic name *Hypericum* originated from the Greek name for the plant, *hyperikon*. Literally translated, the name is an amalgamation of the root words "hyper" (meaning over) and "eikon" (meaning image or apparition), referring to the plant's supposed ability to ward off evil spirits (Foster 2000).

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FIGURE 11.1 Diagrams of St. John's wort (*Hypericum perforatum*). A sketch of the lower stem showing leaf arrangement is to the left, and that of the upper stem with flowers is to the right. An opened capsule is shown in the bottom left, and a flower petal is shown to the bottom right. (From USDA-NRCS PLANTS Database; Britton, N. L., and A. Brown. 1913. *An illustrated flora of the Northern United States, Canada and the British Possessions* 2:533.)

Early Christians also believed the plant had mystical properties. According to one legend, the greatest effect was obtained when the plant was harvested on Saint John's Day (June 24), which is often the time of peak blooming (Foster 2000). Another legend holds that the plant released its blood-red oil on August 29, the day of St. John's beheading (Castleman 2001).

The plant enjoyed continued use as an herbal remedy in the Middle Ages. Sixteenth-century herbalists including Paracelsus, Gerard, and Culpeper all recommended SJW preparations to treat wounds and alleviate pain (Foster 2000; Castleman 2001). In 1525, Paracelsus recommended it for treating depression, melancholy, and overexcitation (Clement et al. 2006). The SJW's use as a medicinal herb continued in Europe, spreading to other continents, between the eighteenth and nineteenth centuries. It was commonly made into teas and tinctures for treatment of anxiety, depression, insomnia, water retention, and gastritis. Over the years, vegetable oil preparations have been used for treatment of hemorrhoids and inflammation. Others have used SJW extracts to treat sores, cuts, minor burns, and abrasions, especially those involving nerve damage (Blumenthal et al. 1998; Foster 2000; Foster and Duke 2000; Castleman 2001).

11.4 CURRENT USAGE AND PREPARATIONS

SJW is currently valued for treating depression and other mood disorders. Products containing SJW in the form of tablets, capsules, teas, and tinctures accounted for an estimated US$6 billion in Europe in the late 1990s (Ernst 1999; Greeson, Sanford, and Monti 2001). In the United States, annual sales reached a peak of US$315 million in 1998, but declined to approximately US$60 million by 2006 (Tilburt, Emanuel, and Miller 2008). SJW has been the subject of several pharmacopoeias and monographs, including the British Herbal Pharmacopoeia (1996), the European Scientific

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Cooperative on Phytotherapy (ESCOP 1996), the American Herbal Pharmacopeia (1997), and the European Pharmacopoeia (European Directorate for the Quality of Medicines (EDQM) 2000), among others (Parfitt 1999; Barnes, Anderson, and Phillipson 2001).

Several pharmaceutical-grade preparations of SJW are commercially available, typically extracted from dried aerial parts. The LI 160, produced by Lichtwer Pharma, is standardized to contain 0.3% hypericin derivatives, and normally comes in 300-mg capsules (Bloomfield, Nordfors, and McWilliams 1996). Another product, Ze 117, produced by Zeller AG, Switzerland, is a 50% ethanolic extract with an herb-to-extract ratio of between 4:1 and 7:1. The hyperforin content of Ze 117 is 0.2%, lower than that of LI 160, whose hyperforin content ranges between 1% and 4%. The dosage of Ze 117 is 500 mg/day (Marquez 2002). The product WS 5570, produced by Dr. Willmar Schwabe Pharmaceuticals, Karlsruhe, Germany, is an 80% ethanolic extract of SJW with a plant-to-extract ratio of between 3:1 and 3:1–7:1 (Barrett 2004). It contains 5–6% hyperforin and 0.12–0.28% hypericin (Lecrubier et al. 2002; Szegedi et al. 2005). Tablets contain 300 or 600 mg of extract. The product WS 5572 has similar hyperforin and hypericin profile to WS 5570, but has a plant-to-extract ratio of between 2.5:1 and 5:1 (Barrett 2004). The product STEI 300, produced by Steiner Arzneimittel of Berlin, contains 0.2–0.3% hypericin and pseudohypericin, and 2–3% hyperforin (Philipp, Kohnen, and Hiller 1999). Capsules contain 350 mg of extract. The German Commission E and European Scientific Cooperative on Phytotherapy (ESCOP) monographs recommend 900 mg of standardized extract per day (ESCOP 1996; Blumenthal et al. 1998). Clinical trials using various *H. perforatum* preparations allow typical dosages in the range of 300–1800 mg/day (Barnes, Anderson, and Phillipson 2001).

In the United States, SJW, like all herbal remedies, is listed as a dietary supplement by the Food and Drug Administration (FDA). Therefore, it is not subject to the strict scrutiny for safety and efficacy that standard pharmaceutical drugs are required to pass (Clement et al. 2006). The FDA mandates that all herbal remedies contain a disclaimer informing the consumer that any claims about the medicine's therapeutic value have not been evaluated by that agency.

11.5 ACTIVE INGREDIENTS

Chemical investigations into the constituents of *H. perforatum* have detected seven groups of medicinally active compounds (Nahrstedt and Butterwick 1997). The most common classes include naphthodianthrones, phloroglucinols, and flavonoids (such as phenylpropanes, flavonol glycosides, and biflavones), as well as essential oils (Bombardelli and Morazzoni 1995; Reuter 1998; Barnes, Anderson, and Phillipson 2001; DerMarderosian and Beutler 2002). Two major active constituents have been identified: hypericin (a naphtodianthrone; Figure 11.2a) and hyperforin (a phloroglucinol; Figure 11.2b). However, roughly 20% of extractable compounds are considered biologically active (Staffeldt et al. 1994; Nahrstedt and Butterwick 1997; Erdelmeier 1998).

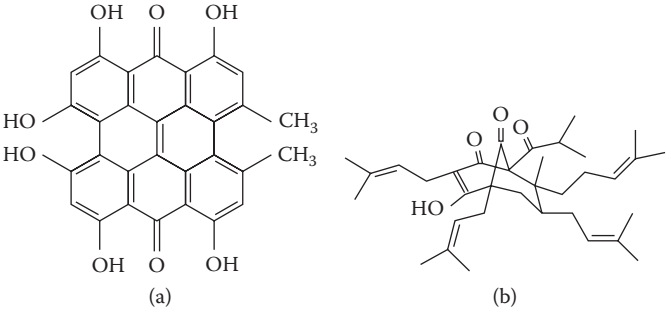


FIGURE 11.2 Chemical structures of (a) hypericin, and (b) hyperforin.

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11.5.1 Naphthodianthrones

The class of compounds isolated from *H. perforatum* that is the most researched is the naphthodianthrones, which have been standardized in concentrations ranging from 0.1% to 0.3% (Robbers and Tyler 1999; Grainger-Bisset and Wichtl 2001; Greeson, Sanford, and Monti 2001). The most common naphthodianthrones include hypericin, pseudohypericin, isophypericin, and protohypericin (Barnes, Anderson, and Phillipson 2001; DerMarderosian and Beutler 2002). Of these, hypericin—an anthraquinone-derived pigment that is responsible for the red color of SJW oils—is the best known. Hypericin is found in the flowers, particularly in the black dots that are located along the petals.

Due to its chemical structure, hypericin is highly photoreactive. Biochemically, hypericin is a polycyclic quinone, possessing four hydroxyl groups that are positioned adjacent to two carbonyl groups (Figure 11.2a). Owing to resonance of the molecule and the relatively short distance between oxygen atoms (approximately 2.5 Å), the hydroxyl hydrogen is capable of transferring between the hydroxyl oxygen and the carbonyl oxygen in the presence of fluorescent light (Petrich 2000). The hydrogen is therefore in constant flux between the two oxygen atoms when exposed to fluorescent light (Smirnov and Fulton 1999). Studies examining the fluorescence spectrum of hypericin and its analogs demonstrate the existence of a "protonated" carbonyl group, supporting the H-atom transition mechanism (Petrich 2000). This hydrogen transfer also causes acidification of the surrounding environment (Fehr, McCloskey, and Petrich 1995; Sureau et al. 1996).

11.5.2 Flavonoids

Flavonoids found in SJW range from 7% in stems to 12% in flowers (DerMarderosian and Beutler 2002) and leaves (Greeson, Sanford, and Monti 2001). Flavonoids include flavonols (kaempferol, quercetin), flavones (luteolin), glycosides (hyperside, isoquercitrin, and rutin), biflavones (biapigenin), amentoflavone, myricetin, hyperin, oligomeric proanthocyanadins, and miquelianin, all of which are biogenetically related (Reuter 1998; Barnes, Anderson, and Phillipson 2001). Rutin concentration is reported at 1.6% (Barnes, Anderson, and Phillipson 2001).

11.5.3 Lipophilic Compounds

Extracts of SJW contain several classes of lipophilic compounds with demonstrated therapeutic value, including phloroglucinol derivatives and oils. Hyperforin, isolated in concentrations of 2–4.5% (Chatterjee, Bhattacharya et al. 1998; Greeson, Sanford, and Monti 2001), is a prenylated phloroglucinol expanded into a bicyclo nonaendionol (2,1), substituted with several lipophilic isoprene chains (Nicolaou, Carenzi, and Jeso 2005). Hyperforin is unstable in the presence of both light and oxygen (Liu et al. 2005). Despite numerous attempts by various researchers, total synthesis of hyperforin has not been accomplished to date (Nicolaou, Carenzi, and Jeso 2005). Other phloroglucinols include adhyperforin (0.2%–1.9%), furohyperforin, and other hyperforin analogs (Hahn 1992; Barnes, Anderson, and Phillipson 2001; Greeson, Sanford, and Monti 2001; DerMarderosian and Beutler 2002). Essential oils are found in concentrations ranging from 0.05% to 0.9% (Greeson, Sanford, and Monti 2001). They consist mainly of mono- and sesquiterpenes, specifically 2-methyloctane, *n*-nonane, α- and β-pinene, α-terpineol, geranil, and trace amounts of myrecene, limonene, and caryophyllene, among others (Hahn 1992; Reuter 1998).

11.5.4 Additional Compounds

Other compounds of various classes have been identified in *H. perforatum*. These include tannins (ranging from 3% to 16%), xanthones (1.28 mg/100 g), phenolic compounds (caffeic acid, chlorogenic acid, and *p*-coumaric acid), and hyperfolin. Additional compounds include, to a lesser extent, acids (nicotinic, myristic, palmitic, and stearic), carotenoids, choline, pectin, hydrocarbons,

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and long-chain alcohols (DerMarderosian and Beutler 2002). Several amino acids that have been isolated from the herb include cysteine, glutamine, leucine, lysine, and GABA (γ-aminobutyric acid; Hahn 1992; Bombardelli and Morazzoni 1995; Greeson, Sanford, and Monti 2001).

11.6 HEALTH EFFECTS: THE SCIENTIFIC EVIDENCE

The widespread popularity of SJW's use as an herbal remedy results from studies that appear to verify its efficacy in treating a variety of diseases, especially depression. In turn, the herb's use has generated widespread interest among scientists seeking to firmly evaluate its effectiveness. Such studies include analyses on the effects of SJW extracts on isolated tissue samples, studies using animal models, and clinical analyses and meta-analyses of humans given SJW extracts.

11.6.1 St. John's Wort and Depression

Mood disorders are common illnesses that force individuals to seek relief offered by physicians and other health-care providers. All over the world, 3–5% of the population requires treatment for depression (Lieberman 1998). The disorder brings with it a series of symptoms such as strong feelings of sadness and guilt, a loss of interest or pleasure, irregular sleeping patterns, a loss of energy, decreased ability to concentrate, and an increase or decrease of appetite. Even more serious symptoms are repeated thoughts of suicide and death (Remick 2002).

Depression is thought to originate from a disruption of normal brain neurochemistry, specifically from a deficiency of amine neurotransmitters like acetylcholine, norepinephrine, dopamine, and serotonin (5-hydroxytryptamine [5-HT]). Antidepression drugs typically raise the levels of those neurotransmitters, especially in nerve–nerve synapses (Remick 2002).

Synthetic antidepressants available currently fall into two categories: (1) tricyclics and (2) selective serotonin reuptake inhibitors (SSRIs). Tricyclics include amitriptyline (brand name: Elavil), desipramine (Norpramin), imipramine (Tofranil), nortriptyline (Aventyl, Pamelor), and trimipramine (Surmentil). Commonly prescribed SSRIs include citalopram (Celexa), fluoxetine (Prozac), paroxetine (Paxil), and sertraline (Zoloft). Monoamine oxidase (MAO) inhibitors (MAOIs) like phenelzine (Nardil) and tranylcypromine (Parnate) are also used to treat depression (American Academy of Family Physicians 2000).

11.6.1.1 Active Principals: In Vitro Studies

Early research suggested that hypericin is the main antidepressant constituent of SJW, stimulating capillary blood flow (DerMarderosian and Beutler 2002). Later, studies on rat brain mitochondria found hypericin to strongly inhibit the enzymes MAO-A and -B (Suzuki et al. 1984; Robbers and Tyler 1999; Barnes, Anderson, and Phillipson 2001). MAO is involved in the degradation of amine neurotransmitters. Inhibiting their degradation boosts their levels in the synapse. However, further studies determined that hypericin's ability to inhibit MAO was lower than was originally estimated. Moreover, the levels of hypericin necessary to obtain significant MAO inhibition were far greater than those likely to be found in human brain tissue at normal doses (Suzuki et al. 1984; Chavez and Chavez 1997). Hypericin has been shown to have a strong affinity for sigma receptors, which regulate dopamine levels. It also acts as a receptor antagonist at adenosine, benzodiazepine, GABA-A, GABA-B, and inositol triphosphate receptors, which regulate action potentials caused by neurotransmitters (Chavez and Chavez 1997; Jellin et al. 2002). Although hypericin has been reported to have antidepressant properties, it cannot by itself completely account for the antidepressant activity of SJW. Recent research focuses on hyperforin as the antidepression agent (Cervo et al. 2002). Hyperforin is a potent reuptake inhibitor of serotonin, dopamine, noradrenaline, GABA, and L-glutamate from the synaptic cleft (Calapai et al. 1999; Vormfelde and Poser 2000; Müller, Singer, and Wonnemann 2001; Zanoli 2004). The IC_{50} values (concentration resulting in 50% inhibition) of approximately 0.05–0.1 μg/mL for neurotransmitters were reported in synaptosomal preparations

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(Chatterjee et al. 1998). Blocking the reuptake of serotonin (5-HT) from the synaptic cleft alleviates symptoms of depression by allowing the serotonin to bind to 5-HT receptors and elicit a greater response (Jones and Blackburn 2002; Molderings 2002).

Unlike synthetic antidepressants that block 5-HT receptors, hyperforin apparently inhibits serotonin uptake by elevating intracellular concentrations of sodium (Na) and calcium (Ca; Singer, Wonnemann, and Muller 1999; Müller, Singer, and Wonnemann 2001; Müller 2003). Treiber et al. (2005) demonstrated that the influx of Na^+ was mediated by nonselective cation channels (NSCCs). They found that hyperforin acts on the subclass NSCC2, increasing intracellular concentrations of both Na^+ and Ca^{2+} ions (Treiber et al. 2005). Leuner et al. (2007) subsequently identified the channel as being the transient receptor potential channel protein 6 (TRPC6). Thus, activation of TRPC6 by hyperforin leads to an increase in sodium uptake by neurons, resulting in a decrease of the sodium gradient between the neuron and the synaptic cleft. The loss of the gradient decreases reuptake of the monoamine neurotransmitters. The mechanism by which hyperforin has been shown to act is therefore different from that used by conventional antidepressants, perhaps pointing the way to a new class of antidepressants (Leuner et al. 2007). Hyperforin also increases the number of 5-HT receptors, as demonstrated by studies on rat brains (Teufel-Meyer and Gleitz 1997), suggesting a possible long-term therapeutic benefit of SJW treatment. Clinical trials also demonstrated that the level of therapeutic effect of SJW extract is directly dependent on the concentration of hyperforin (Laakmann et al. 1998).

11.6.1.2 Clinical Studies

Since the 1980s, dozens of clinical studies have investigated the effectiveness of SJW. Some of the studies compared study populations receiving SJW to those receiving placebo. Others compared study populations receiving SJW to those receiving standard antidepressants. Other studies were three-armed, comparing study populations receiving SJW to a second study population receiving a standard antidepressant and a third receiving placebo.

Several meta-analyses of clinical studies have been conducted since the mid-1990s (Linde et al. 1996; Kim, Streltzer, and Goebert 1999; Linde and Mulrow 1998; Barnes, Anderson, and Phillipson 2001; Linde et al. 2005a,b; Clement et al. 2006; Linde, Berner, and Kriston 2009). Perhaps the most comprehensive—and widely recognized—meta-analyses are the Cochrane Reviews published by Linde and associates (Linde et al. 1996, 1998, 2005a; Linde, Berner, and Kriston 2009). These reviews were performed through computerized searches of several databases, based on published and unpublished trials conducted to that effect. Each review had specific inclusion criteria, with the criteria evolving over time. For example, to be included in their 1998 review, trials had to be randomized, include patients with depressive disorders, compare preparations that included SJW with placebos or other antidepressants, and include an objective clinical assessment of symptoms. Their analysis included 27 trials spanning a total of 2291 patients. Their 2005 review mandated that the trials be double-blind, administration of SJW with other plant extracts be avoided, trials have an SJW intervention of at least 4 weeks, and trials use only standard antidepressants (Linde et al. 2005b). A total of 37 trials, spanning 3405 patients, met their criteria. Conversely, in their 2009 review, trials were restricted to those including patients with major depression. A total of 29 trials with 5489 patients met those criteria.

Linde and Mulrow (1998) found that preparations containing *H. perforatum* extracts were significantly superior to placebo and as effective as standard antidepressants. Based on that evidence, the authors concluded that extracts of *Hypericum* are more effective than placebo for the short-term treatment of mild to moderately severe depressive disorders. However, they did not see sufficient evidence to establish whether SJW is as effective as other antidepressants.

Barnes, Anderson, and Phillipson (2001) reported on seven additional clinical studies examining monopreparations of SJW. In general, patients receiving SJW extracts reported a greater decrease in depression scores than those taking placebo and similar outcomes to those taking synthetic antidepressants.

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Barnes, Anderson, and Phillipson (2001) noted that the trials comparing SJW to synthetic antidepressants were criticized because the dosages of the latter were unrealistically low. Spira (2001) pointed to the study's usage of somewhat outdated tricyclics and the short (6 week) duration of the analysis.

In stark contrast to results of the mostly German studies conducted in the 1990s, two studies conducted in the United States concluded that SJW was ineffective in treating moderate to major depression. An assessment conducted in 11 academic medical centers in the United States. by Shelton et al. (2001) between 1998 and 2000 encompassed 200 adult outpatients who were diagnosed as having major depression. Their study found that remission rates were low: as much as 14.3% in the treatment versus 4.9% in the placebo control. Shelton et al. (2001) agreed that SJW was a safe and well-tolerated herb, with headache being the only adverse reaction that was higher in the herb group than in the placebo group. However, the authors concluded that SJW was not effective for treating major depression, and even questioned its efficacy for treating moderate depression.

A second study, conducted by the *Hypericum* Depression Trial Study Group (2002), was a double-blind multicenter investigation aimed at determining whether SJW (LI-160) was useful in treating major depression. The study involved 340 adult outpatients at 12 academic and community psychiatric research clinics in the United States. Their study had the benefit of being three-armed, involving comparisons against a control group receiving a placebo, and a second receiving the SSRI sertraline. The proportion of patients showing full or partial response to SJW was 47.6%, which was actually lower than those receiving placebo (55.9%) and sertraline (61.1%). Perhaps most noteworthy was the finding that statistically, response rates did not differ significantly between placebo and either SJW or sertraline. The authors concluded that the findings failed to support any claims of efficacy of *H. perforatum* in treating moderately severe depression. They admitted that their results might have been due to the low assay sensitivity of the trial. However, the three-armed design of the test was significant, because without a placebo group one might conclude that SJW was as effective as an established synthetic antidepressant.

The lack of statistical difference between *Hypericum* and placebo contrasted markedly with the findings of previous German studies that did find such a difference. Likewise, the lack of difference between sertraline and placebo was also striking. Rather than showing a lack of efficacy for either SJW or sertraline, Kupfer and Frank (2002) attributed the lack of statistical difference to an unusually high placebo response. They expressed concerns that variability in placebo response from trial to trial may obscure interpretation of controlled experiments on natural and synthetic antidepressants alike. Linde et al. (2002) speculated that the difference between U.S. and German studies may be due to the latter's focus on patients with mild to moderately severe depression. They suggest that studies may be needed to determine whether SJW appears to be particularly effective in Germany, and not elsewhere.

In their subsequent Cochrane Review that included studies up to May 2004, Linde et al. (2005a) observed that the effectiveness of SJW extracts depended on the size of and the setting in which the trial is located. Trials that were more likely to show a clear benefit of SJW over placebo were small (defined as below the median of the variance), excluded patients with major depression, were conducted in German-speaking countries (Germany, Switzerland, or Austria), and were published before 1995. Larger, more recent trials restricted to patients having major depression and those conducted in the United States, the United Kingdom, France, and Sweden revealed only minor benefit of SJW over placebo. No difference between SJW and synthetic antidepressants—tricyclics or SSRIs—was noted. Compared to those receiving synthetics, patients receiving SJW reported fewer adverse effects and stayed in the trials at a higher rate. The latest Cochrane Review, limited to trials with individuals showing symptoms of major depression (Linde, Berner, and Kriston 2009), again showed heterogeneity for individuals given SJW compared to those given placebo. Nonetheless, the results showed a clear benefit to those receiving *Hypericum*. As noted in their previous Cochrane Reviews, the differential was more striking for smaller trials in German-speaking countries than for larger ones conducted in areas where German is not the prevailing language. No difference

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was observed between individuals given extracts of SJW and those given synthetic SSRIs. Linde, Berner, and Kriston (2009) reported few adverse effects of SJW administration, although they cautioned against the interactions of SJW with other prescribed drugs.

Studies reporting clinical trials published since the July 8, 2008 cutoff for the Linde, Berner and Kriston (2009) review also showed an advantage over placebo or similar benefits as observed for synthetic antidepressants (Kasper et al. 2008a,b; Brattström 2009; Rahimi, Nikfar, and Abdollahi 2009).

Thus, despite the negative findings of the *Hypericum* Depression Trial Study Group (2002), there is consensus among the research studies published to date that indicates the administration of *H. perforatum* is clearly helpful for treating mild to moderate depression. The findings of the most recent Cochrane Review (Linde, Berner, and Kriston 2009) also point to SJW's efficacy when the herb is given to patients experiencing major depression. Readers are cautioned, however, that the benefits are only applicable to standardized extracts of SJW such as LI 160, WS 5570/2, and ZE 117. All the recent clinical trials mentioned in the literature suggest that *Hypericum* is more tolerable than synthetic antidepressants as it causes fewer side effects and also shows similar adverse reactions as seen in placebo-controlled groups. This makes SJW an attractive option for treating depression.

11.6.2 Antibacterial and Antiviral Properties

As noted in Section 11.1, extracts of *H. perforatum* have been used over thousands of years to treat cuts, abrasions, and other wounds. Its usefulness in reducing inflammation is well known, and appears to be related, at least in part, to its ability to serve as an antibacterial agent. Recent research also suggests that it is useful in combating viruses.

Antibacterial properties of *H. perforatum* extracts were reported by Russian scientists in 1959 (Schempp et al. 1999). The main antibacterial component was determined to be hyperforin (Bystrov et al. 1975). Studies show that hyperforin inhibits the growth of certain types of microorganisms. Growth inhibition occurred for all gram-positive bacteria tested, although no growth-inhibitory effects were seen in the gram-negative bacteria tested (Bystrov et al. 1975). Methicillin-resistant (MRSA) and penicillin-resistant (PRSA) *Staphylococcus aureus* were especially susceptible to hyperforin. The MRSA strain was shown to be resistant to several types of penicillins, ofloxacin, clindamycin, erythromycin, cephalosporins, and gentamicin (Bystrov et al. 1975).

Extracts of SJW have long been regarded as being effective against various classes of viruses. Studies by Mishenkova et al. (1975) indicated that flavonoid and catechin-containing fractions of SJW are active against influenza virus. Since 1988, the virucidal activities of hypericin extract have been investigated against many other forms of viruses (Diwu 1995). Hypericin compounds are effective against enveloped viruses, but not nonenveloped viruses (Diwu 1995), particularly when activated by light (Carpenter and Kraus 1991; Hudson, Harris, and Towers 1993; Hudson, Graham, and Towers 1994). Hypericin inactivates enveloped viruses at different points in the viral life cycle (Lenard, Rabson, and Vanderoef 1993). Degar et al. (1992) suggested that hypericin inactivates enveloped viruses by altering viral proteins, and not nucleic acids as targeted by antiviral nucleosides. Hypericin also inhibits the ability of viruses to fuse with cell membranes (Degar et al. 1992; Lenard, Rabson, and Vanderoef 1993), which may explain why hypericin inactivates enveloped viruses rather than nonenveloped ones.

These promising in vitro results have begun to promote various in vivo studies of certain viruses in mice. These include LP-BMS murine immunodeficiency viruses, murine cytomegalovirus (MCMV), Sindbis virus, Friend virus, and Ranscher leukemia virus (Hudson, Lopez-Bazzocchi, and Towers 1991; Meruelo 1993; Stevenson and Lenard 1993). Hypericin also shows in vitro activity against influenza and herpes viruses (Tang et al. 1990), vesiculostomatitis and Sendai viruses (Lenard, Rabson, and Vanderoef 1993), and duck hepatitis B virus (Moraleda et al. 1993). Hypericin is used to inactivate several enveloped viruses present in human blood and to treat acquired immunodeficiency syndrome (AIDS) patients (Holden 1991; Meruelo 1993). Working with the human immunodeficiency virus (HIV), Degar et al. (1992) observed changes in the p24 protein and the

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p24-containing *gag* precursor, p55. They also observed that a recombinant p24 formed an anti-p24 immunoreactive material. This indicated the occurrence of alterations of p24, and such alterations may be able to inhibit the release of reverse transcriptase activity. In contrast, in a phase I clinical trial, Gulick et al. (1999) found that hypericin had no beneficial effect when administered to 30 HIV-infected patients with CD4 counts <350 cells/mm^3. More recently, Maury et al. (2009) found that 3-hydroxy lauric acid in field-grown *H. perforatum* has anti-HIV activity. They assert that such anti-HIV activity can be developed into inexpensive therapies, expanding the current arsenal of antiretroviral agents.

Regarding other viruses, a noteworthy finding is that hypericin completely inactivated bovine diarrhea virus (BVDV) in vitro in the presence of light (Prince et al. 2000). Conversely, Jacobson et al. (2001) found that in the doses studied, hypericin demonstrated no effect on hepatitis C virus.

11.6.3 Anticancer Properties

Hyperforin and hypericin have also been examined for their anticancer properties. According to Schempp et al. (2002), hyperforin inhibits tumor cell growth in vitro. The mechanism involves induction of apoptosis (programmed cell death) through the activation of caspases, which are cysteine proteases that trigger a cascade of proteolytic cleavage occurrences in mammalian cells. Hyperforin also causes the release of cytochrome *c* from isolated mitochondria. Mitochondrial activation is an early event in hyperforin-mediated apoptosis, and hyperforin inhibits tumor growth in vivo (Schempp et al. 2002). Schempp and his colleagues agreed that since hyperforin has significant antitumor activity, is readily available in high quantities (since it is naturally occurring in abundance), and has low toxicity in vivo, hyperforin holds promise of being an interesting novel antineoplastic agent. Other in vitro studies demonstrated that hyperforin in conjunction with polyphenolic procyanidin B2 effectively inhibited the growth of leukemia K562 and U937 cells, brain glioblastoma cells LN229, and normal human astrocytes (Hostanska et al. 2003).

Hypericin has also been investigated as an anticancer agent, reportedly inhibiting the growth of cells derived from a variety of neoplastic tissues, including glioma, neuroblastoma, adenoma, mesothelioma, melanoma, carcinoma, sarcoma, and leukemia (Fox et al. 1998). The activity of hypericin is attributed to its photodynamic properties (Agostinis et al. 2002). In the presence of light and oxygen, hypericin acts as a powerful natural photosensitizer, generating superoxide radicals that form peroxide or hydroxyl radicals, or singlet oxygen molecules that kill tumor cells. In this way, hypericin can be used as a component of photodynamic therapy (PDT; Agostinis et al. 2002). At first, PDT was used only for skin lesions, but it is becoming increasingly accepted as a treatment for many types of tumors.

Fox et al. (1998) found that hypericin photoactivated with white light or ultraviolet light or both could induce nearly complete apoptosis (94%) in malignant cutaneous T cells and lymphoma T cells. Similarly, Olivo, Du, and Bay (2006) suggested its applicability in treating nasopharyngeal cancer. In vitro studies have found that hypericin works synergistically with the anticancer agent 5-aminolevulinic acid (5-ALA) to stop formation of human esophageal cancer cells (Höpfner et al. 2003), and human endometrial cancer cells (HEC-1A; Schneider-Yin et al. 2009) when exposed to white light. Hypericin and 5-ALA work synergistically to induce protoporphyrin IX (PpIX), which, after conversion to porphyrin, converts oxygen to more reactive species that kill cancer cells (Schneider-Yin et al. 2009). Exposing tumors cells to hypericin in conjunction with laser irradiation led to toxic effects on human prostatic cancer cell lines (Colasanti et al. 2000), human urinary bladder carcinoma cells (Kamuhabwa et al. 2000), and pancreatic cancer cell lines (Liu et al. 2000) in in vitro systems. Experiments using nude mice receiving implants of pancreatic cancer cells and human squamous carcinoma cells showed decreases in cancer proliferation following laser PDT using hypericin (Chung et al. 2000; Liu et al. 2000; Barnes, Anderson, and Phillipson 2001).

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PDT with hypericin promises to treat both nonmelanoma and melanoma skin cancer cells. A pilot study conducted by Kacerovská et al. (2008) found that topical application of *H. perforatum* extract followed by irradiation with red light induced partial response in patients suffering from actinic keratosis, basal cell carcinoma (BCC), and Bowen's carcinoma in situ. However, all patients complained of burning and pain sensations during irradiation (Kacerovská et al. 2008). When working with cultured melanoma cells, Davids et al. (2008) found that ultraviolet A (UVA)-activated hypericin led to cell death, but only when cells were exposed to a higher dose of 3 μM for 4 hours. Interestingly, hypericin was found to kill melanoma cells via two mechanisms that depended on pigmentation. In pigmented cells, hypericin increases the concentration of reactive oxygen species that cause melanosomes to leak toxic melanin precursors into the cytoplasm, resulting in necrotic death. In contrast, intracellular accumulation of hypericin induces a mitochondrial-associated caspase-dependent apoptotic mode of cell death in nonpigmented melanoma cells (Davids et al. 2008).

Hostanska et al. (2003) showed that hyperforin and hypericin work synergistically to impede the growth of leukemic (K562, U937) cells. Additional studies reveal that use of hypericin alone has only a weak inhibitory effect on cancerous cell growth, whereas methanolic extract of SJW together with hypericin leads to long-lasting inhibition of cell growth, induces apoptosis, and decreases phototoxicity (Roscetti et al. 2004; Schmitt et al. 2006). Although hyperforin and hypericin both show promise as anticancer agents, more research is clearly needed to evaluate their efficacy, mode of action, and adverse interactions.

11.6.4 Antioxidant and Neuroprotective Properties

Recent research shows that extracts of *H. perforatum* decrease oxidative stress and consequently prevent neurotoxicity, inflammation, and gastrointestinal problems. Flavonoid-rich extracts of *H. perforatum* (FEHP) are effective against hydrogen peroxide–induced apoptosis in PC12 cells (a cell line derived from the pheochromocytoma of the rat adrenal medulla). Standard extracts of *H. perforatum* can prevent DNA fragmentation and shrinkage of cells as a result of hydrogen peroxide activity (Lu et al. 2004). Thus, FEHP may effectively treat oxidative stress–related neurodegenerative disorders such as Parkinson's and Alzheimer's diseases (Zou et al. 2010).

Silva et al. (2008) found that the flavonols quercetin and kaempferol provide neuroprotective action by decreasing oxidation of the mitochondrial lipid membrane and maintaining mitochondrial transmembrane electric potential. Conversely, they noted that biapigenin primarily affects mitochondrial bioenergetics and lowers the ability of mitochondria to absorb calcium. Studies performed by Mohanasundari et al. (2006) reported that *H. perforatum* extract led to the inhibition of MAO-B activity and decreased astrocyte activation in striatal area of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced mice. Thus, *H. perforatum* serves as a neuroprotective agent against MPTP-induced Parkinson's disease in mice (Mohanasundari and Sabesan 2007). Additional studies showed that the combined use of bromocriptine and *H. perforatum* extract significantly increased dopamine and 3,4-dihydroxyphenylacetic acid, a metabolite of dopamine, in MPTP-induced Parkinson's disease in male Swiss albino mice (Yoshitake et al. 2004; Mohanasundari et al. 2006).

Extracts of SJW protect against cell death caused by amyloid β peptides (Abeta) that form plaques in the brains of those suffering from Alzheimer's disease. Silva et al. (2004) found that ethanolic extracts and those containing flavonol glycosides, flavonol, and biflavone aglycones decreased lipid peroxidation and cell death in rat cultured hippocampal neurons. Flavonoids also enhance survival of microglia (cells that phagocytize foreign bodies in the central nervous system [CNS]) exposed to Abeta (Kraus et al. 2007). Flavonoids decreased formation of amyloid-induced reactive oxygen species in microglia. The flavanols (+)-catechin and (–)-epicatechin from *H. perforatum* increased cell viability and membrane fluidity of microglia (Kraus et al. 2007). Hyperforin also has been reported to disaggregate amyloid deposits in a dose- and time-dependent manner, thus preventing

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Abeta-induced neurotoxicity (Dinamarca et al. 2006). Hence, various components found in extracts of *H. perforatum* may actively combat Alzheimer's disease by mediating Abeta toxicity.

Rats subjected to chronic restraint stress had better recognition memory and working memory, and had significantly enhanced recall of passive avoidance behavior when administered extracts of *H. perforatum* compared to controls not receiving the extracts (Trofimiuk et al. 2005, 2006; Trofimiuk and Braszko 2008). The herb counteracted the negative effects of stress on cognitive function, and significantly improved hippocampus-dependent spatial working memory (Trofimiuk and Braszko 2008). On the other hand, although *H. perforatum* has an antiamnestic effect, studies conducted by Khalifa (2005) and Tadros et al. (2009) proved that SJW decreases prepulse inhibition (PPI) of startle response. Prepulse inhibition is important to filtering sensory information, and disruption of PPI is evident in schizophrenia and Huntington's disease (Khalifa 2005). The SJW extract decreased PPI response possibly through enhancing the transmission of monoaminergic (dopaminergic, serotonergic, and noradrenergic) neurotransmitters in the brain stem, thalamus, cortex, and/or hippocampus (Khalifa 2005). Disruption of PPI was found when SJW was administered on both acute (500 mg/kg for 1 day) and chronic (200 mg/kg for 3 days) regimens. Administration of hyperforin similarly elicited disruption of PPI (Tadros et al. 2009). These results suggest that SJW may have limited utility in treatment of cognitive disturbance disorders such as Huntington's and other psychotic diseases.

Sanchez-Reus et al. (2007) found that SJW extracts decreased oxidative stress in the brains of rats treated with the pro-oxidant rotenone. The effects were attributed to liposomal quercetin that significantly preserved the activities of antioxidant enzymes found in the brain tissue. These results led Sanchez-Reus et al. (2007) to conclude that standardized extracts of *H. perforatum* could be a treatment of choice for depressed elderly patients showing degenerative disorders associated with elevated oxidative stress.

11.6.5 Anti-Inflammatory Activity

H. perforatum shows promise as an anti-inflammatory agent. Rats fed doses of SJW showed decreased levels of blood and bowel enzymes associated with colonic inflammation (Dost et al. 2009), and had lower incidences of gastric ulcers (Cayci and Dayioglu 2009). Sosa et al. (2007) found that lipophilic extracts of *H. perforatum* had greater anti-inflammatory activity than did ethylacetic or hydroalcoholic extracts. Quercetin and I3,II8-biapigenin, the two major oil extracts of SJW, showed particular anti-inflammatory and gastroprotective activity (Zdunic et al. 2009).

Three mechanisms for the anti-inflammatory response of SJW have been proposed. Tedeschi et al. (2003) found that SJW extracts inhibited the expression of proinflammatory genes like cyclooxygenase-2, interleukin 6, and inducible nitric oxide synthase (iNOS). Detailed experiments on the latter system found that *H. perforatum* extracts decreased the activity of janus kinase 2, leading to a series of reactions that inhibited the downregulation of signal transducer and transcription (STAT)-1 α DNA binding, further disrupting gene transcription (Tedeschi et al. 2003). Hammer et al. (2007) found that pseudohypericin and hyperforin inhibited production of the inflammatory agent prostaglandin E2 (PGE2). A subsequent ethanol fractionation of *H. perforatum* extract revealed that four major compounds—chlorogenic acid, amentoflavone, quercetin, and pseudohypericin—acted together to decrease the inflammation caused by PGE2 (Hammer et al. 2008). This four-component system apparently worked only when pseudohypericin was activated in the presence of light. *H. perforatum* extract also decreased inflammation in experimental mice given carrageenan-induced pleurisy (Menegazzi et al. 2006). Extracts acted at multiple levels, particularly with the inhibition of nuclear factor κB (NF-κB) and STAT-3 (Menegazzi et al. 2006).

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11.6.6 Effects as a Wound-Healing Agent

As noted in Section 11.3, *H. perforatum* extract has been used over thousands of years as a wound-healing agent. Controlled studies investigating this claim are surprisingly sparse. Ozturk et al. (2007) found that chicken embryonic fibroblasts exposed to SJW extract demonstrated enhanced collagen production, followed by the polygonal shape activation of fibroblast cells that is responsible for wound closure.

11.6.7 Effects on Reducing Opium Dependence

Hydroethanolic crude extract of *H. perforatum* demonstrated antinociceptive effect against acetic acid–induced abdominal constriction assay in mice, similar to that of opium. This indicates that *H. perforatum* may act by activating the opioid receptors without causing withdrawal symptoms (Subhan et al. 2007). Feily and Abbasi (2009) reported that *H. perforatum* decreased the symptoms of opiate withdrawal in adult Wistar rats. The effectiveness of *H. perforatum* was determined to be equivalent to clonidine, an FDA-approved medication for treating withdrawal symptoms. Thus, *H. perforatum* may effectively treat opiate withdrawal symptoms in humans (Feily and Abbasi 2009).

11.7 ADVERSE EFFECTS AND INTERACTIONS WITH OTHER DRUGS

As with any pharmacologically active substance, although treatments involving SJW are generally safe, adverse effects can arise either when it is used alone or, especially, in conjunction with other medications.

11.7.1 Adverse Effects

As noted in Section 11.6.1.2, normal dosages of SJW have relatively few side effects. In general, the most common adverse effects are gastrointestinal symptoms, allergic reactions, dizziness, confusion, restlessness, lethargy, and dryness of the mouth (Barnes, Anderson, and Phillipson 2001; Greeson, Sanford, and Monti 2001). These effects are generally mild, moderate, or transient (Ernst et al. 1998). Meta-analyses of clinical trials revealed that reports of adverse effects and drop-out rates were less than 2.5% for patients receiving SJW, far lower than for those receiving conventional antidepressants (Ernst et al. 1998; Schulz 2006). Extracts have also been found to lack genotoxic potential and mutagenic activity, based on in vivo and in vitro studies (Barnes, Anderson, and Phillipson 2001). However, isolated instances of acute toxic neuropathy and induced mania have been reported (Bove 1998; Nierenberg et al. 1999). Hypericin has a unique phototoxic effect that can result in photodermatitis when taken in high doses. The toxic effects are attributed to an acidification of the surrounding environment caused by the transfer of hydrogen between hydroxyl groups on receiving light energy (Fehr, McCloskey, and Petrich 1995; Sureau et al. 1996). Excessive cutaneous phototoxicity was observed with high doses of hypericin (0.5 mg/kg of body weight) associated with AIDS treatments (Gulick et al. 1999). Normal doses of SJW taken for mild depression do not have any significant associated phototoxic effects (DerMarderosian and Beutler 2002; Jellin et al. 2002).

Recent research using in vitro approaches suggests that use of SJW may have phototoxic effects on the eye. Human lens epithelial cells (He et al. 2004) and human retinal pigment epithelial cells (hRPEs; Wielgus et al. 2007) were both damaged by exposure to a combination of hypericin and light in the visible or UV ranges. Schey et al. (2000) found that hypercin and light combined to cause damage to lens α-crystallin proteins. However, no adverse effects of SJW to vision have been reported in a clinical setting. Nonetheless, individuals taking SJW should take normal precautions to protect their eyes against excessive exposure to sunlight.

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11.7.2 Drug Interactions: Overview

Although adverse drug reactions are relatively rare for individuals taking SJW as a stand-alone supplement, the daily dose becomes an issue of concern when the user is also taking other medications that potentially interact with the constituents of SJW. Preparations made from the species have been reported to interact with a diverse selection of drugs that are explored in excellent reviews (Barnes, Anderson, and Phillipson 2001; Greeson, Sanford, and Monti 2001; Izzo 2004; Mannel 2004; Di et al. 2008; Zhou and Lai 2008). The herb in some cases has been shown to increase the effectiveness of other compounds when taken together. This increase may be helpful to the individual or may increase the compound's effects to toxic levels. Conversely, the herb may decrease or even cancel the effects of other drug constituents (Parker et al. 2001). Mechanisms responsible for the interactions have been clarified over the past 15 years. As has been widely reported (e.g., Izzo 2004; Di et al. 2008; Zhou and Lai 2008), SJW extract induces P-glycoprotein (P-gp) along with a series of drug-metabolizing enzymes, the cytochrome P450s (CYPs), including CYP2C9 and CYP3A4.

11.7.2.1 P-glycoprotein

P-glycoprotein is an adenosine triphosphate (ATP)-binding cassette (ABC) transporter belonging to the multidrug resistance/transporter associated with antigen processing (MDR/TAP) subfamily (Higgins 1992; Garrigues, Escargueil, and Orlowski 2002). It is distributed and expressed in the intestinal epithelium, hepatocytes, renal proximal tubular cells, and capillary endothelial cells. As a member of the MDR/TAP subfamily, it is involved in multidrug resistance and transports various molecules across extra- and intracellular membranes (Dean, Hamon, and Chimini 2002). In humans, P-gp is encoded by the *MDR1* (*ABCB1*) gene (Ueda et al. 1987; Zhou 2008). The encoded protein is an ATP-dependent drug efflux pump for xenobiotic constituents with broad substrate specificity.

Extracts of SJW have been widely observed to induce P-gp expression. These studies have been conducted in vitro, in rats and mice, and clinically (Durr et al. 2000; Hennessy et al. 2002; Dresser et al. 2003). Expression has been observed in P-gp from various organs and tissues including intestinal cells (Durr et al. 2000), peripheral blood lymphocytes (Hennessy et al. 2002), and intestinal carcinoma cells (Perloff et al. 2001). Hypericin was the main constituent responsible for the stimulation of P-gp activity (Mannel 2004). Conversely, Tian et al. (2005) found that hyperforin increased the expression of P-gp in LS 180 intestinal carcinoma cells, whereas hypericin had no effect. Removal of SJW resulted in a restoration of normal P-gp level within 48 hours (Tian et al. 2005).

11.7.2.2 Cytochrome P450

The CYP comprises a large and diverse superfamily of hemoprotein enzymes common to nearly all taxa. They metabolize multiple exogenous and endogenous substrates and catalyze a diverse array of reactions, especially in the liver, where they metabolize drugs and toxic compounds (von Moltke et al. 1995; Zhou 2008). A wide variety of CYP isotypes occur within individual organisms and across taxa. The nomenclature involves use of a three-character code following the CYP prefix. The first character is a number that refers to the family, the following capital letter indicates the subfamily, and the second number refers to the specific gene (Nelson et al. 1993). Thus, the common CYP3A4 belongs to family 3, subfamily A, gene 4.

The SJW extract has been widely found to induce a variety of CYP isotypes. Increases in the activity of CYP3A, and especially CYP3A4, are particularly well documented (reviewed by Madabushi et al. 2006; Whitten et al. 2006; Zhou and Lai 2008). Gurley et al. (2002) found increases in CYP3A4 to be especially noticeable in females. Specific cell types in which induction has been noticed include intestinal and hepatic cells from mice, rats, and humans (Durr et al. 2000; Moore et al. 2000; Bray et al. 2002b; Cantoni et al. 2003; Komoroski et al. 2004). The SJW extracts were also shown to induce other CYP isotypes including CYP1A2 (Nebel et al. 1999), CYP2C9 (Obach 2000; Komoroski et al. 2004), CYP2C19 (Wang et al. 2004), CYP2D6 (Obach 2000), and CYP2E1 (Bray 2002b; Gurley et al. 2002). Wenk, Todesco, and Krähenbühl (2004) found that

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extracts successfully induced CYP1A2 in females, but not in males. Conversely, extracts failed to induce or inhibited expression of other isotypes of CYP, including CYP1A or CYP2D6 (Bray et al. 2002b; Komoroski et al. 2004; Wenk, Todesco, and Krähenbühl 2004). Interestingly, Obach (2000) found that SJW inhibited CYP3A4, but only in an in vitro system. When working with isolated hepatocytes, Komoroski et al. (2004) found that acute administration of hyperforin at 5 and 10 μM inhibited CYP3A4 activity.

Of the various SJW constituents, hyperforin appeared to be the most effective in activating CYP isotypes (Komoroski et al. 2004; Pal and Mitra 2006; Whitten et al. 2006). Pal and Mitra (2006) also found that the flavonoids kaempferol and quercetin showed enhanced expression of CYP3A4 messenger ribonucleic acid (mRNA) in Caco-2 cells. In contrast, hypericin had no effect on any of the CYP isotypes tested (Komoroski et al. 2004). The ability of hyperforin to induce CYP3A4 and other isotypes depends on the duration and dosage of exposure. Bray (2002a, b) observed no induction of hepatic CYP2E1 and CYP3A in mice after 4 days of treatment, whereas 3 weeks of dosing led to increased expression of both genes. In humans, the effect of CYP3A induction is strongly influenced by SJW dose (Mueller et al. 2006). Likewise, Mueller et al. (2009) found that administration of an SJW product with a low hyperforin content led to clinically irrelevant levels of induction of CYP3A. In their review, Whitten et al. (2006) recommend more studies should be conducted to determine whether decreased CYP3A induction occurs after giving low-dose hyperforin extracts.

A mechanism by which extracts of SJW, particularly hyperforin, induce some cytochromes has been proposed (Figure 11.3). The pathway starts with the human pregnane X receptor (PXR), which is a member of the orphan nuclear receptor family (Moore et al. 2000; Mannel 2004), and is encoded by the NR1I2 gene (Zhou and Lai 2008). The PXR functions as a transcriptional regulator for several genes, including those that code for the CYP enzymes and various drug transporters such as P-gp, which is involved in the detoxification and transport of multiple xenobiotics (Wang et al.

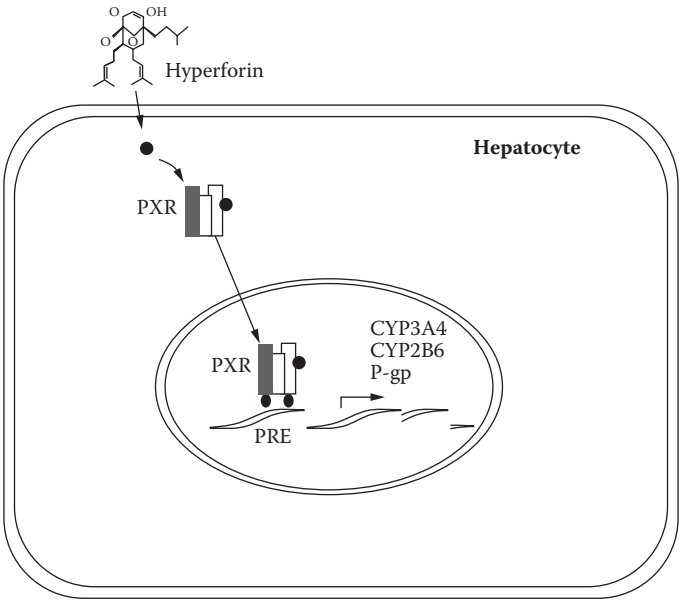


FIGURE 11.3 Diagram of the pathway of gene induction by the St. John's wort constituent, hyperforin, in a hepatocyte. Hyperforin enters the cell and binds to the human pregnane X receptor (PXR) in the cytoplasm. The complex enters the nucleus, binding with the pregnane response element (PRE). The PXR/PRE complex induces the downstream gene targets CYP3A4, CYP2B6, and MDR1, producing the enzymes cytochrome P450 (CYP) 3A4, CYP2B6, and P-glycoprotein (P-gp). (Reprinted from Zhou, S. F., and X. Lai. 2008. *Curr Drug Metab* 9(5):394–409, Bentham Science Publishers Ltd. With permission.)

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2004; Zhou and Lai 2008). Intracellular hyperforin binds with PXR to produce a functional complex that binds to the pregnane response element (PRE) of CYP3A4, CYP2B6, and MDR1 genes. The PXR/PRE complex then activates and initiates the targeted genes, producing active CYP3A4, CYP2B6, and P-gp (Zhou and Lai 2008). Because of this process, SJW has significant interaction with any drug metabolized by CYP3A4 and CYP2B6 or transported by P-gp.

11.7.2.3 Specific Drug Interactions

In this section, specific drugs reported to interact with SJW are reviewed briefly. Readers seeking additional details should consult the excellent reviews available, including, but not limited to, those by Henderson et al. (2002), Izzo (2004), Di et al. (2008), and Zhou and Lai (2008).

Fexofenadine is a nonsedating antihistamine used for treating allergic rhinitis and urticaria (Markham and Wagstaff 1998). Clinical studies have found that the effects of SJW on fexofenadine vary depending on dose and duration. A single treatment caused a 45% increase in the maximum plasma concentration, presumably by inhibiting P-gp (Wang et al. 2002). Conversely, a 12-day treatment caused a 35% decrease in Cmax (maximum plasma concentration; Wang et al. 2002) and a 60% increase in oral clearance of fexofenadine (Dresser et al. 2003), suggesting P-gp induction.

Digoxin and other members of the digitalis family of cardioactive drugs from the *Digitalis* (foxglove) plant also show side effects when used in conjunction with SJW. Whereas a single dose of SJW had no measureable impact on digoxin pharmokinetics, a 10-day treatment of SJW extract showed decreased Cmax and AUC (area under the plasma concentration–time curve) of digoxin compared to placebo (Johne et al. 1999). However, Mueller et al. (2004) and Arold et al. (2005) found that patients who were administered low-hyperforin preparations of SJW had little in the way of decrease in digoxin AUC. Thus, the pharmacokinetic effect is apparently due to the induction of intestinal P-gp caused by multiple doses of SJW high in hyperforin.

The anticancer drug imatinib mesylate (Gleevec) is a strong inhibitor of specific tyrosine kinases that promote tumor cell proliferation (Collins and Workman 2006). Imatinib is transported by P-gp and metabolized by CYP3A4 and CYP3A5 (Peng, Lloyd, and Schran 2005). Two studies have shown SJW interacts with imatinib. Frye et al. (2004) found that 2 weeks of SJW treatment on 10 healthy subjects decreased the AUC by 32%. When working with 12 healthy subjects receiving SJW extracts, Smith et al. (2004), likewise, found increased clearance and reduced AUC and Cmax for imatinib.

SJW has been shown to affect the pharmacokinetics of the drug amitriptyline, a tertiary amine tricyclic antidepressant metabolized in the liver by CYP3A4, CYP2C19, and CYP2D6 (Venkatakrishnan, von Moltke, and Greenblatt 1999). A clinical study of 12 patients taking both SJW and amitriptyline exhibited a decrease in AUC as well as decreases in plasma concentrations (Johne et al. 2002).

Ivabradine is a selective sinus node channel inhibitor that decreases cardiac pacemaker activity, allowing for slower heart contractions and more time for blood flow. Intestinal and hepatic CYP3A4 metabolizes ivabradine (Zhou and Lai 2008). In 12 volunteers given SJW for 14 days, the AUC of ivabradine was lowered by 61.7%, while the maximum plasma concentration was lowered by 52.9% (Portoles et al. 2006).

Cyclosporin and tacrolimus are immunosuppressants commonly used to prevent the body from rejecting transplanted organs (Akhlaghi and Trull 2002; Zhou and Lai 2008). Cyclosporin and tacrolimus are metabolized by CYP3A4 and P-gp (Hebert 1997; Lown et al. 1997; Niwa et al. 2007; Zhou 2008). Concerns over the ability of SJW to interfere with cyclosporin first surfaced with clinical reports of patients receiving transplants (Barone et al. 2000; Mai et al. 2000; Ruschitzka et al. 2000; Ernst 2002). In those cases, patients demonstrated improved immunosuppression once SJW therapy was stopped. Larger studies involving 30 (Breidenbach et al. 2000) and 11 (Bauer et al. 2003) recently transplanted patients found that plasma levels of cyclosporin dropped when receiving SJW treatment, and that cyclosporin levels rose sharply when SJW was stopped. Mai et al. (2003) found that SJW extracts containing high levels of hyperforin induced lower plasma Cmax and AUC

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than extracts having low hyperforin content. Other studies indicate that patients on other immunosuppressants, such as tacrolimus, experience similar effects (Mai et al. 2003).

Antihypertensive agents have also been shown to interact with SJW. The calcium channel blocker, verapamil, is first-pass metabolized by CYP3A4 (Tannergren et al. 2004). Eight males administered SJW for 14 days showed a 78–80% decrease in AUC and a 76–78% decrease in the Cmax of verapamil, compared to the control (Tannergren et al. 2004). Clinical studies on nifedipine (Wang et al. 2007) and talinolol (Schwarz et al. 2007) revealed similar decreases in their AUC and Cmax values, although the latter may be attributed more to P-gp than CYP3A4 induction.

SJW has been shown to interact with benzodiazepine sedatives, including quazepam, alprazolam, and midazolam (Zhou and Lai 2008). All are metabolized by CYP3A4, although other CYPs play a role (Gorski et al. 1994; von Moltke et al. 1996; Miura and Ohkubo 2004). Studies conclude that interactions with SJW from a 14-day treatment decrease the AUC and Cmax values for the drugs as well as reduce oral clearance and bioavailability (Kawaguchi et al. 2004). The proposed mechanism for the decreased pharmacokinetics is CYP3A4 induction, although SJW did not reduce the sedative effect of quazepam.

The use of SJW may decrease concentrations and nullify the effect of steroid contraceptives that are substrates for—and inducers of—CYP3A4 (Thummel and Wilkinson 1998). Moreover, the major component of oral contraceptives, ethinylestradiol, is metabolized by CYP3A4 (Guengerich 1988). An analysis conducted by Hall et al. (2003) found that SJW administered concomitantly with a combination oral contraceptive pill containing ethinylestradiol and norethindrone to 12 healthy premenopausal women caused an increase in the oral clearance of norethindrone and a significant reduction in the half-life of ethinylestradiol, consistent with increased CYP3A activity. However, they found that SJW did not affect the serum concentrations of follicle-stimulating hormones, luteinizing hormones, and progesterone. The authors noted a higher incidence of breakthrough bleeding during the SJW administration phase, a problem also observed by others (Ernst 1999; Yue, Bergquist, and Gerden 2000; Schwarz, Buschel, and Kirch 2003). Breakthrough bleeding often leads to the discontinuation of oral contraceptive use, occasionally resulting in unintended pregnancy (Rosenberg and Waugh 1998). Once SJW was discontinued, menstrual cycles returned to normal; however, alternate forms of birth control are suggested if the use of SJW is continued (Schwarz, Buschel, and Kirch 2003).

SJW can decrease the serum levels of two main classes of anti-HIV compounds: (1) protease inhibitors and (2) nonnucleoside reverse transcriptase inhibitors (NNRTIs), when taken concomitantly. In both cases, the effect is largely due to induction of CYP3A4 (Zhou 2008). An analysis of eight healthy subjects found a 57% decrease in the plasma concentration of the protease inhibitor indinavir (Piscitelli et al. 2000). A follow-up study by Ho et al. (2009) found that SJW extract administered to rats stimulated the ability of hepatic and intestinal CYP3A to lower indinavir plasma levels. Decreases in NNRTIs, particularly nevirapine and lamivudine, were also found when coadministered with SJW. In contrast, a study by L'Homme et al. (2006) found that SJW did not lower the concentration of nevirapine when administered to a small group of healthy women.

Warfarin and related compounds are widely used anticoagulants synthesized from coumarin, a benzopyrone found naturally in several plants (Runciman et al. 2002; Yarnell and Abascal 2009). Warfarin is a vitamin K inhibitor, and is used to treat clotting disorders like thrombosis and embolism, as well as myocardial infarction, atrial fibrillation, and stroke (Bovill, Fung, and Cushman 2004). Claims of interactions between SJW and coumarin-derived anticoagulants were first made in cases observed in Sweden (Ernst et al. 1998 Yue, Bergquist, and Gerden 2000). Subsequent controlled studies found that healthy males given SJW had higher clearances of anticoagulant than the control group (Jiang et al. 2004; Jiang, Blair, and McLachlan 2006). Since at least one enantiomer of warfarin is metabolized by CYP2C9, Zhou and Lai (2008) suggest the induction of that enzyme by either hyperforin or hypericin in SJW contributes to the clearance of warfarin.

The antifungal agent, voriconazole, is metabolized mainly by CYP2C19, and also by CYP3A4 and CYP2C9 (Hyland, Jones, and Smith 2003). In a study of 16 men, concomitant SJW treatment

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for 15 days was found to decrease the AUC of voriconazole by 59%, as well as increasing its oral clearance (Rengelshausen et al. 2005).

SSRIs are commonly used compounds to treat depression, anxiety, and personality disorders, and are primarily metabolized by CYP2D6 (Zhou and Lai 2008). The use of SJW with antidepressants containing paroxetine, sertraline, venlafaxine, nefazodone, clomipramine, and others can create serotonin syndrome, which is characterized by shivering, tachycardia, hypertension, hyperactive bowel sounds, diaphoresis, and hyperthermia (Gordon 1998; Lantz, Buchalter, and Giambanco 1999; Beckman, Sommi, and Switzer 2000; Parker et al. 2001; Boyer and Shannon 2005). Research suggests that CYP and P-gp induction is not the underlying mechanism behind the interaction. The SSRIs are mainly metabolized by CYP2D6, but the cause of the additive effect is uncertain (Zhou and Lai 2008). However, the combination of SSRIs and SJW should be avoided until the safety profile can be fully defined.

The use of SJW concomitantly with other herbs taken as dietary supplements may lead to other interactions. The documented cases of concomitant use of SJW with herbs that have sedative properties where the combination may have enhanced both the therapeutic as well as the adverse effects include interactions with calamus, canendula, California poppy, catnip, capsicum, celery, couch grass, elecampane, Siberian ginseng, German chamomile, goldenseal, gotu kola, hops, Jamaican dogwood, kava, lemon balm, sage, sassafras, scullcap, shepherd's purse, stinging nettle, valerian, wild carrot, wild lettuce, ashwaganda root, and yerba mensa (Jellin et al. 2002).

11.8 FUTURE RESEARCH NEEDS

Despite the many dozens of clinical, in vivo, and in vitro studies conducted on the medicinal attributes of SJW, unanswered questions remain regarding its therapeutic value, mechanisms of action, and adverse interactions. A particularly perplexing question relates to its therapeutic value in treating depression. The literature over the past 15 years contains conflicting evidence regarding the usefulness of the species in treating major depression. What is more, the persistent difference in demonstrated efficacy between studies conducted in German-speaking and non-German-speaking countries remains an issue. We agree with Linde, Berner, and Kriston (2009) that future work must focus on resolving this apparent contradiction. Additional research is needed regarding the therapeutic value of SJW in treating other diseases like cancers, skin wounds, and opium dependence. Moreover, the potential of specific constituents like hypericin, hyperforin, and flavonoids deserve further elucidation.

Regarding adverse impacts, we note that reports of interactions with some drugs (e.g., warfarin) are based on repeated independent studies involving clinical observations, controlled assessments of dozens of individuals, and in vivo tests. Conversely, the evidence for interactions with a few other drugs (e.g., ivabradine, protease inhibitors, vorconizole) appears to be based on a single study of perhaps a dozen individuals. Further work to at least determine replicability seems in order. We also agree with Zhou and Lai (2008) that additional research is needed to investigate whether different formulations of SJW (e.g., low vs. high hyperforin content) result in different adverse interactions.

11.9 CONCLUSIONS

SJW has a long history of use as an herbal treatment for a variety of ailments. Over the past 20 years, it has become a mainstream alternative treatment for depression, as well as holding promise as a therapy for cancer, inflammation, bacterial and viral infections, and other disorders. Thanks to its popularity, the effectiveness of SJW has been intensively studied since the mid-1980s. These studies have focused on the pharmacology of its constituents and on clinical trials. Pharmacological investigations show that extracts of SJW do have neuroactive properties. Interestingly, such properties appear to derive primarily from the constituent hyperforin, rather than from hypericin, which has been investigated for a longer period of time.

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Numerous studies conducted in Germany in the 1990s indicate that individuals showing mild to moderate depression who take SJW show improvement in mood at rates higher than those taking a placebo. Moreover, the rates of improvement were seen as being similar to those experienced by individuals taking synthetic antidepressants. However, the clinical studies comparing the effects of SJW against synthetics were criticized on the basis of their short durations of study and a claim that the dosage of the synthetics was below the typical dosage. Studies conducted in the United States in 2001 and 2002 failed to show a clear benefit of SJW over a synthetic antidepressant and a placebo. However, studies and meta-analyses conducted since then point to the efficacy of SJW in treating all forms of depression. It is interesting to note that the greatest clinical success for the herbal remedy was achieved in Europe, where the use of herbal therapies is standard, compared to the findings for the remedy in the United States, where reliance on synthetic medicines is highest.

SJW does appear to be an effective treatment for other disorders, particularly some skin ailments, inflammation, and certain forms of cancer. It has demonstrated antibacterial and antiviral activity, although its promise as an anti-HIV therapy is questionable. Recently, its antioxidant and neuroprotective properties have become recognized. Most clinical studies show SJW to be relatively safe, especially at typical dosages. However, high dosages might lead to phototoxicity in susceptible individuals. Extracts of SJW do appear to interact with other medications, especially drugs that impact liver and intestinal enzyme function. Therefore, individuals taking SJW along with other medications should be aware of such potential drug interactions, and should report their use to their health-care providers.

As with any herbal remedy, any individual taking SJW is advised to consult his or her physician to ascertain that the herb is the best course of action when seeking the most efficacious remedy for any condition.

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12 Health Benefits of Tea

Mauro Serafini, Daniele Del Rio, Yao Denis N'Dri, Saverio Bettuzzi, and Ilaria Peluso

CONTENTS

12.1 Introduction 239
12.2 Tea: History and Origin 240
12.3 Tea: How Many Use It, Trade Volume, Way of Intake, Preparation, and Processing 240
12.4 Active Ingredients and Mechanisms of Action 240
12.4.1 Catechins in Tea and Their Bioavailability 240
12.4.2 Antioxidant Activity: Human Studies 242
12.4.3 Antioxidant Activity: Molecular Aspects 246
12.4.4 Anti-Inflammatory Activity: Molecular Aspects 248
12.4.5 Anti-Inflammatory Activity: Human Study 250
12.4.6 Antiviral Activity of Green Tea Catechins (Veregen) 251
12.5 Health Effects: The Scientific Evidence 251
12.5.1 Anticancer Activity of Green Tea 251
12.5.2 Green Tea Catechins Extract against Human Prostate Cancer 252
12.5.3 Tea and Cardiovascular Diseases 253
12.6 Adverse Effects of Green Tea Catechins Extract Administration 254
12.7 Research Needs 254
12.8 Conclusions 255
References 255

12. 1 INTRODUCTION

Tea is one of the most popular drinks due to its pleasant taste and perceived health effects. Although health benefits have been attributed to tea consumption since the beginning of its history, scientific investigation of this beverage and its constituents has been under way for about 30 years (McKay and Blumberg 2002; Gardner, Ruxton, and Leeds 2007). Consumption of tea, in particular green tea (GT), has been correlated with low incidence of chronic pathologies in which oxidative stress has been reported to be involved, such as cancer (Chung et al. 2003; Butt and Sultan 2009) and cardiovascular diseases (CVDs; Stangl et al. 2007; Babu and Liu 2008).

The health benefits ascribed to the consumption of teas may be related to the high content of bioactive ingredients such as polyphenols. Polyphenols have been reported to possess antioxidant, antiviral, and anti-inflammatory activities; modulate detoxification enzymes; stimulate immune function and decrease platelet aggregation (Lampe 2003; Frankel and Finley 2008). Among all tea polyphenols, epigallocatechin gallate (EGCG) has been found to be responsible for much of the health-promoting ability of GT (Khan et al. 2006). In general, GT has been found to be superior to black tea (BT) in terms of health effects, owing to the higher content of EGCG, although the role of thearubigins and theaflavins contained in BT have not been properly investigated. In vitro and animal studies provide strong evidence that polyphenols derived from tea possess bioactivity to delay the onset of risk factors associated with disease development (Cabrera, Artacho, and

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Giménez 2006; Wolfram 2007; Yang et al. 2007; Yang et al. 2009; Yang, Lambert and Sang 2009). Studies conducted on cell cultures and animal models indicate a potentially modulating effect of tea on gene transcription, cell proliferation, and other molecular functions (McKay and Blumberg 2002). Over the last few years, clinical studies have revealed several physiological responses to tea that may be relevant to the promotion of health and the prevention or treatment of some chronic diseases (Crespy and Williamson 2004; Cabrera, Artacho, and Giménez 2006). This chapter covers recent findings on the medicinal properties and health benefits of tea with special reference to antioxidant and anti-inflammatory actions as key mechanisms for cancer and CVD prevention.

12.2 TEA: HISTORY AND ORIGIN

The second emperor of China, Shen Nung, is believed to have discovered tea when the leaf of the plant *Camellia sinensis* blew into his cup of hot water (2737 BCE). The first European to encounter tea and write about it was the Portuguese Jesuit missionary Father Jasper de Cruz in 1560. Around 1650, the Dutch introduced several teas and tea traditions to New Amsterdam (which later became New York). The first tea sold as a health beverage was in London, England, at Garway's Coffee House in 1657. In 1826, John Horniman introduced the first retail tea in sealed, lead-lined packages. In 1870, Twinings of England began to blend tea for uniformity. The Englishman Richard Blechynden created iced tea during a heat wave at the St. Louis World Fair in 1904, and the New York tea importer Thomas Sullivan inadvertently invented tea bags in 1908 when he sent tea to clients in small silk bags and they mistakenly steeped the whole bags. Finally, the world's first instant tea was introduced in 1953. Nowadays, BT is consumed principally in Europe, North America, and North Africa, whereas GT is taken throughout Asia.

12.3 TEA: HOW MANY USE IT, TRADE VOLUME, WAY OF INTAKE, PREPARATION, AND PROCESSING

The four types of tea most commonly found in the market are BT, oolong tea, GT, and white tea. The difference among them lies in the different processing or, in the case of white tea, different harvesting times. White tea leaves are picked and harvested before they fully open; this is done when the buds are still covered by fine white hair. In the case of white tea and GT, the leaves are steamed quickly after harvesting to prevent oxidation of polyphenols and then dried. In the production of BT, the leaves are rolled, disrupting the cellular compartment and bringing phenolic compounds into contact with polyphenol oxidases, and the young *C. Sinensis* leaves undergo oxidation for 90–120 minutes before drying. During this process, which is referred to as "fermentation," flavan-3-ols are converted to complex condensation products, such as theaflavins and thearubigins. Oolong tea, which is manufactured mainly in Taiwan and exported to Japan and Germany, is produced with a shorter fermentation period than BT and is said to have a taste and color somewhere between GT and BT (Duthie and Crozier 2003; Del Rio et al. 2004).

12.4 ACTIVE INGREDIENTS AND MECHANISMS OF ACTION

The major active ingredients of tea are catechins, their bioavailability and mechanisms of action are described below.

12.4.1 Catechins in Tea and Their Bioavailability

The major phenolics present in teas are flavan-3-ols (Figure 12.1) and flavonols. The main flavan-3-ols in GT are (–)-epicatechin (EC) and its gallate derivatives. These compounds are present in lower amounts in BT, and are converted by oxidation to theaflavins and thearubigins (Finger, Kuhr, and Engelhardt 1992; Balentine, Wiseman, and Bouwens 1997). Conjugates of quercetin and kaempferol are the main flavonols in tea, with lower levels of myricetin. The conjugating moiety has been

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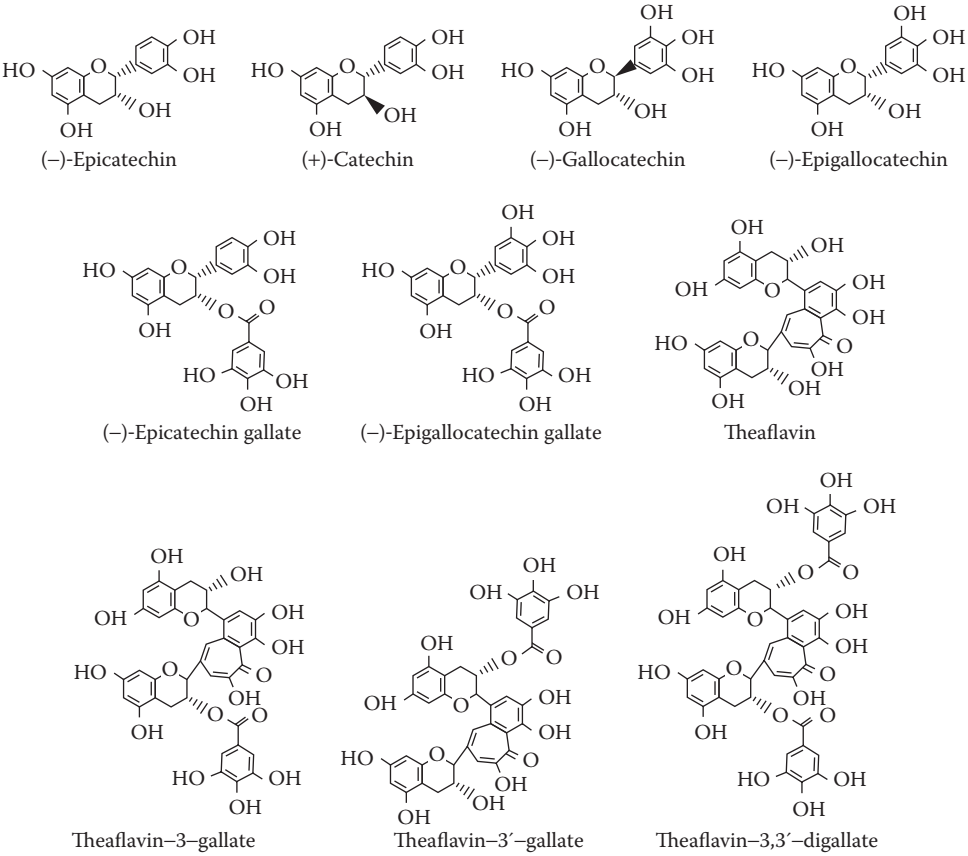


FIGURE 12.1 Structures of the flavan-3-ols found in tea.

reported to vary from mono- to di- and triglycosides (Wang and Sporns 2000; Del Rio et al. 2004). Other related compounds found in tea are gallic acid and quinic esters of gallic, coumaric, and caffeic acids, together with the purine alkaloids theobromine and caffeine, proanthocyanidins, and trace levels of flavones (Crozier, Jaganath, and Clifford 2009).

The literature reporting the bioavailability of GT phenolics shows very different and controversial results (Manach et al. 2005). Urinary excretion reported after drinking different doses of tea or tea extracts ranges from unquantifiable concentrations to levels close to 10% of the ingested amount. A very similar pattern of differences could be observed when bioavailability studies were carried out with chocolate, which constitutes the second greatest source of flavan-3-ols (Baba et al. 2000; Mullen et al. 2009), or when single molecules like (–)-EGCG or (+)-catechin were introduced as supplements (Van Amelsvoort et al. 2001; Goldberg, Yan, and Soleas 2003). Analytical limitations have drastically biased the identification and characterization of flavan-3-ol catabolites; also, the unavailability of pure standards for each specific catabolite has significantly limited the quality of bioavailability studies. However, with all the health benefits attributed to GT, it is necessary to define the absorption and catabolism of its bioactive components with greater clarity with the help of more advanced analytical methodologies that are now readily available in most research laboratories. High-performance liquid chromatography (HPLC) with multistage mass spectrometric (MS/MS) detection has been used to analyze flavan-3-ols in biological fluids, which has facilitated the identification of a range of metabolites (Li et al. 2000; Meng et al. 2001). Stalmach et al. (2009) identified a total of 10 metabolites in human plasma, in the form of O-methylated, sulfated, and glucuronide conjugates of EC and EGC, with 29–126 nM peak-plasma concentrations

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(C_{max}) occurring 1.6–2.3 hours after ingestion. Plasma also contained nonmetabolized (–)-EGCG and (–)-EC-3-gallate with respective C_{max} values of 55 and 25 nM. In the same study, urine excreted up to 24 hours after consumption of GT contained 15 metabolites of EC and EGC, but (–)-EGCG and (–)-EC-3-gallate were not detected. The overall calculated bioavailability was equivalent to 8% of intake (Stalmach et al. 2009). A similar GT-feeding study was carried out using human volunteers with ileostomy (Stalmach et al. 2009). The 24-hour plasma and urinary profiles were very similar to those obtained with subjects having an intact, functioning colon, confirming that the detected flavan-3-ol metabolites were absorbed principally in the small intestine. More recently, a feeding study with 20 volunteers investigated in detail the catabolism of GT catechins (GTCs) by means of HPLC with tandem mass spectrometry (HPLC-MS/MS; Del Rio et al. 2010). A total of 8 and 39 relevant compounds were identified in plasma and urine, respectively (Table 12.1). In particular, metabolites derived by the action of intestinal or hepatic uridine 5'-diphosho-glucuronyltransferases, sulfotransferases, and catechol-O-methyltransferase were identified with the MS detector through the loss of the conjugating groups (i.e., glucuronic acid and sulfate). In addition to the metabolites reported by Stalmach and colleagues (2009), metabolites derived from colonic bacteria ring fission activity have been identified, as described by Sang et al. (2008). In plasma, EGC-gallate was the only unmetabolized compound in this study and the highest in terms of absolute concentration. The EGC catabolites reached their peak-plasma concentration (T_{max}) at 2 hours from ingestion, whereas EC catabolites showed their T_{max} at 1 hour. Among conjugates, the main metabolites excreted in urine were EGC-O-glucuronide and its methoxy counterpart methyl-O-EGC-O-glucuronide. The main EC metabolite was methyl-EC-sulfate, whereas the sulfate metabolite of EGC was almost negligible. The main class of colonic catabolites was represented by (–)-5-(3,4-dihydroxyphenyl)-γ-valerolactone (M6′, which can derive from both EC and EGC, and their epimers) and 5-(3′,5′-dihydroxyphenyl)-γ-valerolactone (M6, which can derive solely from EC and catechins). Further, (–)-5-(3,4,5-trihydroxyphenyl)-γ-valerolactone (M4, which can derive solely from EC and catechins) was a relevant colonic product. These molecules, all in their variously conjugated versions, were by far the main excreted metabolites, and their urinary concentration was, on average, 10 times higher than that of flavanol conjugates. Bioavailability of flavan-3-ols, considering for the first time the whole set of catechin-derived catabolites, was 39.5%.

The identification of most of the metabolites in these studies was possible thanks to the availability of MS/MS detectors, and this is probably the reason why several older studies failed to pinpoint most flavan-3-ol-derived molecules. Previous research mostly dealt with the treatment of samples with deconjugating enzymes, such as microbial glucuronidases and sulfatases, which allowed the detection of aglycons in biological fluids (Lee et al. 2002; Henning et al. 2005) with non-MS/MS detectors. However, this treatment did not help in understanding the true metabolic processes undergone by dietary catechins and, above all, this kind of detection did not consider the methoxy derivatives, which constitute a notable fraction of the total excreted flavanols. Several studies also failed in identifying and quantifying γ-valerolactones, which alone constitute almost 90% of the excreted flavan-3-ol metabolites. Sang et al. (2008) gave the most complete and detailed description of the human urinary metabolite profile of tea polyphenols using HPLC with electrospray ionization tandem MS/MS with data-dependent acquisition, but they did not set up a bioavailability study. Moreover, contrary to their observations, urinary unconjugated valerolactones were not found by Del Rio et al. (2010), and some molecules with double conjugation were observed in urine for the first time (EGC-sulfate glucuronide, methyl-EGC-sulfate glucuronide, M6-/M6′-sulfate glucuronide, and M6-/M6′-disulfate).

12.4.2 Antioxidant Activity: Human Studies

Oxidative stress, which is the imbalance between free-radical species and antioxidant defense, can originate from an increase in free-radical production either by exogenous processes, such as pollution and cigarette smoking, or by endogenous processes, such as inflammation and respiratory

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TABLE 12.1
MS/MS Identification and Location of Flavan-3-ols Catabolites

	Flavan-3-ol Catabolite	M-H (m/z)	MS^2 Fragments (m/z)	Biological Fluid
1	(Epi)gallocatechin-sulfate glucuronide	561	385, 481, 305	Urine
2	M4-glucuronide	399	223	Urine
3	M4-sulfate	303	223	Urine
4	M6′-glucuronide	383	207	Urine
5	Methyl-(epi)gallocatechin-sulfate glucuronide	575	495, 399, 319	Urine
6	M6-sulfate glucuronide	463	287, 207	Urine
7	(Epi)gallocatechin-glucuronide	481	305	Plasma, urine
8	Methyl-(epi)gallocatechin-sulfate glucuronide	575	495, 399, 319	Urine
9	(Epi)catechin-sulfate glucuronide	545	465, 369, 289	Urine
10	M6′-disulfate	367	287, 207	Urine
11	M6′-sulfate	287	207	Urine
12	(Epi)gallocatechin-sulfate	385	305	Urine
13	M6′-sulfate	287	287, 207	Urine
14	Methyl-(epi)gallocatechin-glucuronide	495	319	Plasma, urine
15	(Epi)catechin-sulfate glucuronide	545	465, 369, 289	Urine
16	Methyl-M4-sulfate	317	237	Urine
17	(Epi)catechin-sulfate	369	289	Urine
18	(Epi)catechin-sulfate	369	289	Urine
19	(Epi)catechin-sulfate glucuronide	545	465, 369, 289	Urine
20	M4-sulfate	303	223	Urine
21	M6-glucuronide	383	207	Urine
22	M4-glucuronide	399	223	Urine
23	Methyl-(epi)gallocatechin-sulfate	399	319	Plasma, urine
24	(Epi)catechin-glucuronide	465	289	Plasma, urine
25	Methyl-(epi)gallocatechin-sulfate	399	319	Plasma, urine
26	Methyl-(epi)catechin-sulfate glucuronide	559	479, 383	Urine
27	Methyl-M4-sulfate	317	237	Urine
28	M6-glucuronide	383	207	Urine
29	M6-sulfate	287	207	Urine
30	Methyl-(epi)catechin-glucuronide	479	303	Urine
31	(Epi)catechin-sulfate	369	289	Plasma, urine
32	Methyl-(epi)catechin-sulfate glucuronide	559	479, 383	Urine
33	Methyl-(epi)catechin-sulfate	383	303	Plasma, urine
34	Methyl-(epi)catechin-glucuronide	479	303	Urine
35	(−)-Epigallocatechin-3-gallate	457	169	Plasma
36	Methyl-(epi)catechin-sulfate	383	303	Plasma, urine
37	Methyl-(epi)catechin-sulfate	383	303	Plasma, urine
38	Methyl-(epi)catechin-sulfate	383	303	Plasma, urine
39	(−)-Epicatechin-3-gallate	441	289, 169	Plasma

Source: Adapted from Del Rio, D., L. Calani, C. Cordero et al. 2010. Bioavailability and catabolism of green tea flavan-3-ols in humans. *Nutrition* 26(11-12):1110–6.

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burst (Halliwell and Cross 1994; Serafini and Del Rio 2004; Halliwell 2009). Free radical–initiated auto-oxidation of cellular membrane lipids can lead to cellular necrosis and a variety of pathological conditions such as cancer, CVD, and even aging (Serafini et al. 2006; Halliwell 2009). It has been widely suggested that antioxidant molecules play an important role in the diet-led prevention of oxidative stress–related chronic diseases, and the health benefits associated with tea consumption have been attributed in part to free-radical scavenging and metal-chelating activity (Babu and Liu 2008; Seeram et al. 2008; Sharma and Rao 2009).

In body fluids, total antioxidant capacity (TAC), defined as the moles of oxidants neutralized by 1 L of plasma, assesses the effectiveness of the endogenous nonenzymatic antioxidant network as well as nutritional antioxidant molecules (Serafini and Del Rio 2004). Since the first evidence of how ingestion of GT and BT boosted plasma antioxidant defenses in humans (Serafini, Ghiselli, and Ferro-Luzzi 1994) was obtained, different studies have investigated the ability of diet to modulate plasma TAC following the consumption of tea in human subjects. As presented in Table 12.2, a large majority of the ingestion studies conducted with teas show a clear impact on plasma TAC (Rietveld and Wiseman 2003; Manach et al. 2005; Williamson and Manach 2005; Fernandez-Panchon et al. 2008). A study with three groups of five volunteers drinking water, BT, or GT demonstrated a significant and strong increase in TAC value in the tea groups between 30 and 60 minutes after a single consumption of 300 mL of either GT or BT. The scavenging capacity returned to its initial level after 80 minutes. There was no significant difference between the GT and BT groups (Serafini, Ghiselli, and Ferro-Luzzi 1996). In the same year, Maxwell and Thorpe (1996) measured TAC in 10 healthy subjects following the ingestion of BT and found no significant change. Also, in another tea study conducted by McAnlis et al. (1998), no change in plasma TAC was found. However, in a crossover study with 10 healthy subjects, tea consumption resulted in an increase in TAC 40 minutes after GT ingestion (Benzie et al. 1999). Further, for the same study, a significant increase in TAC value in urine was found. Pietta et al. (1998) found increases in plasma TAC, EGC-gallate, and EC-gallate after ingestion of GT. In agreement with the plasma results, urine samples collected at 6–48 hours contained detectable amounts of final catechin metabolites, including 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, 3-methoxy-4-hydroxy-hippuric acid, and 3-methoxy-4-hydroxybenzoic acid (vanillic acid). In a crossover study with 24 volunteers, ingestion of GT significantly increased plasma TAC (Leenen et al. 2000). In this study, a single dose ingestion of GT or BT again resulted after 60 minutes in a significant increase of catechins in plasma. As expected on the basis of the higher catechin concentration in GT, the rise in total plasma catechins was significantly higher following the consumption of GT as compared with BT. Consumption of BT and GT also resulted in a significant increase in plasma TAC relative to consumption of water (Leenen et al. 2000). Increases in plasma TAC were found also in a randomized crossover study conducted with BT (Langley-Evans 2000), as well as in 10 young, healthy subjects who received GT on three occasions each separated by 1 week, with the amount of tea increasing stepwise from 150 to 300 and 450 mL (Sung et al. 2000). In the first week, nonsignificant increases compared with baseline values were found. After doubling and tripling the initial amount of GT, a positive dose–response relation was found (Sung et al. 2000). In contrast, no significant effect was found on plasma TAC after ingestion of BT and GT in a randomized crossover study in healthy subjects (Hodgson et al. 2000). A double-blind, placebo-controlled, crossover trial in 60 coronary artery disease subjects was performed by Duffy et al. (2001) to determine the effect of tea consumption on antioxidant status, with no effect on plasma TAC observed. Kimura et al. (2002) showed that after acute ingestion of a tea–polyphenol extract by healthy subjects, plasma TAC did not change although an increase in EGCG concentration was observed. However, the number of subjects enrolled in the acute study ($n = 5$) might not have been enough to obtain an adequate sample size of statistical significance. When a long-term supplementation was performed on 16 subjects, plasma-free EGCG concentration and TAC did not change. Similarly, Henning et al. (2005), in an acute ingestion study, found no differences in plasma EGCG concentrations and TAC after administration of EGCG either in purified form or

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TABLE 12.2
Antioxidant Effects of Tea in Human Intervention Studies

Treatment	Days	n	Biomarkers Affected	Unchanged Biomarkers	PP Levels	References
BT	1	10		TAC	Not measured	Maxwell and Thorpe 1996
GT, BT	1	10	↑ TAC		Not measured	Serafini et al. 1996
GTE, BTE	28	45	↑ TAC	MDA	Not measured	Van het Hof et al. 1997
BT	28	10		TAC	Not measured	McAnlis et al. 1998
GTE	1		↑ TAC		↑ Both plasma and urinary catechins	Pietta et al. 1998
GT	1	10	↑ TAC		↑ Urinary PP	Benzie et al. 1999
GT, BT	1	20	↑ TAC		↑ Urinary 4-O-methyl gallic acid	Hodgson et al. 2000
BT	1	8	↑ TAC		Not measured	Langley-Evans 2000
GTE, BTE	1	21	↑ TAC		↑ Catechins	Leenen et al. 2000
GT	1	10	↑ TAC		Not measured	Sung et al. 2000
BT	28	50	↑ TAC		Not measured	Duffy et al. 2001
TE	1	5		TAC	Catechin unchanged	Kimura et al. 2002
TE	7	16	↑ TAC			
GTE	21	16	↑ TAC	Urinary 8-OH-G	↑ Urinary catechins	Young et al. 2002
GT, BT, GTE	1	30	Only GTE ↑ TAC			Henning et al. 2004
GT	24	42	↑ TAC ↓ ROOH		PP unchanged	Erba et al. 2005
GTE	1	20		8-OH-G TAC	EGCG unchanged	Henning et al. 2005
BT	28	44		TAC, urinary 8OH-G, urinary 8-iso PGF	↑ Catechins	Widlansky et al. 2005
BT	1	9	↑ TAC		↑ PP	Kyle et al. 2007
GT	45	100	↑ TAC		Not measured	Bertipaglia de Santana et al. 2008
GT	7	14	↑ TAC ↓ ROOH		↑ PP	Panza et al. 2008

Note: BT: black tea; GT: green tea; GTE: green tea extract; PP: Polyphenols; n: number of total subjects; ↑: increase.

as GT extract (GTE). Previously, Henning et al. (2004) in a crossover design studied the effects of GT, BT, and a GTE supplement. Flavanol absorption was enhanced only when tea polyphenols were administered as a GTE supplement in capsules, which led to a small but significant increase in plasma TAC compared to the increase when BT or GT was administered (Henning et al. 2004). In an acute intervention study, Kyle et al. (2007) showed that consumption of BT was associated with significant increases in plasma TAC and concentrations of total phenols, catechins, and the flavonols quercetin and kaempferol within 80 minutes of ingestion. In a chronic intervention study conducted by Van het Hof et al. (1997), healthy subjects consumed 6 cups of GT, BT, or

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water per day for 4 weeks. A small but significant increase in plasma TAC was observed after 4 weeks with GT but not with BT. Total catechins increased after acute and chronic ingestion of BT, without any effect on plasma TAC and urinary 8-hydroxy-2′-deoxyguanosine and 8-isoprostane levels (Widlansky et al. 2005). Intervention with GTE for 3 weeks increased plasma TAC in a mixed group of smokers and nonsmokers, with a higher excretion of urinary catechins observed at 2 hours (Young et al. 2002).

As far as intervention studies evaluating markers of lipid oxidation are considered, the picture is even more complex than with effects on TAC. In a recent study conducted by Bertipaglia de Santana et al. (2008), 100 dyslipidemic individuals were asked to ingest 50 g of soy, 3 g of GT, or 50 g of soy and 3 g of GT daily, whereas the control group ingested a hypocholesterolemic diet. All the groups that used soy or GT or both showed increased plasma TAC, but no statistically significant difference occurred in the plasma levels of lipid hydroperoxides after 45 days of supplementation. Most intervention studies do not show an effect of tea ingestion on markers of lipid oxidation in healthy subjects (McAnlis et al. 1998; Princen et al. 1998; Cherubini, Beal, and Frei 1999; O'Reilly et al. 2001; Hodgson et al. 2002; Mukamal et al. 2007; Bertipaglia de Santana et al. 2008), and only few studies indicate that tea consumption may be effective in reducing lipid peroxidation (Ishikawa et al. 1997; Freese et al. 1999; Inami et al. 2007). Controversial results from intervention studies may be due to wide differences in the target population (i.e., dietary habit and lifestyle) and/or in experimental protocols (i.e., dose and length of treatment); however, a major point to be considered is that the majority of such studies were conducted on healthy subjects for whom oxidative stress markers are at lower levels than in subjects suffering from oxidative stress–related pathologies or chronic inflammation.

12.4.3 Antioxidant Activity: Molecular Aspects

Experimental data indicate that tea polyphenols may offer indirect protection by activating endogenous defense systems (Rahman, Biswas, and Kirkham 2006). Many antioxidant genes are regulated at transcriptional levels by cellular redox status (Liu et al. 2005). Several lines of evidence suggest a tight connection between exogenous and endogenous antioxidants that appear to act in a coordinated fashion. It is reasonable to hypothesize that this link is achieved through antioxidant responsive elements (AREs) present in the promoter regions of many of the genes inducible by oxidative and chemical stresses (Rahman, Biswas, and Kirkham 2006). Studies strongly suggest that tea polyphenols can stimulate antioxidant transcription and detoxify defense systems through ARE (Rahman, Biswas, and Kirkham 2006).

An ARE, also referred to as the "electrophile response element," is a *cis*-acting transcriptional regulatory element involved in the activation of coding of genes for a number of antioxidant proteins and phase II detoxifying enzymes, including glutathione peroxidase (GPx), heme oxygenase 1 (HO-1), γ-glutamylcysteine synthetase (γ-GCS), superoxide dismutase (SOD), and glutathione reductase (GR; Lee and Johnson 2004; Chen et al. 2006; Mann, Niehueser-Saran et al. 2007; Mann, Rowlands et al. 2007; Gopalakrishnan and Kong 2008). Nuclear factor-erythroid 2-related factor 2 (Nfr2) is the transcription factor that is responsible for both constitutive and inducible expression of ARE-regulated genes (Chen et al. 2006; Gopalakrishnan and Kong 2008). Under normal physiological conditions, Nfr2 is bound to kelch-like ECH-associated protein-1 (Keap1; Figure 12.2) and thereby sequestered in the cytoplasm in association with the actin cytoskeleton (Mann, Niehueser-Saran et al. 2007; Gopalakrishnan and Kong 2008). Under conditions of increased oxidative or xenobiotic stress, Nfr2 dissociates from Keap1, translocates to the nucleus, and binds to ARE sequences in association with other members of the basic leucine zinc zipper transcription factor family, such as Maf, resulting in the transcriptional activation of phase II detoxifying enzymes and antioxidant genes (Na and Surh 2008).

Nuclear translocation and export of Nfr2 have been reported to be modulated by phosphorylation via mitogen-activated protein kinases (e.g., extracellular regulated kinase [ERK]1/2, p38 MAPK)

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and/or protein kinase C (PKC; Mann, Niehueser-Saran et al. 2007). It was found that PKC was inhibited in vitro by tea flavonoids (Middleton, Kandaswami, and Theoharides 2000). Phenolic compounds have been found to modulate the MAPK pathway by acting on several steps of the activation cascade (Park and Dong 2003; Santangelo et al. 2007). Recent proteomic investigations have identified a large number of proteins interacting with EGCG; nearly all of them are hypothesized to mediate GTCs action (Patra et al. 2008). Membrane lipid rafts and sphingolipid- and cholesterol-enriched membrane microdomains assemble important signaling proteins into complexes prone to be activated by molecular triggers (Brown 2006; Sengupta, Baird, and Holowka 2007). It has been demonstrated that the lipid raft is used as a platform by a 67-kDa laminin receptor (LamR). The LamR may systematically reshape the rafts and affect the uptake of EGCG (Patra et al. 2008; Tachibana 2009). The EGCG is first incorporated into the plasma membrane, and then reloaded into the rafts where LamRs are present (Figure 12.2). In the rafts, EGCG interacts with many receptors, and the nonspecific binding of EGCG in membrane lipid rafts destabilizes the rafts, structure and inactivates MAPK signaling. The structure–activity relationship analysis of major GTCs (and their epimers) on cell-surface binding suggests that the binding activities of pyrogallol-type catechins (EGCG and GCG) are higher than those of catechol-type catechins (ECG and CG). The mechanism of endocytosis of

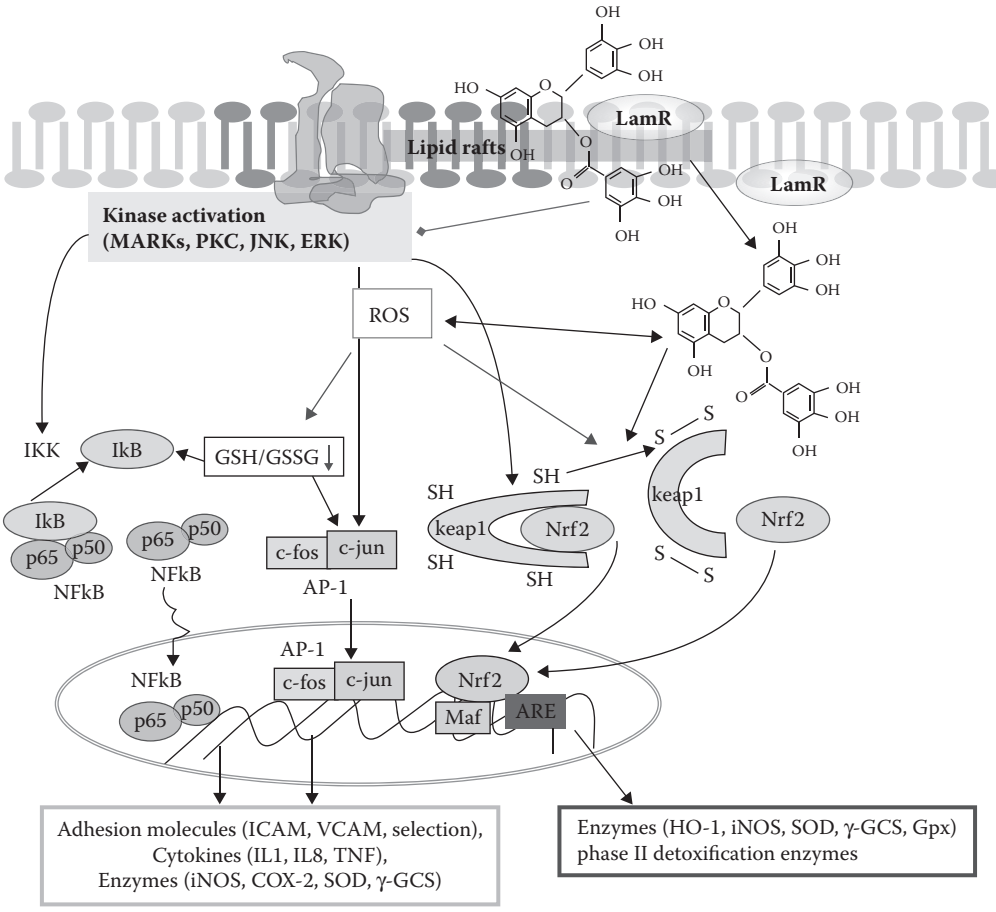


FIGURE 12.2 Interaction of EGCG with lipid rafts, inactivating mitogen-activated protein kinase and consequently downregulating nuclear factor κB, activating protein 1, and Nfr2 pathways. A laminin receptor in lipid rafts mediates EGCG transport to cytosol, where EGCG autooxidation or its quinone derivative may activate antioxidant responsive element pathway via oxidation of sulphidryl (SH) residues of kelch-like ECH-associated protein-1.

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EGCG has not yet been experimentally dissected. However, it is reasonable to believe that LamR, in association with rafts, transports EGCG to the cytosol (Patra et al. 2008). It was previously known that expression of 67 LamR confers EGCG responsiveness to tumor cells and that LamR is usually upregulated in cancer cells (Patra et al. 2008). This may explain observations about the markedly different mechanisms of EGCG action in normal and transformed cells (Balasubramanian, Efimova, and Eckert 2002). Despite the inhibitory effects of EGCG on the MAPK pathway, the GT polyphenol extract, and EGCG in particular, stimulates the transcription of phase II detoxifying enzymes through the ARE (Yu et al. 1997; McKay and Blumberg 2002). Low and high concentrations of EGCG lead to different effects. Higher concentrations of EGCG result in the sustained activation of MAPK, especially JNKs, that ultimately leads to apoptosis (Bode and Dong 2003). Depending on the concentration, EGCG is auto-oxidized under cell-culture conditions (Hou et al. 2005; Surh, Kundu, and Na 2008; Yang et al. 2008), exerting pro-oxidant activity and decreasing the glutathione (GSH) concentration in some cell types (Saeki et al. 2002). It is not clear whether EGCG auto-oxidation induces the occurrence of effects inside animal tissues, because these tissues are endowed with antioxidative enzymes and are usually under lower oxygen partial pressure than the cell-culture medium. However, the polyphenol-related ARE-mediated upregulation of MnSOD expression seems to be mediated by nitric oxide (NO) and/or reactive oxygen species (ROS) generation (Mann, Niehueser-Saran et al. 2007; Mann, Rowlands et al. 2007). It has been suggested that some quinone derivatives of polyphenols can oxidize two highly reactive cysteine thiol groups of Keap1, resulting in disulfide bond formation and Nfr2 release (Surh, Kundu, and Na 2008). It is paradoxical, but the activation of Nfr2/ARE signaling by antioxidant polyphenols to induce cytoprotective enzymes is attributed to their pro-oxidant activity. The versatility of EGCG, which makes it able to interact with so many targets, also makes the proposition of a unified mechanism of action very difficult.

12.4.4 Anti-Inflammatory Activity: Molecular Aspects

Inflammation is a normal host defense mechanism that protects the host from infection and other insults. Where an inflammatory response does occur, it is normally well regulated so that it does not cause excessive damage to the host, is self-limiting, and resolves rapidly (Calder et al. 2009). This self-regulation involves the activation of negative feedback mechanisms such as the secretion of anti-inflammatory cytokines, inhibition of proinflammatory signaling cascades, shedding of receptors for inflammatory mediators, and activation of regulatory cells. Pathological inflammation involves a loss of tolerance and/or a loss of regulatory processes. Where inflammation is excessive, irreparable damage to host tissues and disease can occur. Inflammation is considered a critical factor in many human diseases and conditions, including obesity, CVDs, neurodegenerative diseases, diabetes, aging, and cancer.

Although inflammation-induced tissue damage occurs in an organ-specific manner in different diseases or conditions, there is some commonality among the responses seen in the different organs. There are many mediators, such as adhesion molecules (AMs) including intercellular AM 1 (ICAM 1) and vascular AM 1 (VCAM-1); lipid-derived eicosanoids, including prostaglandin (PG) E2 (PGE2), PGI2, leukotriene (LT) B4, and LTC4; cytokines, including tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), IL-6, and IL-10; and chemokines, including IL-8, monocyte-chemoattractant protein-1 (MCP-1), and macrophage inflammatory molecule 1α (MIP1α). These mediators coordinate the events of acute inflammation, regulate vascular changes, and perform inflammatory cell recruitment (Santangelo et al. 2007). The anti-inflammatory activities of catechins may be due to their suppression of leukocyte adhesion to endothelium and subsequent transmigration through inhibition of transcriptional factors–mediated production of cytokines and AMs in both endothelial and inflammatory cells (Surh et al. 2005; Biesalski 2007; Babu and Liu 2008). It has also been suggested that the molecular mechanisms involved in the anti-inflammatory activities of tea polyphenols include the inhibition of proinflammatory enzymes, such as cyclooxygenase 2 (COX-2), lipoxygenase (LOX), and inducible NO synthase (iNOS), and the modulation of signal transduction and transcription factors, including nuclear factor κB (NF-κB), activating protein 1 (AP-1), Nfr2, MAPK, and PKC (Frei

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and Higdon 2003; Santangelo et al. 2007). Among inflammatory cells, polymorphonuclear leukocytes are particularly adept at generating and releasing ROS and reactive nitrogen species (RNS). Among the proinflammatory enzymes, iNOS and COX are responsible for increasing the levels of NO and PGE2. A number of studies have found that flavonoids inhibit production of NO and expression of iNOS messenger ribonucleic acid (mRNA) by macrophages, and it is implied that they therefore might have anti-inflammatory properties (Crouvezier et al. 2001). It has also been observed that several flavonoids are able to decrease the expression of different proinflammatory cytokines/chemokins, which include TNF-α, IL-1β, IL-6, IL-8, and MCP-1, in many cell types (Santangelo et al. 2007). The ECG, EGC, and EGCG enhanced the production of the anti-inflammatory cytokine, IL-10, whereas EC and theaflavins had no effect. (Santangelo et al. 2007). Molecular mechanisms, including catechin-mediated inhibition of transcription factors NFκB and AP-1 and reduction of MAPK activity, have been suggested as relevant anti-inflammatory pathways for tea (Figure 12.2; Sueoka et al. 2001; Lambert and Yang 2003; Park and Dong 2003; Tipoe et al. 2007).

The NFκB is a dimer that classically consists of a p50 subunit and a transactivating subunit p65 (or relA; Santangelo et al. 2007; Gopalakrishnan and Kong 2008). In unstimulated cells, NFκB is sequestered in the cytoplasm as an inactive non-DNA-binding form, associated with inhibitor κB proteins (IκBs). On cell stimulation with various NFκB inducers, IκB proteins are rapidly phosphorylated by IκB kinase (IKK) complex and subsequently degraded by the ubiquitin proteasome pathway. The released NFκB dimer can then move into the nucleus, where it induces the expression of various genes (Santangelo et al. 2007; Gopalakrishnan and Kong 2008). The influence of EGCG on the NFκB pathway has been extensively studied, and studies demonstrate its inhibitory effects on NFκB obtained by counteracting the activation of IKK and the phosphorylation and degradation of IκBα (Sueoka et al. 2001; Lambert and Yang 2003; Park and Dong 2003; Wheeler et al. 2004, Santangelo et al. 2007; Tipoe et al. 2007). Importantly, the gallate group is functionally necessary for the inhibition of IKK activity, and the presence of the catechin structure dramatically enhances this effect (Santangelo et al. 2007). The EGCG inhibits phosphorylation of p65, thus providing an additional mechanism for the inhibition of NFκB activation; this effect could be the result of IKK inhibition, because IKK can phosphorylate the p65 subunit in vitro (Wheeler et al. 2004). In addition, theaflavins block phosphorylation and/or degradation of IκB (Bode and Dong 2003). Despite the central role of NFκB in inflammation-associated genes expression, this transcription factor requires assistance from MAPK (Santangelo et al. 2007). The EGCG prevents IL-12 production and the expression of COX-2 by inhibiting phosphorylation of p38 MAPK, augmenting phosphorylation of ERK and nuclear-protein binding to NFκB site (Yoon and Baek 2005; Santangelo et al. 2007).

The AP-1 transcription factors and the AP-1 factor–associated signal transduction, implicated in inflammatory response, are important targets of EGCG action (Sueoka et al. 2001; Balasubramanian, Efimova, and Eckert 2002; Lambert and Yang 2003; Park and Dong 2003; Tipoe et al. 2007). The AP-1 proteins consist of homodimers of Jun proteins and heterodimers of Jun and Fos factors (Gopalakrishnan and Kong 2008). The exact subunit composition is influenced by the nature of the extracellular stimulus and the MAPK signaling pathway that is activated (JNK, ERK, etc.). On stimulation, regulation of AP-1 activity occurs through activating transcription of these genes as well as through phosphorylation of existing Jun and Fos proteins at specific serine and threonine sites (Sueoka et al. 2001; Lambert and Yang 2003; Park and Dong 2003; Tipoe et al. 2007). Similar to the mechanism described for antioxidant enzyme regulation, EGCG produces a reduction in MAPK activity and reduces AP-1 factor level and activity in immortalized and transformed keratinocytes; however, it increases AP-1 factor levels in normal keratinocytes (Balasubramanian, Efimova, and Eckert 2002). Both EGCG and theaflavin-3,3′-digallate were found to inhibit phosphorylation of ERKs, and theaflavin-3,3′-digallate inhibited p38 kinase phosphorylation (Bode and Dong 2003). Both ERKs and p38 kinase phosphorylation are implicated in AP-1 activation. In addition, induction of Nfr2 and overexpression of HO-1 (ARE-mediated) suppressed MCP-1 and VCAM-1 expression, suppressed monocyte adhesion to endothelial cells and transmigration, suppressed activation of p38 MAP kinase, and inhibited atherosclerotic lesion formation (Chen et al. 2006). Induction of other

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antioxidant genes such as MnSOD may involve rapid phosphorylation of ERK1/2 and IκB, translocation of the p50 subunit of NFκB to the nucleus, and transactivation of MnSOD expression (Mann, Niehueser-Saran et al. 2007; Mann, Rowlands et al. 2007), indicating an interplay between red-ox and inflammatory transcription factors. In addition, interaction with lipid rafts may account for flavonoid anti-inflammatory activity, because in T lymphocytes lipid rafts are implicated in signaling from the T-cell antigen receptor (TCR; Kabouridis and Jury 2008).

However, the large body of in vitro and cellular evidence can be influenced somehow by the concentrations utilized, which range from 2 to 100 μM, in contrast to physiological levels in plasma that are not higher than 1 μM, following the ingestion of flavonoid-rich food (Manach et al. 2004). Moreover, in vivo, catechins are extensively metabolized and transformed into molecules having different chemical structures and activities compared to those originally present in the food. A large majority of in vitro and cellular experiments have not been performed with the metabolites present in body fluids, which further increases the chance of misinterpretation of results.

12.4.5 Anti-Inflammatory Activity: Human Study

In spite of a large amount of in vitro evidence, consumption of BT and GT in humans had only slight effects on inflammation markers such as IL-6, IL-1β, TNF-α, and C-reactive protein (CRP; de Maat et al. 2000; Widlansky et al. 2005; Ryu et al. 2006; Mukamal et al. 2007), as summarized in Table 12.3. Administration of BT, GT, and GTE for 4 weeks had no effect on the inflammatory markers IL-6, IL-1β, TNF-α, CRP, and fibrinogen (de Maat et al. 2000); however, BT lowered P-selectin levels concomitantly with an increase of 4-O-methyl gallic acid (Hodgson et al. 2001). Contrary to these findings, Wildansky et al. (2005) observed in patients with coronary artery disease an increase in plasma catechins after 4 weeks of 900 mL of BT per day; however, this was not accompanied by a reduction in CRP. Further, CRP, as well as IL-6, was unaffected by GT administration in diabetic patients (Ryu et al. 2006), and fibrinogen, CRP, IL-6, TNF-α, ICAM, and VCAM were all unaffected by 6 months of BT consumption in diabetic subjects (Mukamal et al. 2007). However, Steptoe et al. (2007) revealed a reduction in CRP levels after 6 weeks of BT consumption.

TABLE 12.3
Overview of Human Intervention Studies on the Anti-Inflammatory Effects of Tea

Treatment	Days	n	Biomarkers Affected	Unchanged Biomarkers	PP Levels	References
BT	28	21	↓ P-selectin	E-selectin, ICAM-1, VCAM-1	↑ Urinary 4-O-methyl gallic acid	Hodgson et al. 2001
BT	28	66		CRP	Unchanged: ECG, EGCG, EGC ↑ EC, total	Widlansky et al. 2005
BT	180	28		Fibrinogen, CRP, IL-6, TNF-α, ICAM, VCAM	↑ Urinary 4-O-methylgallic acid	Mukamal et al. 2007
BT	42	75	↓ CRP	P-selectin	Not measured	Steptoe et al. 2007
BT, GT, GTE	28	64		IL-6, IL-1β, TNF-α, CRP, fibrinogen	Not measured	de Maat et al. 2000
GT	28	55		CRP and IL-6	Not measured	Ryu et al. 2006

Note: BT: black tea; GT: green tea; GTE: green tea extract; EC: epicatechin; ECG: epicatechin gallate; EGC: epigallocatechin; EGCG: epigallocatechin gallate; PP: Polyphenols; n: number of total subjects; ↓: decrease; ↑: increase.

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The assumption that tea flavonoids are responsible for anti-inflammatory action cannot be fully justified on the basis of current in vivo evidence. Studies investigating the effect of catechins on the markers of inflammation are scarce and do not focus on pure molecules. Moreover, most of the studies do not assess flavonoid absorption, or they fail to associate the anti-inflammatory effect following tea ingestion with changes in the circulating levels of tea constituents or their metabolites.

12.4.6 Antiviral Activity of Green Tea Catechins (Veregen)

External genital warts are very common and represent a significant health problem, particularly for young adults. One review revealed that the efficacy of all treatments is less than optimal and multiple therapies may be necessary for complete resolution of the condition (Mayeaux and Dunton 2008). Recently, the Food and Drug Administration (FDA) approved the marketing of sinecatechins (Veregen, Bradley/MediGene, AG, D-82152 Planegg/Martinsried, Germany), a botanical drug product, for the treatment of external genital and perianal warts. Sinecatechins is a water extract of GT leaves from *C. sinensis*. This is the first case of an herbal extract being approved as a drug for clinical therapy. The novel drug, produced from GT, now represents a real alternative to conventional therapy and demonstrates how well-designed clinical trials for the investigation of the therapeutic features of GT and catechins may lead to important formulations beneficial to health (Stockfleth et al. 2008; Tatti et al. 2009).

12.5 HEALTH EFFECTS: THE SCIENTIFIC EVIDENCE

Tea polyphenols found in black and green tea may have a protective effect against heart disease and some cancers, as described below.

12.5.1 Anticancer Activity of Green Tea

Cancer is not one disease but a plethora of different diseases with important differences in terms of lethality, which have much to do with individual response. In many cases, the efficacy of the weapons we have against cancer is limited. Some cancers may turn very aggressive, and when this happens only palliative therapy is available for the patient. Important examples are aggressive lung, breast, pancreatic, and prostate cancers. Lifestyle has always been considered a fundamental risk factor for cancers; the diseases do not have a prevalent genetic imprinting. Therefore, although unfortunately it was recognized only quite recently, the prevention or inhibition of progression of subclinical cancer toward more aggressive stages is considered in many cases to be the most effective therapy. The definition of chemoprevention is as follows: a strategy for pharmacological intervention with natural or synthetic compounds that may prevent, inhibit, delay, or reverse carcinogenesis (Sporn et al. 1976; William et al. 2009).

Hypotheses and studies on the possible anticancer activity of GT go back a long time and have been extensively reviewed (Bode and Dong 2009; Boehm et al. 2009; Yang et al. 2009; Butt and Sultan 2009). There are three main streams of information leading to the final conclusion that active compounds such as catechins, found in high quantities in GT, may be beneficial in this regard. The first is epidemiological evidence; the second is preclinical data; and the third stream, still in its infancy, is based on clinical trials. In all these branches, although discussion is still rather open, epidemiology supports the increasing consensus that GT consumption decreases cancer risk (Yoshizawa et al. 1987; Dreosti, Wargovich, and Yang 1997; Katiyar and Mukhtar 1996. Bode and Dong 2009; Boehm et al. 2009; Yang et al. 2009; Butt and Sultan 2009). Studies involving many in vitro and in vivo experimental systems provide convincing evidence that supports epidemiological findings (Yang and Wang 1993; Yang 1997; Yang, Maliakal, and Meng 2002). Anticancer activity of GT has been demonstrated in many cancer models, including lung, mammary gland, skin, esophagus, stomach, liver, pancreas, intestine, and colon (Wang et al. 1989; Huang et al. 1992; Yang and Wang 1993; Gensler et al. 1996; Dreosti, Wargovich, and Yang 1997; Huang et

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al. 1997; Yang 1997; Chung et al. 1998). These studies raise considerable interest in this issue, although the precise mechanism of action of GTCs and EGCG, which seems to be the most powerful biologically active catechin, is still unclear. At the moment, several hypotheses have been made and experimental data suggest that catechins may interact directly with several molecular targets (Tachibana 2009) and affect gene expression and signaling by epigenetic mechanisms (Patra et al. 2008). However, the most relevant issue faced as of now is how to collect clinical evidence of the anticancer activity of catechins by performing pilot or definitive clinical trials targeting specific preneoplastic or cancer lesions. Section 12.5.2 addresses this issue by providing a paradigmatic example of chemoprevention by means of catechins in an important cancer model: prostate cancer.

12.5.2 Green Tea Catechins Extract against Human Prostate Cancer

Prostate cancer is the second leading cause of cancer-related death among men in Western countries, representing at the moment a major health problem that is slowly but constantly growing as the population ages (Haas and Sakr 1997). The threat is particularly growing in Italy, where it was recently announced that prostate cancer is now the most lethal cancer prevalent among the male population. This finding can certainly be correlated with the fact that Italians are now the oldest population in Europe.

Prostate cancer represents an ideal candidate for chemoprevention because of its high incidence and long latency period before the development of clinically evident disease. Several potential chemopreventive agents have already been tested, including COX-2 inhibitors (Basler and Piazza 2004), 5-α-reductase inhibitors (Thompson et al. 2003), and vitamin D analogs (Packianathan et al. 2004). Among natural compounds, GTEs very rich in catechins (GTCs) have been recently used because results from epidemiological and case control studies support the idea of a chemopreventive effect of bioactive compounds extracted from GT, such as catechins (Jian et al. 2004). The possible mechanism of anticancer activity of GTCs has been extensively reviewed (Khan et al. 2006). Although the molecular mechanisms of GTC action are still unclear, supporting evidence suggests that GTCs induce apoptosis in cancer cells by a mechanism that is not related to altered activity of the members of the B cell lymphoma 2 (BCL-2) family.

The most biologically active of the catechins, EGCG, has been found to inhibit angiogenesis, causing nutritional deficiency in tumor cells. The GTCs have also been found to induce the synthesis of some hepatic phase II enzymes involved in the detoxification of xenobiotics and chemical carcinogens (Khan et al. 2006). In addition, GTCs were found to possess antimetastatic potential by inhibiting urokinase, metalloproteinase 2 (MMP-2), and MMP-9. The EGCG has been found to downregulate the androgen receptor in human prostate cancer cells in culture. Therefore, a plethora of scientific papers have created the rationale for a strong potential antiproliferative effect of GTCs in cultured human prostate cancer cells. The antiproliferative effect was demonstrated specifically against cancer cells in vitro (Caporali et al. 2004). In this work, a gene named clusterin (CLU), recently proposed as a novel tumor suppressor for prostate cancer (Bettuzzi et al. 2009), was found to mediate GTC activity (Caporali et al. 2004). This result is remarkable because catechins often inhibit protein activity and gene expression; CLU seems to be one of the rare genes upregulated by GTCs. In the same work, it was demonstrated that oral administration of a 0.3% solution of GTCs in drinking water was effective in vivo in inhibiting prostate cancer progression in a well-known animal model of this disease, that is, the transgenic adenocarcinoma of mouse prostate (TRAMP). All male TRAMP mice spontaneously develop prostate cancer as a function of age, but only 20% of the animals developed prostate cancer when the GTC solution was given right after weaning as the sole source of drinking water. The possibility that GTC action is mediated by CLU was also confirmed in vivo because mice responding to GTCs showed recovery of CLU expression (which is downregulated during prostate cancer progression) immediately followed by reactivation of caspase-9 expression.

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Mice refractory to GTC treatment did not express either CLU or caspase-9 (Caporali et al. 2004; Scaltriti et al. 2006).

On the basis of these findings, a pilot clinical trial was recently conducted to assess the efficacy of GTCs in real clinical settings. In this study (Bettuzzi et al. 2006), 60 patients bearing pure HGPIN, the most likely preinvasive stage of prostate cancer, were randomly divided into two groups, one taking 600 mg/day of GTCs and the other taking placebo for a duration of 1 year. The primary aim of the study was to determine the impact of administration of GTCs on prevalence/progression of prostate cancer. At the end of the study, only 3% of the patients who received GTCs were diagnosed with cancer by repeated needle biopsy, as compared to 30% of the placebo arm. More data were gathered later in the same cohort of patients. In a follow-up study (Brausi, Rizzi, and Bettuzzi 2008), it was found that the clinical conditions of patients were stable even 2 years after suspension of chemoprevention with GTCs. This result suggests the hypothesis that the clinical benefit consisting of a powerful inhibition of prostate cancer progression achieved in subjects after 1 year of GTC administration is a stable condition. In this paradigmatic example, natural compounds extracted from GT were found to be very effective, representing a new hope of curing prostate cancer at least in its early phases.

12.5.3 Tea and Cardiovascular Diseases

Half of the mortality in Western populations over 40 years of age is due to diseases of the cardiovascular system, of which the main pathophysiological factor is atherosclerosis. Atherogenesis is a chronic inflammatory process that involves a complex interplay between circulating cellular and blood elements within the cells of the artery wall (Steinberg and Witzum 1990). This process occurs at a young age in the arteries as accumulation of lipids in the subintimal area, known as "fatty streaks." Fatty streaks, consisting of subendothelial aggregates of lipid-laden foam cells, predominantly macrophages, may progress to fibrous plaques, which represent the characteristic lesions of advancing atherosclerosis. The fibrous plaque comprises mainly smooth muscle cells and is the product of GTC action cytokines and growth factors. Fibrous plaques may undergo calcification, necrosis, hemorrhages, ulceration, or thrombosis to form a complex lesion that is most commonly associated with clinical atherosclerosis (Steinberg and Witzum 1990).

Lipid metabolism is usually impaired (Briel et al. 2009) in CVD, and increased oxidative stress (Molavi and Mehta 2004) has also been reported to be involved in CVD development. Other disturbances associated with CVD include inflammation (Fearon and Fearon 2008), platelet aggregation (Caslake and Packard 2003), and impaired endothelial function (Constans and Conri 2006). Tea flavan-3-ols have been reported to affect these disturbances. Besides being antioxidant molecules, flavan-3-ols can modulate the lipid profile (Richard et al. 2009) and blood coagulation (Vita 2005). In humans, significant decreases (approximately 10 mg/dL [0.25 mmol/L]) in total and low-density lipoprotein cholesterol were reported after consumption of a GTE (Nantz et al. 2009). A similar decrease was achieved by a capsule containing theaflavin-enriched GTE (375 mg) taken daily (Maron et al. 2003) and with 2 cups of GT, containing approximately 250 mg of total catechins (Erba et al. 2005).

Endothelial function is being increasingly recognized as a biomarker of cardiovascular health, and dysfunction of the endothelial layer is recognized as an early etiological factor in atherogenesis (Hunt 2000). There is accumulating evidence to indicate that GTCs can positively impact endothelial and vascular functions in animals and humans (Ihm et al. 2009; Park et al. 2009), and a number of plausible molecular mechanisms have been proposed (Moore, Jackson, and Minihane 2009). However, the literature on the topic is not entirely consistent, and there is also a strong concern regarding the physiological relevance of in vitro and cellular findings that utilized GTC at far higher concentrations than those achieved through diet.

Different studies have investigated the association between GT consumption and CVD mortality with contrasting results (Sato et al. 1989; Nakachi et al. 2000; Iwai et al. 2002). An inverse

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association between tea intake and mortality from CVD was observed in a large epidemiological study (Kuriyama 2008) and was found to be more pronounced in women, with a multivariate hazard ratio (95% confidence intervals) of CVD mortality in the highest quartile of intake equal to 0.69 (0.53–0.90; $p < 0.05$). In a cohort of 76,979 individuals aged 40–79 years who were free of stroke, coronary heart disease, and cancer at entry, it was shown that the multivariable hazard ratios for those drinking 1–6 cups/week, 1–2 cups/day, 3–5 cups/day, and 6 cups/day were, respectively, 0.34 (0.06–1.75), 0.28 (0.07–1.11), 0.39 (0.18–0.85; $p < 0.05$), and 0.42 (0.17–0.88; $p < 0.05$) for CVD among women compared to nondrinkers of tea (Mineharu et al. 2009). It must be pointed out that most of the published epidemiological studies were conducted in Asian countries, where consumption of GT is far higher compared to the rest of the world.

12.6 ADVERSE EFFECTS OF GREEN TEA CATECHINS EXTRACT ADMINISTRATION

In the literature, more than 30 cases of hepatitis caused by GTE (GTCs) supplementation have been reported (Adachi et al. 2003; Garcia-Moran et al. 2004; Abu el Wafa et al. 2005; Gloro et al. 2005; Bonkovsky 2006). In all cases, symptoms were similar, and occurred about 30 days after the beginning of treatment. Abdominal pain, elevated transaminase levels, and jaundice were the main symptoms observed. In most cases, suspension of GTC administration and hospitalization was followed by slow recovery, and enzyme levels returned to normal within 3 months; however, two cases had complete liver failure leading to transplantation, which was successful (Gow et al. 2004). The fact that in at least three cases the symptoms returned in patients who resumed the supplement following recovery from the first attack is proof that GTE was the cause of the liver failure (Molinari et al. 2006). The first reports were from people in France taking an extract known as Exolise from Arkopharma (BP 28–06511 *CARROS* Cedex - FRANCE), which was sold as an aid for weight loss (Seddik et al. 2001; Sgro et al. 2002; Vial et al. 2003). Exolise was removed from the market by the French authorities.

The connection between GTE consumption and idiopathic hepatitis is hard to explain. It seems more likely that instead of toxic exposure, the condition may rather be a triggered reaction similar to other cases of idiopathic hepatitis. The paradox is that there is no hint in the literature that indicates whole GT causes liver toxicity. Therefore, adverse effects may be specifically related only to the extracts. Although the total risk is considered to be quite low, these extracts have to be taken with caution, and automedication is not always a good idea. Liver function has to be checked soon after beginning the treatment. As in the case of all medications, the risk–benefit calculation must be favorable.

12.7 RESEARCH NEEDS

Future research on the health effects of tea in humans should focus on the biological significance of its in vivo catabolism. As stated in Section 12.4.1, most polyphenols present in tea, with probably the only exception of EGCG, undergo drastic modification as a result of reaction with human and microbial enzymes. The molecules generated by this interaction are concentrated in biological fluids and should be studied for their long-terms effects in human intervention studies. Moreover, gut microflora differ greatly among subjects, and this feature could mean different microbial catabolism and, consequently, different biological effects. Therefore, future intervention studies should consider the colonic microflora profile of each volunteer to check for possible interactions. The observed antioxidant effect of tea ingestion in vivo requires more evidence about the mechanism of action through which this effect is delivered.

Possible mechanisms of antioxidant action such as induction of endogenous redox-controlled pathways or direct effect of polyphenols metabolites should be unraveled, providing clear-cut evidence in long-term intervention studies to isolate the molecules responsible for the effect. The

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experimental evidence in humans suggests a potential role for GT in modulating inflammatory response in vivo. However, the limited number of studies and the contrasting results obtained suggest a strong need for increasing the body of evidence in tailored human intervention studies before drawing final conclusions about the anti-inflammatory role of tea polyphenols.

12.8 CONCLUSIONS

In conclusion, scientific evidence of the health effects of tea ingestion on CVD and cancer is mounting. However, no clear-cut conclusion has been reached on the mechanism of action of the molecules involved in this effect, although anti-inflammatory, antioxidant, and endothelial function effects seem to play a key role. Despite the lack of convincing evidence in long-term intervention studies, tea catechins are still the major player in the biological activity of teas. However, tailored human trials with proper placebo or pure molecules are needed to clarify whether catechins represent ancillary ingredients or key molecules involved in the biological properties of GT. In the meantime, an increase in the consumption of tea, with a negligible calorie load, should be encouraged.

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13 Turmeric, the Golden Spice
From Traditional Medicine to Modern Medicine

Sahdeo Prasad and Bharat B. Aggarwal

CONTENTS

13.1 Introduction 263
13.2 Origin, Nomenclature, History, Cultivation, and Processing of Turmeric 264
13.3 Composition of Turmeric 269
13.4 Consumption and Importance of Turmeric 269
13.5 Turmeric as a Traditional Medicine 270
13.6 From Traditional Medicine to Modern Medicine 271
13.6.1 In Vitro Studies with Turmeric 271
13.6.2 In Vivo Studies with Turmeric 273
13.6.3 Clinical Studies Using Turmeric 279
13.7 Studies with Turmeric Oil 281
13.8 Safety, Efficacy, and Contraindications 282
13.9 Conclusions 282
References 282

13.1 INTRODUCTION

Natural plant products have been used throughout human history for various purposes. Having co-evolved with animal life, many of the plants from which these natural products are derived are billions of years old. Tens of thousands of these products are produced as secondary metabolites by higher plants as a natural defense mechanism against disease and infection. Many of these natural products have pharmacological or biological activity that can be exploited in pharmaceutical drug discovery and drug design. Medicines derived from plants have played a pivotal role in the health care of many cultures, both ancient and modern (Newman, Cragg, and Sander 2003; Butler 2004; Balunas and Kinghorn 2005; Gurib-Fakim 2006; Newman and Cragg 2007). The Indian system of holistic medicine known as "Ayurveda" uses mainly plant-based drugs or formulations to treat various ailments, including cancer. Of the at least 877 small-molecule drugs introduced worldwide between 1981 and 2002, the origins of most (61%) can be traced to natural products (Newman and Cragg 2007). Although many synthetic drugs are produced through combinatorial chemistry, plant-based drugs are more suitable, at least in biochemical terms, for human use. Nonetheless, modern medicine has neither held in very high esteem nor encouraged the medicinal use of natural products.

Turmeric is a plant that has a very long history of medicinal use, dating back nearly 4000 years. In Southeast Asia, turmeric is used not only as a principal spice but also as a component in religious ceremonies. Because of its brilliant yellow color, turmeric is also known as "Indian saffron." Modern medicine has begun to recognize its importance, as indicated by the over 3000 publications dealing with turmeric that came out within the last 25 years. This review first discusses in vitro

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studies with turmeric, followed by animal studies, and finally studies carried out on humans; the safety and efficacy of turmeric are further addressed.

13.2 ORIGIN, NOMENCLATURE, HISTORY, CULTIVATION, AND PROCESSING OF TURMERIC

The use of turmeric dates back nearly 4000 years to the Vedic culture in India, where it was used as a culinary spice and had some religious significance. It probably reached China by 700 AD, East Africa by 800 AD, West Africa by 1200 AD, and Jamaica in the eighteenth century. In 1280, Marco Polo described this spice, marveling at a vegetable that exhibited qualities so similar to that of saffron. According to Sanskrit medical treatises and Ayurvedic and Unani systems, turmeric has a long history of medicinal use in South Asia. Susruta's Ayurvedic *Compendium*, dating back to 250 BC, recommends an ointment containing turmeric to relieve the effects of poisoned food.

Today, turmeric is widely cultivated in the tropics and goes by different names in different cultures and countries (Table 13.1). In North India, turmeric is commonly called "haldi," a word derived from the Sanskrit word *haridra*, and in the south it is called "manjal," a word that is frequently used in ancient Tamil literature. The name turmeric derives from the Latin word *terra merita* (meritorious earth), referring to the color of ground turmeric, which resembles a mineral pigment. It is known as *terre merite* in French and simply as "yellow root" in many languages. In many cultures, its name is based on the Latin word *curcuma*. In Sanskrit, turmeric has at least 53 different names,

TABLE 13.1
Various Names of Turmeric/Curcumin in Different Languages

Language	Name
Arabic	Kurkum, Uqdah safra
Armenian	Toormerik, Turmerig
Assamese	Halodhi
Bengali	Halud
Bulgarian	Kurkuma
Burmese	Hsanwen, Sanwin, Sanae, Nanwin
Catalan	Cúrcuma
Chinese	Yu chin, Yu jin, Wohng geung, Geung wohng, Wat gam, Huang jiang, Jiang huang, Yu jin, Yu jin xiang gen
Croatian	Indijski šafran, Kurkuma
Czech	Kurkuma, Indický Šafrán, Žlutý kořen, Žlutý zázvor
Dhivehi	Reen'dhoo
Danish	Gurkemeje
Dutch	Geelwortel, Kurkuma Tarmeriek, Koenjit, Koenir
English	Indian saffron
Esperanto	Kurkumo
Estonian	Harilik kurkuma, Kurkum, Pikk kollajuur, Lõhnav kollajuur, Harilik kurkuma, Kurkum, Pikk kollajuur, Lõhnav kollajuur
Farsi	Zardchubeh
Finnish	Kurkuma, Keltajuuri
French	Curcuma, Safran des Indes, Terre-mérite, Souchet des Indes
Galician	Cúrcuma
German	Curcuma, Sárga gyömbérgyökér
Greek	Kitrinoriza, Kourkoumi, Kourkoumas
Gujarati	Halad, Haldar
Hebrew	Kurkum

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TABLE 13.1 (*Continued*)
Various Names of Turmeric/Curcumin in Different Languages

Language	Name
Hindi	Haldi
Hungarian	Kurkuma, Sárga gyömbérgyökér
Icelandic	Túrmerik
Indonesian	Kunyit, Kunir; Daun kunyit
Italian	Curcuma
Japanese	Ukon, Tamerikku
Kannada	Arishina, Arisina
Khmer	Romiet, Lomiet, Lamiet
Korean	Kang-hwang, Keolkuma Kolkuma, Sim-hwang, Teomerik, Tomerik, Tumerik, Ulgum, Ulgumun
Laotian	Khi min khun, Khmin khŭn
Latvian	Kurkuma
Lithuanian	Ciberžole, Kurkuma, Dažine ciberžolé
Malay	Kunyit basah
Malayalam	Manjal
Marathi	Halad
Nepali	Haldi, Hardi, Besar
Norwegian	Gurkemeie
Pahlavi	Zard-choobag
Pashto	Zarchoba
Polish	Kurkuma, Ostryź długi, Szafran indyjski
Portuguese	Açafrão da Índia, Curcuma
Punjabi	Haldi
Romanian	Curcumă
Russian	Koren, kurkumy, Kurkuma
Sanskrit	Ameshta, bahula, bhadra, dhirgharaja, gandaplashika, gauri, gharshani, haldi, haridra, harita, hemaragi, hemaragini, hridayavilasini, jayanti, jwarantika, kanchani, kaveri, krimighana, kshamata, kshapa, lakshmi, mangalaprada, mangalya, mehagni, nisha, nishakhya, nishawa, patavaluka, pavitra, pinga, pinja, pita, pitika, rabhangavasa, ranjani, ratrimanika, shifa, shiva, shobhana, shyama, soubhagaya, suvarna, suvarnavarna, tamasini, umavara, vairagi, varavarnini, varnadatri, varnini, vishagni, yamini, yoshitapriya, yuvati
Singhalese	Kaha
Slovak	Kurkuma
Slovenian	Kurkuma
Spanish	Cúrcuma, Azafrán arabe
Swahili	Manjano
Swedish	Gurkmeja
Tagalog	Dilaw
Tamil	Manjal
Telugu	Haridra, Pasupu
Thai	Kha min chan, Kha min; Wanchakmadluk
Tibetan	Gaser, Sga ser
Turkish	Hint safranı, Sarı boya, Zerdeçal, Safran kökü, Zerdali, Zerdeçöp, Zerdecube
Ukrainian	Kurkuma
Urdu	Haldi, Zard chub
Vietnamese	Bot nghe, Cu nghe, Nghe, Uat kim, Khuong hoang
Yiddish	Kurkume

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including *anestha* (not offered for sacrifice or homa), *bhadra* (auspicious or lucky), *bahula* (plenty), *dhirgharaja* (long in appearance), *gandhaplashika* (which produces good smell), *gauri* (to make fair), *gharshani* (to rub), haldi (that draws attention to its bright color), *haridra* (dear to hari, Lord Krishna), *harita* (greenish), *hemaragi* (exhibits golden color), *hemaragini* (gives the golden color), *hridayavilasini* (gives delight to heart, charming), *jayanti* (one that wins over diseases), *jawarantika* (which cures fevers), *kanchani* (exhibits golden color), *kaveri* (harlot), *krimighni* or *kashpa* (killer of worms), *kshamata* (capability), *laxmi* (prosperity), *mangalprada* (who bestows auspiciousness), *mangalya* (auspicious), *mehagni* (killer of fat), *nisha* (night), *nishakhya* (known as night), *nishawa* (clears darkness and imparts color), *patwaluka* (perfumed powder), *pavitra* (holy), *pinga* (reddish-brown), *pinja* (yellow-red powder), *pita* (yellow), *pitika* (which gives yellow color), *rabhangavasa* (which dissolves fat), *ranjani* (which gives color), *ratrimanika* (as beautiful as moonlight), *shifa* (fibrous root), *shobhna* (brilliant color), *shiva* (gracious), *shyama* (dark colored), *soubhagaya* (lucky), *survana* (golden color), *survanavara* (which exhibits golden color), *tamasini* (beautiful as night), *umavara* (Parvati, wife of Lord Shiva), *vairagi* (who remains free from desires), *varavarnini* (which gives fair complexion), *varna datri* (enhancer of body complexion), *varnini* (which gives color), *vishagni* (killer of poison), *yamini* (night), *yoshitapriya* (beloved of wife), and *yuvati* (young girl).

Turmeric is a product of *Curcuma longa*, a rhizomatous herbaceous perennial plant belonging to the ginger family Zingiberaceae, which is native to tropical South Asia. As many as 133 species of *Curcuma* have been identified worldwide (Table 13.2). Most of them have common local names

TABLE 13.2
List of Curcuma Species

1. *Curcuma aeruginosa* (pink and blue ginger)
2. *Curcuma albicoma*
3. *Curcuma albiflora*
4. *Curcuma alismatifolia* (summer tulip)
5. *Curcuma amada* (mango ginger)
6. *Curcuma amarissima*
7. *Curcuma Americana*
8. Curcuma angustifolia (tall hidden ginger)
9. *Curcuma aromatica*
10. *Curcuma attenuata*
11. *Curcuma aurantiaca* (rainbow curcuma)
12. *Curcuma aurantiflora*
13. *Curcuma australasica* (Cape York turmeric)
14. *Curcuma bakeriana*
15. *Curcuma bicolo*r (candy corn)
16. *Curcuma brog*
17. *Curcuma burttii*
18. *Curcuma caesia*
19. *Curcuma cannanorensis*
20. *Curcuma cannanorensis* var. *Lutea*
21. *Curcuma caulina*
22. *Curcuma careyana*
23. *Curcuma certothecca*
24. *Curcuma chuanezhe*
25. *Curcuma chuanhuangjiang*
26. *Curcuma chuanyujin*
27. *Curcuma cochinchinensis*
28. *Curcuma codonantha*
29. *Curcuma coerulea*
30. *Curcuma colorata*
31. *Curcuma comosa*
32. *Curcuma cordata* (jewel of Thailand)
33. *Curcuma cordifolia*
34. *Curcuma coriacea*
35. *Curcuma decipiens*
36. *Curcuma domestica* (Emperor variegated)
37. *Curcuma ecalcarata*
38. *Curcuma ecomata*
39. *Curcuma elata* (giant plume)
40. *Curcuma erubescens*
41. *Curcuma euchroma*
42. *Curcuma euclroma*
43. *Curcuma exigua*
44. *Curcuma ferruginea*
45. *Curcuma flaviflora* (red fireball ginger)
46. *Curcuma glans*
47. *Curcuma glaucophylla*
48. *Curcuma gracillima* (chocolate zebra)
49. *Curcuma grahamiana*
50. *Curcuma grandiflora*
51. *Curcuma haritha*
52. *Curcuma harmandii* (emerald pagoda ginger)
53. *Curcuma heyneana*
54. *Curcuma inodora* (pink ginger)

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TABLE 13.2 (*Continued*)
List of Curcuma Species

55. *Curcuma karnatakensis*
56. *Curcuma kudagensis*
57. *Curcuma kwangsiensis*
58. *Curcuma kwangsiensis* var.*affinis*
59. *Curcuma kwangsiensis* var. *puberula*
60. *Curcuma lanceolata*
61. *Curcuma latiflora*
62. *Curcuma latifolia*
63. *Curcuma leopoldi*
64. *Curcuma leucorhiza*
65. *Curcuma loerzingii*
66. *Curcuma lillicina* (pink cloud)
67. *Curcuma longa* (turmeric)
68. *Curcuma longiflora*
69. *Curcuma longi spica*
70. *Curcuma lutea*
71. *Curcuma malabarica*
72. *Curcuma mangga*
73. *Curcuma meraukensis*
74. *Curcuma montana*
75. *Curcuma musacea*
76. *Curcuma mutabilis*
77. *Curcuma neilgherrensis*
78. *Curcuma nilamburensis*
79. *Curcuma ochrorhiza*
80. *Curcuma officinalis*
81. *Curcuma olena*
82. *Curcuma oligantha* (white turmeric)
83. *Curcuma oligantha* var. *lutea*
84. *Curcuma ornate* (ornate plume ginger)
85. *Curcuma pallida*
86. *Curcuma parviflora* (white angel)
87. *Curcuma parvula*
88. *Curcuma peethapushpa*
89. *Curcuma petiolata* (Temu Puteri in Java)
90. *Curcuma phaeocaulis*
91. *Curcuma pierreana* (sleeping princess)
92. *Curcuma plicata*
93. *Curcuma porphyrotaenia*
94. *Curcuma prakasha*
95. *Curcuma pseudomontana*
96. *Curcuma purpurascens*
97. *Curcuma purpurea*
98. *Curcuma raktakanta*
99. *Curcuma ranadei*
100. *Curcuma reclinata*
101. *Curcuma rhabdota*
102. *Curcuma rhomba*
103. *Curcuma roscoeana* (pride of Burma)
104. *Curcuma rotunda*
105. *Curcuma rubescens* (wine red plume)
106. *Curcuma rubricaulis*
107. *Curcuma rubrobracteata* (fire plug)
108. *Curcuma sessilis*
109. *Curcuma siamensis*
110. *Curcuma sichuanensis*
111. *Curcuma singularis* (Easan white)
112. *Curcuma soloensis*
113. *Curcuma sparganifolia*
114. *Curcuma speciosa*
115. *Curcuma spicata*
116. *Curcuma stenochila*
117. *Curcuma strobilifera*
118. *Curcuma sulcata*
119. *Curcuma sumatrana* (Sumatra ginger)
120. *Curcuma sylvatica*
121. *Curcuma sylvestris*
122. *Curcuma thalakaveriensis*
123. *Curcuma thorelii* (Chiang Mai Snow)
124. *Curcuma trichosantha*
125. *Curcuma vamana*
126. *Curcuma vellanikkarensis*
127. *Curcuma viridiflora*
128. *Curcuma wenchowensis*
129. *Curcuma wenyujin*
130. *Curcuma xanthorrhiza*
131. *Curcuma yunnanensis* (Yunnan plume ginger)
132. *Curcuma zanthorrhiza* (temulawak)
133. *Curcuma zedoaria* (zedoary white turmeric)
134. *Curcuma zerumbet*

and are used for various medicinal formulations. Some specific turmeric species are shown in Figure 13.1. The turmeric plant needs temperatures between 20°C and 30°C and a considerable amount of annual rainfall to thrive. Individual plants grow to a height of 1 m, and have long, oblong leaves. Plants are gathered annually for their rhizomes, and are reseeded from some of those rhizomes in the following season. The rhizome, from which the turmeric is derived, is tuberous, with a rough and segmented skin. The rhizomes mature beneath the foliage in the ground. They are yellowish brown with a dull orange interior. The main rhizome is pointed or tapered at the distal end and measures 2.5–7.0 cm (1–3 inches) in length and 2.5 cm (1 inch) in diameter, with smaller tubers

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FIGURE 13.1 (See color insert.) Different species of *Curcuma* that are used traditionally as a spice or as medicine.

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branching off. When the turmeric rhizome is dried, it can be ground to a yellow powder with a bitter, slightly acrid, yet sweet, taste.

India produces nearly all of the world's turmeric crop and consumes 80% of it. With its inherent qualities and high content of the important bioactive compound curcumin, Indian turmeric is considered to be the best in the world. Erode, a city in the South Indian state of Tamil Nadu, is the world's largest producer of and the most important trading center for turmeric. It is also known as "Yellow City," "Turmeric City," or "Textile City." Sangli, a city of Maharashtra, is second only to Erode in size and importance as a production and trading site for turmeric.

Before turmeric can be used, the turmeric rhizomes must be processed. Rhizomes are boiled or steamed to remove the raw odor, gelatinize the starch, and produce a more uniformly colored product. In the traditional Indian process, rhizomes were placed in pans or earthenware filled with water and then covered with leaves and a layer of cow dung. The ammonia in the cow dung reacted with the turmeric to give the final product. For hygienic reasons, this method has been discouraged. In present-day processing, rhizomes are placed in shallow pans in large iron vats containing 0.05–0.1% alkaline water (e.g., solution of sodium bicarbonate). The rhizomes are then boiled for between 40–45 minutes (in India) or 6 hours (in Hazare, Pakistan), depending on the variety. The rhizomes are removed from the water and dried in the sun immediately to prevent overcooking. The final moisture content should be between 8% and 10% (wet basis). When finger tapping of the rhizome produces a metallic sound, it is sufficiently dry. The dried rhizomes are polished to remove the rough surface. Sometimes, lead chromate is used to produce a better finish, but for obvious reasons this practice should be actively discouraged. The powder maintains its coloring properties indefinitely, although the flavor may diminish over time. Protecting the turmeric powder from sunlight retards the rate of deterioration.

13.3 COMPOSITION OF TURMERIC

More than 100 components have been isolated from turmeric. The main component of the root is a volatile oil, containing turmerone, and there are other coloring agents called curcuminoids in turmeric. Curcuminoids consist of curcumin demethoxycurcumin, 5′-methoxycurcumin, and dihydrocurcumin, which are found to be natural antioxidants (Ruby et al. 1995; Selvam et al. 1995). In a standard form, turmeric contains moisture (>9%), curcumin (5–6.6%), extraneous matter (<0.5% by weight), mould (<3%), and volatile oils (<3.5%). Volatile oils include d-α-phellandrene, d-sabinene, cinol, borneol, zingiberene, and sesquiterpenes (Ohshiro, Kuroyanag, and Keno 1990). There are a variety of sesquiterpenes, like germacrone; termerone; ar-(+)-, α-, and β-termerones; β-bisabolene; α-curcumene; zingiberene; β-sesquiphellanderene; bisacurone; curcumenone; dehydrocurdione; procurcumadiol; bis-acumol; curcumenol; isoprocurcumenol; epiprocurcumenol; procurcumenol; zedoaronediol; and curlone, many of which are specific for a species. The components responsible for the aroma of turmeric are turmerone, arturmerone, and zingiberene. The rhizomes are also reported to contain four new polysaccharides-ukonans along with stigmasterole, β-sitosterole, cholesterol, and 2-hydroxymethyl anthraquinone (Kapoor 1990; Kirtikar and Basu 1993). Nutritional analysis showed that 100 g of turmeric contains 390 kcal, 10 g total fat, 3 g saturated fat, 0 mg cholesterol, 0.2 g calcium, 0.26 g phosphorous, 10 mg sodium, 2500 mg potassium, 47.5 mg iron, 0.9 mg thiamine, 0.19 mg riboflavin, 4.8 mg niacin, 50 mg ascorbic acid, 69.9 g total carbohydrates, 21 g dietary fiber, 3 g sugars, and 8 g protein (Balakrishnan 2007). Turmeric is also a good source of the ω-3 fatty acid and α-linolenic acid (2.5%; Goud, Polasa, and Krishnaswamy 1993).

13.4 CONSUMPTION AND IMPORTANCE OF TURMERIC

Turmeric has been put to use as a foodstuff, cosmetic, and medicine. It is widely used as a spice in South Asian and Middle Eastern cooking. It lends curry its distinctive yellow color and flavor. It is used as a coloring agent in cheese, butter, and other foods (Govindarajan 1980; Ammon and

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Wahl 1991). As a result of Indian influence, turmeric has made its way into Ethiopian cuisine. In South Africa, turmeric is traditionally used to give boiled white rice a golden color. Turmeric is also used in manufactured food products such as canned beverages, dairy products, baked products, ice cream, yellow cakes, yogurt, orange juice, biscuits, popcorn, sweets, cake icings, cereals, sauces, and gelatins. It is a significant ingredient in most commercial curry powders. Turmeric has numerous uses in Asian cuisine. It is used in savory and sweet dishes, and is widely used in Eastern specialties such as fresh turmeric pickle. The reported consumption of turmeric in Asian countries in humans is in the range of 200–1000 mg/day (Thimmayamma, Rau, and Radhaiah 1983; Polasa et al. 1991) or 160–440 g/person/year (Krishnaswamy 1996). Intake in urban areas is lower (200 mg/day) than in rural areas (600 mg/day/person; Thimmayamma, Rau, and Radhaiah 1983).

According to some estimates, as much as USD $10 billion is spent every year on alternative therapies. Over USDA $650 million is spent on botanical supplements that are used for chronic inflammatory diseases such as chronic obstructive airways disease (COPD), asthma, and rheumatoid arthritis. Botanical supplements have been used for centuries in traditional medicine, including Ayurveda (science of long life), Chinese medicine, Kampo (Japanese medicine), and Egyptian medicine. Several of the medicines that are traditionally used exhibit anti-inflammatory activities (Garodia et al. 2007; Aggarwal et al. 2006). Turmeric is one such herb.

Turmeric is used as an herbal medicine for rheumatoid arthritis, chronic anterior uveitis, conjunctivitis, skin cancer, small pox, chicken pox, wound healing, urinary tract infections, and liver ailments (Dixit, Jain, and Joshi 1988). It is also used for digestive disorders; to reduce flatus, jaundice, menstrual difficulties, and colic; for abdominal pain and distension (Bundy et al. 2004); and for dyspeptic conditions including loss of appetite, postprandial feelings of fullness, and liver and gallbladder complaints. It has anti-inflammatory, choleretic, antimicrobial, and carminative actions (Mills and Bone 2000). The main clinical targets of turmeric are the digestive organs: in the intestine, for treatment of diseases such as familial adenomatous polyposis (Cruz-Correa et al. 2006); in the bowels, for treatment of inflammatory bowel disease (Hanai and Sugimoto 2009); and in the colon, for treatment of colon cancer (Naganuma et al. 2006). For arthritis, dosages of 8–60 g of fresh turmeric root three times daily have been recommended (Fetrow and Avila 1999). For dyspepsia, 1.3–3.0 g of turmeric root is recommended. No known interaction of drugs with turmeric has been reported by the monographs of the German regulatory authority, Commission E (Blumenthal, Goldberg, and Brinckmann 2000).

13.5 TURMERIC AS A TRADITIONAL MEDICINE

In folk medicine, turmeric has been used in therapeutic preparations over the centuries in different parts of the world. In Ayurvedic practices, turmeric is thought to have many medicinal properties including strengthening the overall energy of the body, relieving gas, dispelling worms, improving digestion, regulating menstruation, dissolving gallstones, and relieving arthritis. Many South Asian countries use it as an antiseptic for cuts, burns, and bruises, and as an antibacterial agent. In Pakistan, it is used as an anti-inflammatory agent, and as a remedy for gastrointestinal discomfort associated with irritable bowel syndrome and other digestive disorders. In Pakistan and Afghanistan, turmeric is used to cleanse wounds and stimulate their recovery by applying it on a piece of burnt cloth that is placed over a wound. Indians use turmeric, in addition to its Ayurvedic applications, to purify blood and remedy skin conditions. Turmeric paste is used by women in some parts of India to remove superfluous hair. Turmeric paste is applied to the skin of the bride and groom before marriage in some parts of India, Bangladesh, and Pakistan, where it is believed to make the skin glow and keep harmful bacteria away from the body. Turmeric is currently used in the formulation of several sunscreens. Several multinational companies are involved in making face creams based on turmeric.

In Ayurvedic medicine, turmeric is a well-documented treatment for various respiratory conditions (e.g., asthma, bronchial hyperactivity, and allergy), as well as for liver disorders, anorexia, rheumatism, diabetic wounds, runny nose, cough, and sinusitis (Araujo and Leon 2001). In traditional Chinese medicine, it is used to treat diseases associated with abdominal pain (Aggarwal, Ichikawa,

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and Garodia 2004). From ancient times, as prescribed by Ayurveda, turmeric has been used to treat sprains and swelling (Araujo and Leon 2001). In both Ayurvedic and traditional Chinese medicine, turmeric is considered a bitter digestive and a carminative. Unani practitioners also use turmeric to expel phlegm or *kapha*, as well as to open blood vessels in order to improve blood circulation. It can be incorporated into foods, including rice and bean dishes, to improve digestion and reduce gas and bloating. It is a cholagogue, stimulating bile production in the liver and encouraging excretion of bile via the gallbladder, which improves the body's ability to digest fats. Sometimes, turmeric mixed with milk or water is taken to treat intestinal disorders as well as colds and sore throats.

13.6 FROM TRADITIONAL MEDICINE TO MODERN MEDICINE

Although modern medicine has been routinely used in treatment of various diseases, it is less than 100 years old. Traditional medicine, in comparison, has served mankind for thousands of years, is quite safe and effective. The mechanism or the scientific basis of traditional medicine, however, is less well understood.

13.6.1 In Vitro Studies with Turmeric

Throughout the Orient, turmeric is traditionally used for both prevention and therapy of diseases. Modern in vitro studies reveal that turmeric is a potent antioxidant, anti-inflammatory, antimutagenic, antimicrobial, and anticancer agent (Table 13.3). Turmeric, used in cooking and in home remedies, has significant antioxidant abilities at different levels of action. Studies indicate that sufficient levels of turmeric may be consumed from curries in vivo to ensure adequate antioxidant protection. (Tilak et al. 2004). As an antioxidant, turmeric extracts can scavenge free radicals, increase antioxidant enzymes, and inhibit lipid peroxidation. Turmeric (100 μg/mL) inhibits lipid peroxidation in renal cells against hydrogen peroxide–induced injury when incubated with cells for 3 hours (Cohly et al. 1998). Using *Salmonella typhimurium* strains TA 100 and TA 1535, a mutagenicity

TABLE 13.3
In Vitro Effects of Turmeric against Various Diseases/Disorders

Disease/Disorder	Dose	Cells/Organisms	References
		Cancer	
Cell proliferation	0.4 mg/mL[a]	CHO and lymphoma	Kuttan et al. 1985
		Antioxidant	
Lipid peroxidation	100 μg/mL[b]	Renal epithelial cell	Cohly et al. 1998
Free-radicals level	ND[c]	In vitro	Betancor-Fernández et al. 2003
HNE modification	12.5–50.0 μg/mL[b]	In vitro	Kurien and Scofield 2007
		Mutagenic	
Mutation	2 μg/plate[a]	*S. typhimurium* TA98 and TA100	Soni et al. 1997
Mutation	2 mg/plate[d]	*S. typhimurium* TA102	Kuttan et al. 2004
Micronucleus formation	100–500 μg/mL[b]	CHO cells	Araújo, Dias, and Takahashi 1999
DNA damage	0.33 g/10^6 cfu[c]	*E. coli, B. megaterium, B. pumilusspores*	Sharma, Gautam, and Jadhav 2000
DNA damage	ND[e]	*E. coli*	Pal and Pal 2005
Micronucleus formation	200 μg/mL	Human lymphocyte	Ghaisas and Bhide 1994

(*Continued*)

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TABLE 13.3 (*Continued*)
In Vitro Effect of Turmeric against Various Diseases/Disorders

Disease/Disorder	Dose	Cells/Organisms	References
		Inflammation	
TNF-α, PGE2 level	50 µg /mL[a] (IC_{50} = 15.2 and 0.92 µg/mL)	HL-60 cells	Lantz et al. 2005
Dendritic cell activation	ND[a]	Dendritic cells	Krasovsky et al. 2009
		Viral	
Epstein–Barr virus early antigen	10 µg/mL[a]	Raji cells	Kapadia et al. 2002
HBV replication	200 or 500 mg/L[c]	HepG 2.2.15 cells	Kim et al. 2009
		Fungal	
Multiplication	MIC 7.8 µg/mL[a]	Dermatophytes	Wuthi-udomlert et al. 2000
Cell viability	LD50 33 and 109 µg/mL[a]	*Lemma minor, T. longifusus*	Khattak et al. 2005
		Microbial	
Multiplication	6.25–50.00 µg/mL[f]	*H. pylori*	Mahady et al. 2002
Multiplication	0.1%–10.0%[e]	*S. typhimurium*	Thongson et al. 2004
Multiplication	5%[c]	Foodborne pathogen	Yano, Satomi, and Oikawa 2006
Multiplication	5%[c]	Histamine-producing bacteria	Paramasivam, Thangaradjou, and Kannan 2007
Growth of mycobacteria	6%[a]	*M. tuberculosis*	Leal et al. 2003
Pathogens viability	ND[g]	Foodborne pathogenic bacteria	Thongson et al. 2005
Bacterial growth and adhesion	50 mg/mL[c]	*H. pylori*	O'Mahony et al. 2005
Infection and pathogenesis	ND[a]	*Schistosoma mansoni cercariae*	Shoheib et al. 2008
		Other	
ATPase level	131 mg/mg protein	Rat jejunal cells	Kreydiyyeh, Usta, and Copti 2000
Procarcinogens activation	IC_{50} = 0.24[b]	Caco-2 cells	Naganuma et al. 2006
Oxidative off-flavors	ND[b]	Pickles	Cleary and McFeeters 2006
Hemolysis	1–100 µg/mL[a]	Human RBC	Mathuria and Verma 2007

TNF = tumor necrosis factor; PGE2 = prostaglandin E2; IC_{50} = median inhibitory concentration; NO = nitric oxide; PI = parainfluenza; AD = adenovirus; CHO = Chinese hamster ovary; HBV = hepatitis B virus; HNE = 4-hydroxy-2-nonenal; LD_{50} = median lethal dose; MIC = minimum inhibitory concentration; ND = not defined; RS = Rous sarcoma.

[a] Ethanolic extract of turmeric
[b] Turmeric powder
[c] Aqueous extract of turmeric
[d] CO^2 gas extract of turmeric
[e] Hexane extract of turmeric
[f] Methanolic extract of turmeric
[g] Turmeric oil

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study showed that turmeric inhibits the mutagenicity produced by direct-acting mutagens such as *N*-methyl *N*'-nitro-*N*-nitrosoguanidine and sodium azide. Turmeric extracts were found to inhibit microsomal activation–dependent mutagenicity of 2-acetamidofluorene (Soudamini et al. 1995).

Numerous lines of evidence suggest that turmeric exhibits anti-inflammatory activity. In one study, crude organic extracts of turmeric were found to inhibit lipopolysaccharide (LPS)-induced production of tumor necrosis factor (TNF)-α (median inhibitory concentration [IC_{50}] value = 15.2 μg/mL) and prostaglandin E2 (PGE2; IC_{50} value = 0.92 μg/mL) from HL-60 cells. A combination of several fractions that contained the turmeric oils was more effective than curcuminoids in inhibiting PGE2 production (Lantz et al. 2005). A hydroethanolic extract of turmeric was recently found to inhibit activation of human dendritic cells in response to inflammatory cytokines (Krasovsky et al. 2009).

Besides these properties, turmeric has strong antimicrobial properties. The growth of histamine-producing bacteria (*Vibrio parahaemolyticus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*) was inhibited by garlic and turmeric extracts at a 5% concentration (Paramasivam, Thangaradjou, and Kannan 2007). Turmeric was also found to inhibit histamine production in *Morganella morganii* (potent histamine-producing bacteria). However, inhibition of histamine production and histidine decarboxylase activity of turmeric is less than that of clove and cinnamon (Shakila, Vasundhara, and Rao 1996). Turmeric extract was found to inhibit growth of the foodborne pathogen *V. parahaemolyticus* with good sensitivity (Yano, Satomi, and Oikawa 2006). A methanolic extract of turmeric inhibited the growth of different strains of *Helicobacter pylori* with a minimum inhibitory concentration range of 6.25–50.0 μg/mL (Mahady et al. 2002). Among the various plant extracts that killed *H. pylori*, such as cumin, ginger, chili, borage, black caraway, oregano, and licorice, turmeric was found to be the most efficient (O'Mahony et al. 2005).

Ethanolic extracts of *C. longa* have good antifungal activity against *Trichophyton longifusus* (Khattak et al. 2005). Tests using the agar disc diffusion method for detecting antifungal activity showed that a crude ethanolic extract of turmeric killed all 29 tested clinical strains of dermatophytes. This extract exhibited an inhibition zone range of 6.1–26.0 mm (Wuthi-udomlert et al. 2000).

The anticancer activities of turmeric include inhibiting cell proliferation and inducing apoptosis of cancer cells. Ar-turmerone, which is isolated from turmeric, induced apoptosis in human leukemia Molt 4B and HL-60 cells by fragmenting DNA to oligonucleosome-sized fragments, a known step in the process of apoptosis (Aratanechemuge et al. 2002). Moreover, the nucleosomal DNA fragmentation induced by ar-turmerone was associated with induction of Bax and p53 proteins, rather than B cell lymphoma 2 (Bcl-2) and p21, and activation of mitochondrial cytochrome *c* and caspase-3 (Lee 2009). This study showed that turmeric extract repressed the production and secretion of hepatitis B surface antigen from HepG 2.2.15 cells, an activity that is mediated through the enhancement of cellular accumulation of p53 protein by transactivating the transcription of the p53 gene as well as increasing the stability of the p53 protein (Kim et al. 2009).

13.6.2 In Vivo Studies with Turmeric

Both the preventive and therapeutic effects of turmeric have been examined in animal models (Table 13.4). These studies report that this yellow spice exhibits anticancer (Azuine and Bhide 1994; Deshpande, Ingle, and Maru 1997; Garg, Ingle, and Maru 2008), hepatoprotective (Miyakoshi et al. 2004), cardioprotective (Mohanty, Arya, and Gupta 2006), hypoglycemic (Kuroda et al. 2005; Honda et al. 2006), and antiarthritic properties (Funk et al. 2006).

In various models, turmeric has been reported to exhibit activity against the development of skin cancer (Villaseñor, Simon, and Villanueva 2002), breast cancer (Deshpande, Ingle, and Maru 1998a), oral cancer (Azuine and Bhide 1992a), and stomach cancer (Azuine and Bhide 1992b). It prevents carcinogenesis at various steps, including inhibiting mutation (Polasa et al. 1991), detoxifying carcinogens (Thapliyal, Deshpande, and Maru 2001), decreasing cell proliferation, and inducing apoptosis of tumor cells (Garg, Ingle, and Maru 2008). Turmeric extract prevents animal tumors induced by

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TABLE 13.4
In Vivo Effect of Turmeric against Development of Various Diseases/Disorders

Disease	Route	References
	Cancer	
Oral carcinogenesis[a]	5% in diet[h]	Azuine et al. 1992
Buccal pouch carcinogenesis[b]	In diet[h]	Krishnaswamy et al. 1998
Buccal pouch carcinogenesis[b]	1% in diet[h]	Garg, Ingle, and Maru 2008
Forestomach tumors[a]	In diet[h]	Nagabhushan, Amonkar, and Bhide 1987
Forestomach tumors[a]	1 mg oral[i]	Azuine et al. 1992
Forestomach and oral[a]	5% in diet[h]	Azuine and Bhide 1994
Forestomach papillomas[a]	0.2%–5.0% oral[i]	Deshpande, Ingle, and Maru 1997
Skin tumor[a]	5 mg topical[i]	Villaseñor, Simon, and Villanueva 2002
Mammary tumorigenesis[a]	5% in diet[h]	Bhide et al. 1994
Mammary tumorigenesis[c]	0.5%–1.0%[i,j]	Deshpande et al. 1998
Preneoplastic lesions of liver	0.05% in diet[h]	Soni et al. 1997
Hepatocarcinogenesis[c]	0.2%–5.0% in diet[h]	Thapliyal et al. 2003
Lymphoma formation and survival[a]	10–40 mg/animal, i.p.[j]	Kuttan et al. 1985
	Cytotoxicity	
Lipid peroxidation[a]	1% in diet[i]	Asai et al. 1997
Lipid peroxidation[c]	0.1% in diet[h]	Kaul and Krishnakantha 1997
Lipid peroxidation[d]	NC	Quiles et al. 1998
Cholestrol and triglyceride level[c]	In diet[j]	Dixit, Jain, and Joshi 1988
LDL oxidation and cholestrol level[d]	1.66–3.2 mg/kg orally[j]	Ramirez-Tortosa et al. 1999
Cell proliferation[c]	20 mg/mL oral[i]	Zhang et al. 1999
CYP 1A1 and 1A2 level[c]	1% in diet[h]	Thapliyal, Deshpande, and Maru 2001
BaP–DNA adducts formation[a]	1% in diet[h]	Thapliyal Deshpande, and Maru 2002
Micronuclei formation[a]	1000 mg/kg intragastric[i]	Chandra Mohan, Abraham, and Nagini 2004
Oxidative damage[a]	1% and 5% in diet[h]	El-Ashmawy et al. 2006
Apoptosis[c]	5% in diet[h]	Hashem, Soliman, and Shaapan 2008
Urinary mutagens level[c]	0.5% in diet[h]	Polasa et al. 1991
BaP–DNA adducts formation[c]	0.1%–3% for 4 weeks	Mukundan et al. 1993
Xenobiotic-metabolizing enzymes[c]	0.5%–10.0% in diet[h]	Goud, Polasa, and Krishnaswamy 1993
Membrane oxidation[d]	1.6 mg/kg in diet[h]	Mesa et al. 2003
Inflammation[b]	NC	Boonjaraspinyo, Boonmars, and Aromdee 2009
	Hepatic Disorder	
Cholesterol, bilirubin, AST, ALT, AP[c]	5% in diet[h]	Deshpande et al. 1998
Focal dysplasia, GGT[c]	0.2%–5.0% in diet[h]	Thapliyal et al. 2003
LDH, ALT, AST[c]	1% in diet[i]	Miyakoshi et al. 2004
	Diabetes	
Hypoglycemia[a]	0.2–1.0 g in diet[j]	Arun and Nalini 2002; Kuroda et al. 2005; Nishiyama et al. 2005
Diabetic cataract[c]	0.5% in diet[h]	Suryanarayana et al. 2005
Oxidative stress[c]	0.5% in diet[h]	Suryanarayana et al. 2007
VEGF expression in diabetes[c]	0.5% in diet[h]	Mrudula et al. 2007
	Wound Healing	
Healing of ulcer[c,d]	Topical application[L]	Gujral, Chowdhury, and Saxena 1953
Wound covering[d]	Paste topically	Kundu et al. 2005
Inflammation and wound repaire	25% mixed with ghee topical[h]	Habiboallah et al. 2008
Renal injury[c]	5% in diet[h]	Hashem, Soliman, and Shaapan 2008
	Neuroprotection	
Depression[a]	140–560 mg/kg oral[i]	Yu, Kong, and Chen 2002
Depression[a]	25–100 mg/kg gavage[i]	Xia et al. 2007

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TABLE 13.4 (*Continued*)
In Vivo Effect of Turmeric against Development of Various Diseases/Disorders

Disease	Route	References
	Cardiac Disorder	
Cardiovascular performance[c]	100 mg/kg oral[i]	Mohanty, Arya, and Gupta 2004, 2006
Arterial blood pressure and heart rate[c]	NC	Adaramoye and Medeiros 2008
	Arthritis	
Adjuvant arthritis[c]	NC	Arora et al. 1971
Freund's adjuvant induced arthritis and acute edema[c]	NC	Chandra and Gupta. 1972
Edema[c]	NC	Ghatak and Basu 1972; Yegnanarayan, Saraf, and Balwani 1976
Joint inflammation and destruction[c]	46 mg/kg, i.p.[i]	Funk et al. 2006
	Hepatitis	
Hepatobiliary clearance[c]	1% in diet[i]	Deshpande, Joseph, and Samuel 2003
	Atherosclerotic	
Lipid peroxidation[d]	In diet[i]	Quiles et al. 1998
LDL oxidation[d]	1.66–3.2 mg/kg oral[i]	Ram"rez-Tortosa et al. 1999
VSMC proliferation[c]	10% oral[i]	Zhang et al. 1999
Membrane oxidation[d]	1.66 mg/kg oral[i]	Mesa et al. 2003
Vasorelaxation[c]	0.5% in diet[i]	Zahid Ashraf, Hussain, and Fahim 2005
	Respiratory Disorders	
Relaxation of aorta or atria[c,d,f]	Orally[k]	Gilani et al. 2005
Hypotensive, bradycardic, and vasodilative[c]	10–30 mg/kg, i.v.[k]	Adaramoye and Medeiros 2008
	Other	
Mucin contents of gastric juice[d]	500 mg/kg in diet[h]	Mukerji, Zaidi, and Singh 1961
Retinol deficiency[c]	0.1% in diet[h]	Kaul and Krishnakantha 1997
Rancidity of sunflower oil	2000 mg/kg[i]	Beddows, Jagait, and Kelly 2000
Hypothyroidism[c]	In diet[i]	Deshpande et al. 2002
Indigestion[c]	In diet[h]	Platel et al. 2002
Feed intake, weight gain[g]	0.5% in diet[h]	Gowda et al. 2008
Skin thickness, wrinkles, melanin[a]	300–1000 mg/kg topical[j]	Sumiyoshi and Kimura In press
Spermatogenesis and fertility[a]	600 mg/kg orally[i]	Mishra and Singh 2009

AP = alkaline phosphatase; ALT = alanine aminotransferase; LDH = lactate dehydrogenase; AST = aspartate aminotransferase; CYP = cytochrome enzymes; GGT = gamma glutamyl transpeptidase; GSH = glutathione; LDL = low-density lipoprotein; LPO = lipid peroxidation; NC = not clear; VEGF = vascular endothelial growth factor; VSMC = vascular smooth muscle cell.

[a] Mice
[b] Hamster
[c] Rat
[d] Rabbit
[e] Dog
[f] Guinea pig
[g] Chicks
[h] Turmeric powder
[i] Aqueous extract of turmeric
[j] Ethanolic extract of turmeric
[k] Methanolic extract of turmeric
[L] Not clear

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Dalton's lymphoma (Kuttan et al. 1985). In this study, mice were injected with Dalton's lymphoma cells intraperitoneally and treated with turmeric extract (10–40 mg/animal) for 10 days. After 30 days, the authors found up to 80% decrease in tumor formation in comparison with nontreated mice (Figure 13.2a). They also observed that up to 75% of animals survived after 30 days and 50% after 60 days of treatment (Figure 13.2b). In a 7,12-dimethylbenz(*a*)anthracene (DMBA)-induced hamster buccal pouch model of carcinogenesis, dietary turmeric (1%) decreased tumor burden and multiplicity and enhanced the latency period in parallel. The mechanisms of anticarcinogenesis were mediated through inhibition of DMBA-induced expression of the *ras* oncogene product, induction of p21 and its downstream targets, mitogen-activated protein kinases, and reduction of proliferating cell nuclear antigen and Bcl-2 expression. Turmeric also enhanced apoptosis (increased expression of Bax, caspase-3, and apoptotic index), decreased inflammation (levels of cyclooxygenase [COX]-2, the downstream target of activator protein-1/nuclear factor κB [NF-κB], and PGE2), and induced aberrant expression of known differentiation markers, that is, cytokeratins (Garg, Ingle, and Maru 2008).

Topical application of turmeric was found to decrease multiplicity and onset of skin tumors (Villaseñor, Simon, and Villanueva 2002). Dietary administration of 1% turmeric per 0.05% ethanolic turmeric extract was found to inhibit DMBA-induced mammary tumorigenesis in female Sprague–Dawley rats (Deshpande, Ingle, and Maru 1998a). Dietary turmeric inhibited ethyl(acetoxymethyl) nitrosamine-induced oral carcinogenesis in Syrian hamsters. However, the inhibitory effect of a combination of turmeric and betel leaf extract was found to be higher than that of the individual constituents (Azuine and Bhide 1992a). Administration of turmeric extract at a dose of 3 mg/animal 18 hours prior to intraperitoneal (i.p.) injection of benzo[a]pyrene (BaP; 250 mg/kg) significantly inhibited bone marrow micronuclei formation in female Swiss mice. Moreover, the incidence and multiplicity of BaP-induced forestomach tumors in female Swiss mice were significantly inhibited by turmeric extract (Azuine, Kayal, and Bhide 1992). Chandra Mohan, Abraham, and Nagini (2004) also showed that pretreatment with turmeric alone and in combination with tomato and garlic extract significantly lowered the

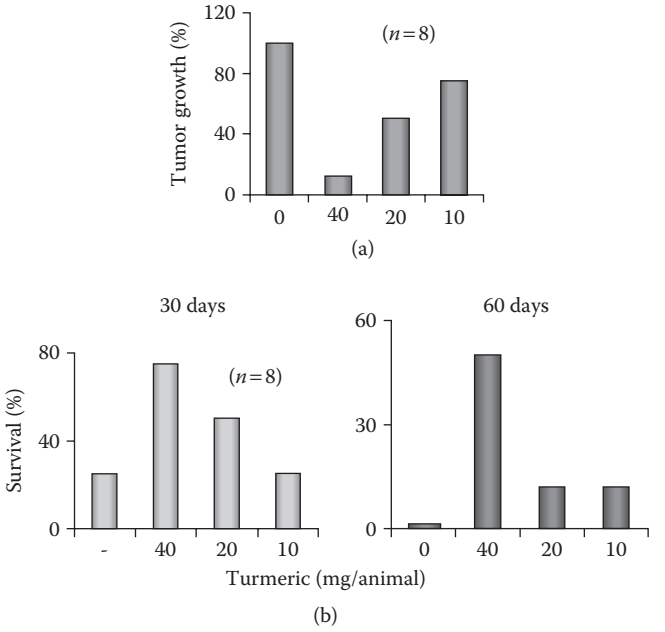


FIGURE 13.2 Inhibition of tumor growth in mice by turmeric extracts in a dose-dependent manner. Mice were injected with Dalton's lymphoma cells (1 million) intraperitoneally. After randomization, turmeric was given to the mice (n = 8) at indicated concentration for 10 days. Controlled animals received saline only. (a) Percent of tumor formation after 30 days. (b) Number of animals surviving at 30 days and 60 days. (Redrawn from Kuttan, R., P. Bhanumathy, K. Nirmala, and M. C. George. 1985. *Cancer Lett* 29:197–202.)

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frequencies of DMBA-induced bone marrow micronuclei, as well as the extent of lipid peroxidation. They revealed that these changes may be mediated by the antioxidant-enhancing effects of the dietary agents. Combined treatment of urethane, a well-known mutagen, and turmeric displayed an inhibition of the genotoxic effect of urethane by turmeric (el Hamss et al. 1999). Decrease in tumorigenesis caused by turmeric is also associated with inhibition of DNA adduct formation. Turmeric inhibited the levels of BaP-induced DNA adducts in the livers of rats. Inclusion of turmeric at 0.1%, 0.5%, and 3.0% in the diet for 4 weeks significantly decreased the level of BaP–DNA adducts, including the major adduct dG-N2-BaP, formed within 24 hours in response to a single i.p. BaP injection (Mukundan et al. 1993). Irrespective of whether turmeric was included in the diet or applied locally, it significantly decreased DMBA-induced DNA adducts at the target site and consequently lowered the number of tumors and tumor burden in the studied animals (Krishnaswamy et al. 1998). Turmeric contains several substances capable of inhibiting chemical carcinogenesis. It enhanced the xenobiotic-metabolizing enzymes in the hepatic tissue of rats fed with 0.5–1.0% turmeric in the diet. Detoxifying enzymes such as uridine diphosphate (UDP), glucuronyl transferase, and glutathione-S-transferase significantly increased in turmeric-fed mice as compared with control animals (Goud, Polasa, and Krishnaswamy 1993).

Ethanolic turmeric extract was found to have opposing actions on murine lymphocytes and on Ehlrich ascitic carcinoma cells. Turmeric enhances lymphocyte viability and blastogenesis, but induces formation of cytoplasmic blebs and plasma membrane disintegration of tumor cells. Thus, it is suggested that turmeric is a conducive agent for lymphocytes and inhibitory as well as apoptosis-inducing for tumor cells (Chakravarty and Yasmin 2005). A comparative study of edible plants like C. *longa* and *F. caraica*, and herbaceous plants like *Gossypium barbadense* and *Ricinus communis* extracts for their antitumor activities showed that the edible plant extracts exhibited higher antitumorigenic activities. Thus, edible plants that show in vivo antitumor activities may be recommended as safe sources of antitumor compounds (Amara, El-Masry, and Bogdady 2008).

Turmeric showed antioxidant potential by lowering oxidative stress in animals. A study showed that a diet containing 0.1% turmeric fed for 3 weeks to retinol-deficient rats lowered lipid peroxidation rates by 22.6% in liver, 24.1% in kidney, 18.0% in spleen, and 31.4% in brain (Kaul and Krishnakantha 1997). A study conducted on mice showed that turmeric extract inhibited membrane phospholipid peroxidation and increased liver lipid metabolism, which indicates turmeric extract has the ability to prevent the deposition of triacylglycerols in the liver. Dietary supplementation for one week (1% w/w of diet) with a turmeric extract showed lower phospholipids hydroperoxide level in mice red blood cells (RBC). The liver lipid peroxidizability induced with Fe^{2+}/ascorbic acid was effectively suppressed by dietary supplementation with turmeric (Asai, Nakagawa, and Miyazawa 1999). Oral administration of a nutritional dose of turmeric extract decreased susceptibility to oxidation of erythrocyte and liver microsome membranes in vitro. When turmeric hydroalcoholic extract (1.66 mg/kg of body weight) was given to rabbits fed a high-fat diet, oxidation of erythrocyte membranes was found to be significantly lower than that in membranes of control animals. Levels of hydroperoxides and thiobarbituric acid–reactive substances in liver microsomes were also low (Mesa et al. 2003). Turmeric also seems beneficial in preventing diabetes-induced oxidative stress. In diabetic rats, an AIN93 diet containing 0.5% turmeric was found to control oxidative stress by inhibiting increases in thiobarbituric acid–reactive substances and protein carbonyls and reversing altered antioxidant enzyme activities without altering the hyperglycemic state (Arun and Nalini 2002; Suryanarayana et al. 2007). This diet also inhibited expression of vascular endothelial growth factor in diabetic rats (Mrudula et al. 2007). Further, it suppressed increase in blood glucose level in type 2 diabetic KK-Ay mice. A dose of 0.2 or 1.0 g of ethanol extract, 0.5 g of hexane extract, and 0.5 g of hexane-extraction residue per 100 g of diet in the mice feed suppressed significant increase in blood glucose levels. The ethanol extract of turmeric also stimulated human adipocyte differentiation, and it showed human peroxisome proliferator-activated receptor-gamma (PPAR-γ) ligand-binding activity (Nishiyama et al. 2005). Further, turmeric appeared to minimize osmotic stress. Most importantly, aggregation and insolubilization of lens proteins due to hypergly-

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cemia was prevented by turmeric, indicating that it prevents or delays the development of cataracts (Suryanarayana et al. 2005).

Turmeric has been reported to be hepatoprotective. Diets containing turmeric extract suppressed increases in lactate dehydrogenase (LDH), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels caused by D-galactosamine-induced liver injury in rats (Miyakoshi et al. 2004). A 5% turmeric extract decreased carbon tetrachloride–induced increases in serum levels of bilirubin, cholesterol, AST, ALT, and alkaline phosphatase (ALP) in mice (Deshpande et al. 1998b). In female Wistar rats fed a diet containing 0%, 0.2%, 1.0%, or 5.0% turmeric, nitrosodiethylamine-induced hepatocarcinogenesis was inhibited. This effect was detected by measuring the numbers of γ-glutamyl transpeptidase–positive foci, a marker of hepatocarcinogenesis (Thapliyal et al. 2003).

Turmeric is also effective against neuronal, cardiac, and kidney disorders. The effect of turmeric on myocardial apoptosis and cardiac function was examined in an ischemia and reperfusion model of myocardial injury. Turmeric at 100 mg/kg administered for 1 month afforded significant cardioprotection and functional recovery that was attributed to reduction in cell death (Mohanty, Arya, and Gupta 2006).

Turmeric is also useful against depression (Yu, Kong, and Chen 2002; Xia et al. 2006; Xia et al. 2007). Its ethanolic extract markedly attenuated swim stress–induced decreases in serotonin, 5-hydroxyindoleacetic acid, and noradrenaline and dopamine concentrations, as well as increases in serotonin turnover. Also, this extract significantly reversed swim stress–induced increases in serum corticotropin-releasing factor and cortisol levels and thus regulated neurochemical and neuroendocrine systems in mice (Xia et al. 2007). In another study, administration of aqueous extracts of turmeric to mice (140–560 mg/kg for 14 days) reduced immobility in the tail suspension test and the forced swimming test (Yu, Kong, and Chen 2002). The effects of 560-mg/kg turmeric were found to be more potent than those of the antidepressant fluoxetine. The extracts significantly inhibited brain monoamine oxidase (MAO)-A activity at a low dose, but at a higher dose, they inhibited brain MAO-B activity. In comparison, fluoxetine showed only a tendency to inhibit MAO-A and -B activity in animal brains. These results demonstrate that turmeric has specific antidepressant effects in vivo. However, since curcumin is not water soluble, the agent in aqueous extracts of turmeric responsible for this activity is not clear.

The antiarthritic effects of turmeric include inhibition of joint inflammation and periarticular joint destruction. In vivo treatment with turmeric extract prevented local activation of NF-κB and the subsequent expression of NF-κB-regulated genes mediating joint inflammation and destruction, including chemokines, COX-2, and the receptor activator of NF-κB ligand (RANKL). It also inhibited inflammatory cell influx, joint levels of PGE2, and periarticular osteoclast formation in rats (Funk et al. 2006). Turmeric was found to be effective against carrageenan-induced edema in rats (Yegnanarayan, Saraf, and Balwani 1976), and water extracts of turmeric were more active than alcohol extracts in the inhibition of carrageenan-induced edema. Turmeric extract, when given intraperitoneally, was found to be more active than hydrocortisone (Ghatak and Basu 1972). The yellow powder of turmeric is known to have potent vasorelaxant activity and to decrease the atherogenic properties of cholesterol. A study showed that supplementation of turmeric in the diet controlled arterial blood pressure in animals and enhanced vasorelaxant responses to adenosine, acetylcholine, and isoproterenol (Zahid Ashraf, Hussain, and Fahim 2005). Turmeric's antiatherosclerotic effect is associated with inhibition of low-density lipoprotein oxidation, prevention of lipoperoxidation, and reduction in levels of cholesterol (Quiles et al. 1998; Ramírez-Tortosa et al. 1999). A study showed that feeding an ethanolic extract of turmeric to rats elevated the high-density lipoprotein (HDL)-cholesterol/total cholesterol ratio. The extract also caused a significant decrease in the ratio of total cholesterol/phospholipids. Turmeric extract exhibited better cholesterol and triglyceride lowering (85% and 88%, respectively) as compared to *Nardostachys jatamansi* extract in triton-induced hyperlipidemic rats (Dixit, Jain, and Joshi 1988). Turmeric suppresses Freund's adjuvant-induced arthritis and acute edema in rats, and it has also been reported that oil extract of turmeric is more active than cortisone (Chandra and Gupta 1972).

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Another interesting property of turmeric is its wound-healing ability. Gujral, Chowdhury, and Saxena (1953) found that turmeric has the property of healing wounds and ulcers in rats and rabbits. Other studies in rabbits revealed that stimulation of mucin secretion could protect the stomach from ulcer (Mukerji, Zaidi, and Singh 1961).

Besides causing these effects, addition of turmeric to the diet significantly improved weight gain of broiler chicks and reduced their relative liver weight. Turmeric also ameliorated the adverse effects of aflatoxin on some serum chemistry parameters (total protein, albumin, cholesterol, calcium) in broiler chicks and restored antioxidant functions in terms of level of peroxides, superoxide dismutase activity, and total antioxidant concentration in their livers (Gowda et al. 2008).

Turmeric acts as a digestive stimulant. As a dietary supplement, it favorably enhanced the activities of pancreatic lipase, chymotrypsin, and amylase. Moreover, turmeric mixed with other spices such as coriander, red chili, black pepper, and cumin brought about a pronounced stimulation of bile flow and bile acid secretion (Platel et al. 2002). Mukerji, Zaidi, and Singh (1961) showed that turmeric increases the mucin content of gastric juice in rabbits. Studies conducted by Farnsworth and Bunyapraphatsara (1992), Supniewski and Hano (1935), and Prucksunand et al. (2001) explain that turmeric has local anesthetic action. After eating turmeric, secretion of gastrin hormone from the antrum of the stomach may be inhibited. Turmeric may possess local membrane-anesthetizing activity at the antrum of the stomach, which then inhibits secretion of gastrin in the same way as oxethazaine, the active ingredient of strocain (Masuda 1973). This is the reason turmeric is administered before meals.

TABLE 13.5
Human Studies with Turmeric

Diseases	Dose	Response	References
Asthma	NC	Relief from bronchial asthma and cough	Jain, Bhatnagar, and Parsai 1979
Cancer	Topical application	Reduction in itching, pain, and size in external cancerous lesions	Kuttan, Sudheeran, and Joseph 1987
Mutation	1500 g/day × 30 days	Reduction in urinary excretion of mutagens in smokers	Polasa et al. 1992
Micronuclei	600 mg/day	Inhibition in micronuclei formation in oral mucosal cells and lymphocytes	Hastak et al. 1997
Abdominal pain	TRE	Reduction of dumpy and colicky pain	Niederau and Göpfert 1999
Ulcer	1500 mg	Reduction of peptic ulcer	Prucksunand et al. 2001
IBS	144 mg/daily × 8 weeks	Reduction of abdominal pain	Bundy et al. 2004
Infection	Topical application	Umbilical cord care after cutting	Alam et al. 2008
Breathing	500 mg in diet	Activation of hydrogen-producing bacterial flora in the colon, increase in breath hydrogen	Shimouchi et al. 2008

IBS = irritable bowel syndrome; TRE = turmeric root extract; NC = not clear.

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13.6.3 Clinical Studies Using Turmeric

Turmeric has been tested against various diseases in humans (Table 13.5). In one study, the antimutagenic effects of turmeric were examined in 16 chronic smokers (Polasa et al. 1992). Turmeric was given in doses of 1.5 g/day for 30 days, and this was found to significantly reduce the urinary excretion of mutagens in these smokers. In six nonsmokers, on the other hand, no change in urinary excretion of mutagens was noted. These results suggest that dietary turmeric is an effective antimutagen and may be useful in chemoprevention. In another study, the effect of turmeric was examined on patients with irritable bowel syndrome. When 1 or 2 tablets of a standardized turmeric extract were given daily for 8 weeks, the prevalence of irritable bowel syndrome was significantly decreased, as was the abdominal pain/discomfort score (Bundy et al. 2004). Alcoholic extract of turmeric offered protection against BaP-induced increase in micronuclei in circulating lymphocytes of healthy individuals (Hastak et al. 1997). In a subsequent study, the authors treated patients suffering from oral submucous fibrosis (OSF) with turmeric extract (3 g/day) for 3 months. The number of micronuclei from oral exfoliated cells of OSF patients before and after treatment with turmeric extract was recorded. They found that the number of micronuclei in oral exfoliated cells decreased substantially and was comparable with that of normal, healthy individuals (Figure 13.3).

Turmeric was also found useful in healing peptic ulcers. In a phase II clinical trial, 45 patients with peptic ulcer received capsule-filled turmeric orally in the dose of 2 capsules (300 mg each) five times daily. After 4 weeks of treatment, ulcers were found to be absent in 48% of cases. After 12 weeks of treatment, ulcer-free cases increased to 76% (Prucksunand et al. 2001). A double-blind trial found turmeric to be helpful for people with indigestion and for people with stomach or intestinal ulcers, but it was shown to be less effective than antacids (Kositchaiwat, Kositchaiwat, and Havanondha 1993). An ethanol extract of turmeric was found to produce remarkable symptomatic relief in patients with external cancerous lesions. In a study of 62 patients, reduction in smell was noted in 90% of the cases and reduction of itching in almost all cases. Some patients (10%) had a reduction in lesion size and pain (Kuttan, Sudheeran, and Joseph 1987).

A study on eight healthy subjects showed that the presence of turmeric in curry increases bowel motility and activates hydrogen-producing bacterial flora in the colon, thereby increasing the concentration of breath hydrogen (Shimouchi et al. 2008). Turmeric paste is used to heal wounds or to

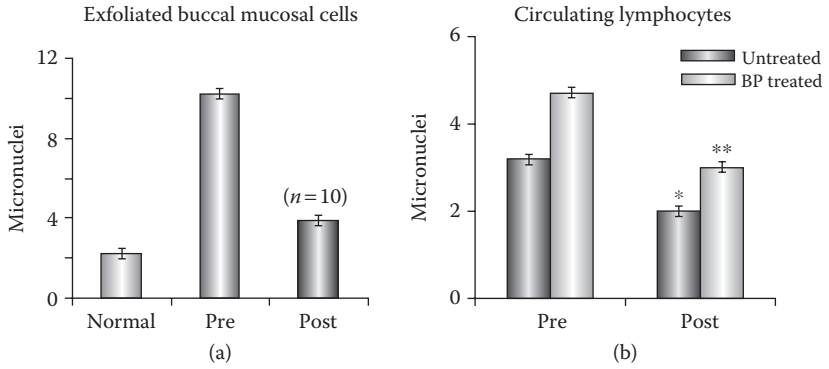


FIGURE 13.3 Inhibition of micronuclei formation in oral submucous fibrosis (OSF) patients: (a) Incidence of micronuclei in exfoliated buccal mucosal cells of OSF patients before and after treatment with turmeric and of normal healthy individuals. (b) Incidence of micronuclei in circulating lymphocytes of OSF patients before and after turmeric treatment. (n = 10). The symbol * indicates statistical significance when compared with untreated pretreatment group ($p < .001$). The symbol ** indicates statistical significance when compared with benzo[a]pyrene-treated pretreatment group ($p < .001$). (Redrawn from Hastak, K., N. Lubri, S. D. Jakhi et al. 1997. *Cancer Lett* 116:265–9.)

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protect against infection. In certain parts of Bangladesh, turmeric is the most common application on the cut umbilical cord after delivery (Alam et al. 2008).

13.7 STUDIES WITH TURMERIC OIL

Turmeric has medicinal properties due to its bioactive components. One of the important components of turmeric is its volatile oil. The role of turmeric oil in in vitro animals and in human is shown in Table 13.6. Turmeric oil inhibits *Trichophyton*-induced dermatophytosis in guinea pigs. Apisariyakul, Vanittanakom, and Buddhasukh (1995) showed that 15 different isolates of dermatophytes are inhibited by turmeric oil at dilutions of 1:40 to 1:320. Interestingly, none of the dermatophyte isolates was inhibited by curcumin. Studies of the antiviral effects of the zedoary turmeric oil spray in the respiratory tract showed that whereas influenza virus, parainfluenza viruses I and III, respiratory syncytial virus, and adenoviruses 3 and 7 were inhibited slightly, parainfluenza virus II was significantly inhibited by this turmeric compound (Huang et al. 2007). Curcuma oil ameliorated the ischemia-induced neurological functional deficits and the infarct and edema volumes in rats. It downregulated inducible nitric oxide (NO) synthase (iNOS)-derived

TABLE 13.6
Studies with Turmeric Oil

Response	Dose	References
	In Vitro	
Mutation in *S. typhimurium*	TO[a]	Jayaprakasha et al. 2002
NO and PGE2 production in rat microglial cells	0.1–1.0 μM *C. comosa*[b]	Thampithak et al. 2009
Influenza, PI, RS, AD virus inhibition	TO[a]	Huang et al. 2007
Multiplication of dermatophytes	1:40–1:320 TO[a]	Apisariyakul, Vanittanakom, and Buddhasukh 1995
	In Vivo	
Disaccharidases level[c]	10% spent turmeric in diet	Kumar, Shetty, and Salimath 2005
Sulfation of glycoconjugates level[c]	10% spent turmeric in diet	Kumar, Vijayalakshmi, and Salimath 2006
Blood glucose, abdominal fat level[d]	0.5%–2.0% turmeric oleoresin in diet	Honda et al. 2006
Total sugar content and uronic acid level[c]	10% spent turmeric in diet	Vijayalakshmi, Kumar, and Salimath 2009
LPO, GSH, free-radicals level[d]	200 mg/kg *C. comosa* orally	Jariyawat et al. 2009
Repellency of mosquito	0.1 mL/3 × 10 cm TO topically	Tawatsin et al. 2001
Neuronal cell death of rat brain	500 mg/kg TO, i.p	Rathore et al. 2008
	Human Studies	
Micronuclei in oral mucosal cells and lymphocytes	Turmeric oleoresin and TO (600 mg/day)	Hastak et al. 1997
OSF	0.6 mL of TO three times/day for 1 month	Joshi et al. 2003
Sputum	Volatile oil	Li et al. 1998

TO = turmeric oil; PI = parainfluenza; RS = Rous sarcoma; AD = adenovirus; LPO = lipid peroxidation.

[a] Not defined.

[b] Hexane extract.

[c] Rat.

[d] Mice.

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NO produced during ischemic injury, which coincided with an increased survival rate of neurons (Dohare et al. 2008). The neuroprotective activity of curcuma oil against cerebral ischemia is associated with its antioxidant activities. Further, cucurma oil attenuated delayed neuronal death via a caspase-dependent pathway. Thus, curcuma oil appears to be a promising agent for the treatment of not only cerebral stroke but also other disorders associated with oxidative stress (Rathore et al. 2008). A study revealed that ingestion of turmeric oleoresin and essential oil inhibits the development of increased blood glucose and abdominal fat mass in obese, diabetic rats (Honda et al. 2006).

Turmeric volatile oil is effective against disorders of the respiratory tract. The volatile oil is active in removing sputum, relieving cough, and preventing asthma. Thus, turmeric volatile oil may be an efficacious drug in the treatment of respiratory diseases (Li et al. 1998). This oil acts as a repellent against both day- and night-biting mosquitoes (Tawatsin et al. 2001).

Hexane extracts of *C. comosa*, an indigenous plant of Thailand that is traditionally used for the treatment of uterine inflammation, at concentrations of 0.1, 0.5, and 1 μM were found to significantly decrease LPS-induced NO and PGE2 production. It also decreased the expression of iNOS and COX-2 (Thampithak et al. 2009).

13.8 SAFETY, EFFICACY, AND CONTRAINDICATIONS

The use of turmeric as a spice and as a household remedy has been known to be safe for centuries. To date, no studies in either animals or humans have discovered any toxic effects associated with the use of turmeric (Lao et al. 2006), and it is clear that turmeric is not toxic even at very high doses. The U.S. Food and Drug Administration (FDA) has conducted its own clinical trials with turmeric and published a 300-page monograph. The FDA has declared turmeric and its active component curcumin as GRAS (generally regarded as safe). Thus, in the United States, turmeric and its components are currently being used in mustard, cereals, chips, cheese, butter, and other products (http://www.kalsec.com/products/color). In a phase I clinical study on the safety and tolerance of turmeric oil use, the oil was administered orally to healthy volunteers for 3 months. No side effects of turmeric oil intake were observed in 3 months on body weight, blood pressure, and hematological, renal, or hepatic toxicity (Joshi et al. 2003).

13.9 CONCLUSIONS

The beneficial effects of turmeric are traditionally achieved through dietary consumption, even at low levels, over long periods of time. A precise understanding of effective dose, safety, and mechanism of action is required for the rational use of turmeric in the treatment of human diseases. Further clinical studies are warranted if turmeric is to be employed in meeting human needs and improving human welfare. The activities of turmeric include antibacterial, antiviral, anti-inflammatory, antitumor, antioxidant, antiseptic, cardioprotective, hepatoprotective, nephroprotective, radioprotective, and digestive activities. Phytochemical analysis of turmeric has revealed a large number of compounds, including curcumin, volatile oil, and curcuminoids, which have been found to have potent pharmacological properties.

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14 Biomolecular and Clinical Aspects of Chinese Wolfberry

Peter Bucheli, Qiutao Gao, Robert Redgwell, Karine Vidal, Junkuan Wang, and Weiguo Zhang

CONTENTS

14.1 Introduction 290
14.2 Description and Traditional Use of Wolfberry Fruit 291
14.2.1 Origin, Identification, and Characterization of Wolfberry 291
14.2.2 Use of Wolfberry in Traditional Chinese Medicine 292
14.2.3 Use of Wolfberry in Foods, Beverages, and Nutricosmetics 292
14.2.4 Magnitude of Wolfberry Usage 292
14.2.5 Evidence for Safety and Health Concerns from Diverse Human Experience 293
14.3 Identification of Bioactives 293
14.3.1 Arabinogalactan-Proteins: Bioactive Glycoconjugates in *Lycium barbarum* L. 293
14.3.1.1 Isolation and Structural Features of Glycoconjugates 293
14.3.1.2 Optimizing *Lycium barbarum* Polysaccharide Recovery from Wolfberry 295
14.3.1.3 Bioactive Properties 295
14.3.2 Zeaxanthin 296
14.3.2.1 Estimated Zeaxanthin Intake: Potential Contribution from Wolfberry 297
14.3.2.2 Mechanism of Intestinal Zeaxanthin Absorption 297
14.3.3 The Vitamin C Precursor 2-*O*-(β-D-Glucopyranosyl) Ascorbic Acid 298
14.4 Bioavailability of Wolfberry Bioactives and Effects of Processing 299
14.4.1 Lacto-Wolfberry: The Generation of a Natural, Stable, and Bioavailable Formulation 299
14.4.2 Bioavailability of Zeaxanthin from Wolfberry 299
14.5 Immunomodulating Activity 301
14.5.1 Role of *Lycium barbarum* Polysaccharides 301
14.5.2 Influence of Glycan Composition and Protein Content of *Lycium barbarum* Polysaccharide 302
14.5.3 Indirect Immune Benefits of *Lycium barbarum* Polysaccharide 303
14.6 Antidiabetic Activity of Wolfberry 304
14.7 Cardiovascular Benefits of Wolfberry 305
14.8 Improvement of Sexual Function by Wolfberry 306
14.9 Cognitive Benefit of Wolfberry 307
14.10 Vision Benefits of Wolfberry 307
14.11 Conclusion 308
References 308

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14.1 INTRODUCTION

"Wolfberry" and "goji berry" are synonyms for one of the best-known and admired berries from China, called *gou qi* (枸杞) or *kei tze* in Chinese. The wolfberry fruit has been used in traditional Chinese medicine (TCM) for more than 2000 years, and its use was first recorded around 200 BCE in *Shen Nong Ben Cao Jing* (神农本草经), an ancient book detailing the medicinal and agricultural knowledge of the mythical Chinese emperor Shen Nong. The *Shen Nong Ben Cao Jing* is the oldest book on Chinese herbs, and records 365 traditional herbs that are classified into three grades: (1) top, (2) middle, and (3) low. Wolfberry is one of the 120 herbs belonging to the top grade, which was believed to have remarkable health benefits and to be harmless to humans. Long-term use of wolfberry was considered beneficial for strengthening the body, keeping fit, prolonging life, and easing life through all the seasons (久服, 坚筋骨, 轻身不老, 耐寒暑). Another classic TCM treatise, the *Compendium of Materia Medica* (本草纲目), a collection of books by Li Shi-Zhen from the sixteenth century, is considered the first pharmacopoeia in the world and the most important one ever written on TCM; in this work, the morphological identification, health benefits, indications, and relevant prescriptions of wolfberry are particularly elaborated. Besides the fruit, use of other parts of the wolfberry plant, including flower, leaf, seed, and root bark, are also recorded. From a TCM point of view, the nature of wolfberry is "calm," and its flavor is "sweet." According to TCM theory and practice, wolfberry can act on both the "liver channel" and the "kidney channel," and the major health benefits of wolfberry are its ability to nourish and tonify liver and kidney, improve *jing* (精, the basic elements that constitute the body and maintain life activities), and improve eye function (Chinese Pharmacopoeia 2005). It should be noted that wolfberry is used not only as a drug in TCM prescriptions to treat diseases but also as a popular food by Chinese people in their daily life for promotion of general health. According to the regulations of the China State Food and Drug Administration, wolfberry is one of the 87 TCM ingredients that can be used as both normal food and functional food.

The health benefits of wolfberry, which have been recorded from the empirical insights of Chinese doctors over hundreds of years, are today perceived by Chinese consumers as centered on age-related conditions, with benefits primarily for eyesight. This public perception is also reflected in the numerous scientific studies that have been conducted in China in the last 30 years. The earliest work to this effect was published in 1998 by Professor S. Bai of Yinchuan, Ningxia, China, in two books consisting of a total of 462 Chinese research articles on wolfberry. More recent reviews (Gross, Zhang, and Zhang 2006; Sze et al. 2008) discuss the scientific evidence for wolfberry's bioactivities in the international literature published after the year 2000. Gaps in scientific evidence were identified, and it was cautioned that no evidence exists "to support claims for a quasi-miraculous potion for longevity" (Potterat and Hamburger 2008, 399). This is particularly true for the rather poorly defined wolfberry fraction of polysaccharides and glycoconjugates often referred to as *Lycium barbarum* polysaccharide (LBP), which is promoted as a unique bioactive with immuno-modulating, antitumor, and antioxidant activity.

Although the benefits of wolfberry are well known and highly thought of in TCM, scientific evidence regarding its benefits is unclear. The objective of this review is to highlight the numerous health benefits of wolfberry from a rigorous scientific and clinical perspective. Arguments and evidence are given in this chapter indicating that LBP is not that unique, and that many of the potential benefits are related to indirect evidence (Sze et al. 2008). We also draw attention to new and refreshing initiatives of researchers, such as Chang and So (2008) who are trying to identify new ways to conduct research on wolfberry, for instance, by looking at its effects in relation to preventing neuronal death in neurodegenerative diseases. New ideas regarding the delivery of bioactives from wolfberry, and other TCM herbs in general (Wang et al. 2005), are also a part of this review.

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14.2 DESCRIPTION AND TRADITIONAL USE OF WOLFBERRY FRUIT

Most of the wolfberry fruit produced is dried in the sun, and in China dried berries are consumed as a usual part of the diet. Dried wolfberry is a common ingredient in commercial food products, supplements, and TCM. Wolfberry is frequently added to soups, hot pots, and herbal teas, and is also popularly soaked in wines alone or together with other TCM ingredients to make functional wines.

14.2.1 Origin, Identification, and Characterization of Wolfberry

Wolfberry occurs as a common shrub with delicate, edible leaves and small purple flowers (Figure 14.1). It grows naturally throughout the northern and western regions of China, and when cultivated, the plant reaches a height of about 2 m. The fruit is roughly spindle shaped or ellipsoidal (elliptical in long section and circular in cross section), 6–20 mm long, and 3–10 mm in diameter (Figure 14.2). The fruit contains 20–50 seeds and has a sweet and fruity taste (Zhao 2004). The two main species cultivated in China are *L. barbarum* L. and *L. chinense* Miller. The species *L. barbarum* is widely cultivated for medicine in north and northwest China, especially along the Yellow River in Ningxia Province. Almost half of the commercial wolfberry fruit produced in China is from Ningxia, and most of it is *L. barbarum*. According to TCM insight, among all regions of China, Ningxia Province has long been considered the site of "authentic origin" (道地产区) of wolfberry. Generally, authentic origin means that a given region has the most suitable growing environment (e.g., soil and climate), a long production history, and a large production volume of a specific herb. Accordingly, an herb produced under such conditions is called an "authentic herb" (*daodi* herb in Chinese, 道地药材) and is considered to be of higher quality than the same type of herb produced in other regions. The reference for Ningxia as the authentic origin of wolfberry can be traced back to the *Compendium of Materia Medica*. This traditional knowledge has been confirmed by modern analysis; as it has been demonstrated that wolfberry from Ningxia contains higher amounts of



FIGURE 14.1 **(See color insert.)** Fresh, mature wolfberry fruit.



FIGURE 14.2 Wolfberry fruit drying in the sun.

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active ingredients and nutrients (Wang 2003; Li and Zhang 2007). The species *L. barbarum* is also indigenous to Europe as described in *Flora for Germany, Austria, and Switzerland* (Thomé 1885), although there is no evidence for a traditional consumption of its fruits in Europe. *L. chinense*, which is a smaller relative, is widely found in China and grows also in Nepal, Pakistan, Thailand, and Europe (Wu and Raven 1994).

14.2.2 Use of Wolfberry in Traditional Chinese Medicine

Wolfberry is mainly used in TCM for treating "yin deficiency" in liver and kidney. The dried fruit is commonly used in TCM preparations at a dose of 6–15 g, taken twice or thrice daily. It is used as a decoction in formulas for treating yin deficiency and liver "qi stagnation" (Liu and Tseng 2005). Wolfberry can also be a part of a mix of Chinese herbs that is ground to a fine powder and used in honey pills (a traditional TCM formulation in which honey is used as main excipient to make pills) of 15 g each. One of these pills is taken with bland soup in the morning and another at night on an empty stomach (Liu and Tseng 2005).

14.2.3 Use of Wolfberry in Foods, Beverages, and Nutricosmetics

Wolfberry is widely used in herbal teas aimed at weight control (with cassia, poria cocos, chrysanthemum, and other herbs), antiaging (with astragalus, ganoderma, and others), and liver protection (with licorice, ganoderma, gynostemna pentaphylla, and others). Consumer survey studies conducted among urban Chinese indicate that tea is one of the most preferred carriers for wolfberry. In South China, where awareness of Chinese herbs and their health benefits is more pronounced than in the rest of the country, wolfberry is commonly used as a tonic ingredient in soups and to make sweet or savory porridge. Wolfberry is also frequently added to traditional hot pot (spicy and nonspicy), a very popular form of healthy eating in southwest China (Sichuan, Hunan) and the big cities (Shanghai, Beijing, Guangzhou). The use of wolfberry has become prevalent in processed foods and beverages, and new product launches have multiplied in recent years in China. Even more surprising are similar product launches in the United States, where wolfberry was rather unknown before 2005 (Table 14.1).

Wolfberry is one of the most popular TCM herbs regulated as a foodstuff that is used in nutricosmetic products in China. Nutricosmetics are used for the promotion of skin and hair health. Only angelica and pearl powder are more frequently found in nutricosmetic products in China.

14.2.4 Magnitude of Wolfberry Usage

In TCM practice, one can note that herbal formulas with wolfberry do not normally contain more than 15 g of wolfberry fruit, and these preparations are taken a maximum of three times a day. Wolfberry is generally washed and cooked together with food (as soup, porridge, hot pot) or mixed with boiling or hot water to make herbal tea. It is difficult to know how much wolfberry is consumed on an individual basis. As an indication, total wolfberry fruit production in China was estimated in 2004 at 95,000 tons

TABLE 14.1
Number of Product Launches in Different Years for Food Products Containing Wolfberry in China and the United States

	2001	2002	2003	2004	2005	2006	2007	2008
China	5	1	3	5	9	39	89	49
United States	0	0	0	0	27	24	97	202

Note: Data from the Global New Products Database (GNPD).

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of dried fruit. According to Chinese practice, wolfberry is more often used by people over 50 years, as it is considered to have antiaging properties, to support immune function, and to protect eyesight. As wolfberry is one of the best-known Chinese herbs and food ingredients, and is commonly found for sale in supermarkets all over the country, it is likely that a large part of the Chinese adult population consumes some wolfberry fruit. Furthermore, wolfberry is very popular among Chinese living outside China. A study among Chinese in the Chinatowns of Oakland and San Francisco in the United States revealed that wolfberry fruit was used by 11% of respondents. It was the third most widely consumed Chinese herb among the 378 persons interviewed in this study, behind only ginseng and a popular cooling herb decoction called *qing bu liang* (a mixture of herbs cooked in soup that consists generally of seven standard herbs: dioscorea, lily bulb, dried polygonatum, fox nut, pearl barley, dried lotus seed, and dried longan; Cheng et al. 2004).

14.2.5 Evidence for Safety and Health Concerns from Diverse Human Experience

In the very long history of the traditional use of wolfberry, there are practically no reports of adverse effects. Published human safety data are rare. Wolfberry contains betaine, which is a known liver protectant, but can also be used to induce menstruation and abortion, so its use should be avoided by pregnant women. Chinese herbalists suggest abstaining from using wolfberry during times of cold and flu. Wolfberry has an estrogen-mimicking effect (Zhao 1998), so it should not be used by people who are pregnant or have diseases that are sensitive to estrogen.

14.3 IDENTIFICATION OF BIOACTIVES

The scientific literature of the last 20 years has documented that wolfberry fruit contains several potentially bioactive components (Gross, Zhang, and Zhang 2006; Potterat and Hamburger 2008; Sze et al. 2008). In Sections 14.3.1 through 14.3.3, we focus on three of these: arabinogalactan-proteins (AGPs), the carotenoid zeaxanthin, and the vitamin C precursor 2-*O*-(β-D-glucopyranosyl) ascorbic acid, and discuss their potential functionality and nutritional relevance.

14.3.1 Arabinogalactan-Proteins: Bioactive Glycoconjugates in *Lycium barbarum* L.

The literature documents that wolfberry fruit contains soluble macromolecules that exhibit a range of bioactivities. These properties are attributed to soluble glycoconjugates that were identified as "arabinogalactan-proteins" (AGPs), but are mostly referred in the Chinese literature as "*L. barbarum* polysaccharide" (LBP). Different molecular forms of wolfberry glycoconjugates (LbGp) have been partially characterized and their structural features and immunomodulating properties described.

14.3.1.1 Isolation and Structural Features of Glycoconjugates

Isolation of glycoconjugates is normally done from air-dried wolfberry fruit. There are no reports of their isolation from freshly harvested fruit. This may impact the reported structural features, as the postharvest history of fruit is known to be a factor in the metabolic status of endogenous enzymes. These can modify the structure of the polysaccharides during extraction unless precautions are taken to inactivate them early in the extraction process. In several publications, LbGps are reported to be extracted by a procedure developed by Huang et al. (1998). Dried and powdered wolfberries were stirred overnight in water and the supernatant concentrated and treated with four volumes of absolute alcohol to precipitate the polysaccharides. Proteins were removed by several washes with chloroform:butanol (4:1 v/v). The crude polysaccharide fraction (LBP) was then subjected to anion exchange chromatography on diethylaminoethyl (DEAE)-cellulose and gel permeation chromatography on Sepharose 4B to yield three glycoconjugates: (1) LbGp3, (2) LbGp4, and (3) LbGp5 (Huang et al. 1998). Their molecular weights were 925, 215, and 24 kDa, respectively. The glycoconjugate

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LbGp3 was composed of arabinose and galactose in a ratio of 1:1. Carbohydrate accounted for 94% of the fraction. LbGp4 was composed of arabinose, galactose, rhamnose, and glucose in a molar ratio of 1.5:2.5:0.43:0.23. Carbohydrate accounted for 86% of this fraction. Finally, LbGp5 was composed of rhamnose, arabinose, xylose, galactose, mannose, and glucose in a molar ratio of 0.33:0.52:0.42:0.94:0.85:1.0. The carbohydrate content was 9% in this fraction. The elemental N content of LbGp3, LbGp4, and LbGp5 indicated protein levels of around 5%, 10%, and 60%, respectively. Subsequently, the authors investigated in more detail the structural features of each of the three glycoconjugates as well as reporting their bioactive properties. Peng, Huang et al. (2001) examined the structural features of LbGp4 by methylation analysis to identify linkage types and ^{1}H- and ^{13}C-NMR to establish the anomeric configuration of the sugars. The glycan moiety (LbGp4-OL) was released by β-elimination, and the monosaccharide composition showed arabinose, galactose, and rhamnose in the molar ratio of 1.33:1.0:0.05. A putative repeat unit was proposed (Figure 14.3).

A similar approach was adopted with LbGp3 (Huang, Tian, and Zheng 1999), but this time the glycan was released by a pronase treatment. The glycan contained arabinose and galactose in a molar ratio of 1:1. A structure was proposed for which, although the detailed structural features of the side chains differed, the essential features were the same as the LbGp4-OL, as shown in Figure 14.4. That is, a backbone of β-(1→4)galactosyl residues with mainly arabinosyl substituents attached at O-3. Peng, Qi et al. (2001) separated the glycoconjugate LbGp5 into three additional fractions: Lbp5A, Lbp5B, and Lbp5C. No structural features were reported by the authors, but LbGp5B, which contained rhamnose, arabinose, glucose, and galactose in the molar ratio 0.1:1.0:1.0:1.2:0.3, also contained 9% galacturonic acid. The amino acid composition of the protein moiety consisted of 17 amino acids, but the presence of hydroxyproline was not reported. However, a more recent paper did report the presence of hydroxyproline in the protein moiety of a glycoconjugate isolated from wolfberry (Zhang and Chen 2006). This finding is significant as hydroxyproline is a common amino acid component of the protein associated with AGPs.

The results suggest that the water-soluble polysaccharides in wolfberry are a complex mixture of several different types of polysaccharides. However, the chromatographic methods used to separate these different polymers are insufficient to completely purify the glycoconjugates. This is indicated by the presence of additional monosaccharides such as xylose, mannose, glucose, and galacturonic acid, which are not commonly found in AGPs, in some of the glycoconjugates. The cell walls of most dicotyledonous plants are rich in pectic polysaccharides and it would be expected that wolfberry also contains moderate amounts of soluble pectic polymers. A recent publication by Zhang and Zhang (2007) reported the presence of 56% galacturonic acid in LBP, as well as six different monosaccharides. It is likely that pectic polysaccharides are present in LBP and other glycoconjugates that have been prepared in the past. A matter of perhaps greater significance is that in this paper it is reported that the backbone of the polymer is composed of (1→3) glycosidic bonds (Zhang and Zhang 2007). The study of the structural features of LbGp4 conducted by Peng, Huang et al. (2001) reported that that "glycans of the arabinogalactan-protein under investigation is based on highly branched 3,4-linked galactans, a finding in contrast to most of the known AGPs which carry 3,6-galactans. It therefore represents a novel structure …" In light of the report by Zhang and Zhang

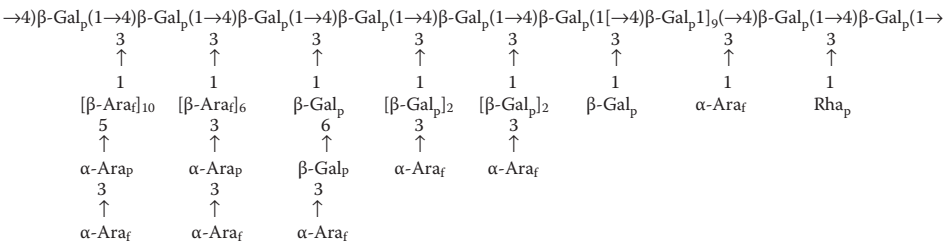


FIGURE 14.3 A reported repeat unit of the wolfberry glycan chain LbGp4-OL. (From Peng, X. M., L. J. Huang, C. H. Qi, Y. K. Zhang, and G. Y. Tian. 2001. *Chinese J Chem* 19:1190–7.)

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(2007), unpublished data from our laboratory, and current knowledge on the structure of AGPs, the stated novelty of *L. barbarum* AGPs in possessing a (1→4)-linked galactosyl backbone must be doubted.

The Yariv reagent (Jermyn and Yeow 1975) is a compound capable of specifically interacting with AGPs (Van Holst and Clarke 1985). Experiments in our laboratory used the reagent to precipitate the AGP fraction from a water-soluble extract of wolfberry. The recovered polysaccharide contained arabinose, galactose, rhamnose, and uronic acid in the molar proportions of 1.0:1.0:0.075:0.2, respectively. Most of the uronic acid was identified as glucuronic acid, which is known to occur as terminal residues on the side chains of some AGPs. Linkage analysis indicated a backbone of (1→3)-linked galactosyl residues, some of which carried substituents at the O-6 position. This structure is in line with the established structural features of many of the type II Arabinogalactans (AG) s characterized in AGPs to date. The non-Yariv precipitable polysaccharide fraction contained 40% galacturonic acid, indicating the predominant presence of pectic polysaccharides. This fraction also contained the xylose, mannose, and glucose contents of the original water-soluble fraction, evidence that they were not structural features of wolfberry AGPs.

14.3.1.2 Optimizing *Lycium barbarum* Polysaccharide Recovery from Wolfberry

Experiments conducted in our laboratory showed that cold water extraction can solubilize 2–3% of dried wolfberries as high-molecular-weight polysaccharides. Of this amount, around 0.4% is present as a Yariv-precipitatable fraction and, therefore, represents the glycoconjugates identified as AGPs. Pectic polysaccharides accounted for most of the remaining soluble polysaccharide. The cold water–insoluble polysaccharide fraction accounted for approximately 20% of the wolfberries and consisted of a mixture of cellulose and pectic and hemicellulosic polysaccharides, commonly occurring in the cell walls of dicotyledonous fruit. Increased yields of LBP have been reported by prolonged high-temperature extraction (Yin and Dang 2008) or enzymatic treatment (Yan et al. 2007). Since the AGPs are readily dissolved in cold water, it is unlikely that significantly greater amounts of the glycoconjugates will be solubilized by a more severe extraction procedure. Most of the increased levels of polysaccharides recovered by these approaches are likely to be pectic and hemicellulosic polymers derived from the breakdown and solubilization of the insoluble cell walls of the wolfberry fruit.

14.3.1.3 Bioactive Properties

The bioactive properties of arabinogalactans and AGPs are, in general, well documented (Redgwell and Fischer 2005). Larch arabinogalactan (not an AGP) is marketed as ImmunEnhancer AG (Larex, Inc., Minnesota). Apart from its role in digestive health promotion (prebiotic properties resulting from it being fermented to short-chain fatty acids, butyrate, and propionate), ImmunEnhancer can enhance immune system performance (D'Adamo 1996). It functions in this capacity by preventing bacteria and viruses from attaching to cell membranes on the liver and other organs, thereby stopping infections from becoming established. Tumor metastasis to the liver has been reported to be impaired by Larch AG (Beuth et al. 1987; Hagman et al. 1991).

The benefits of AG and AGPs do not appear to be related to a source but seem to be a consequence of the structural features they have in common. Therefore, it is logical that wolfberry AGPs demonstrate the same bioactive properties as those documented for AGs and AGPs from other plant sources. Since the mid-1980s, more than 200 papers have been published on the aspects of chemistry and health benefits of the fruit of *L. barbarum*. More than 50 of these refer to functionalities specifically related to the polysaccharide content. However, many of these studies have used a "soluble polysaccharide" extract and not a purified glycoconjugate. Therefore, not all reported bioactivities can necessarily be attributed solely to the AGP content of the fruit.

The research focus has been on mainly three interrelated areas: (1) modulation of the immune system, (2) antitumor activity, and (3) antioxidant activity. An early study (Geng et al. 1989) reported that LBPs stimulated T lymphocytes to produce interleukin (IL)-2 in the elderly. Interleukin-2 was one of the first soluble "hormonelike" mediators of the immune system discovered, and it fueled interest in the

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field of immunology, as the vital role of cytokines had not been realized prior to its discovery. Using a partially purified glycoconjugate (LBP_{3p}), Gan et al. (2003, 2004) confirmed the earlier study that showed the glycoconjugate's effectiveness in increasing, in a dose-dependent manner, the expression of the cytokines IL-2 and tumor necrosis factor α (TNF-α) at both messenger ribonucleic acid (mRNA) and protein levels. In a clinical trial conducted on 75 cancer patients, LBP was orally administered in combination with lymphokine-activated killer cells and IL-2. This treatment led to a significant regression of cancer compared to treatment in the absence of the lycium polysaccharides (Gau, Yang and Du 1994). Peng, Huang et al. (2001) demonstrated the high immunoactivity of the purified glycoconjugate LbGp4. The mechanism was investigated using a tritium thymidine incorporation assay, flow cytometry, and electrophoretical mobility assays. The glycoconjugate LbGp4 and its glycan promoted splenocyte proliferation in mice, and the effects of the glycan chain were stronger than those of the glycoconjugate. Zhang et al. (2005) showed a positive effect of an ill-defined "polysaccharide–protein complex" by lessening proliferation and increasing apoptosis of the human hepatoma cell line QGY7703 associated with liver cancer. More recently, the effect of an LBP on the growth of human prostate carcinoma PC-3 cells was studied (Luo et al. 2008). Results indicated that LBP can inhibit the growth of PC-3 cells and induce apoptosis. LBP-induced breakage of DNA strands in PC-3 cells and the ratio of Bcl-2/Bax protein decreased. The expression of Bax is upregulated by the tumor suppressor protein p53, and Bax has been reported to be involved in p53-mediated apoptosis.

Evidence for the antioxidative ability of lycium polysaccharides is documented in several publications based on in vitro and several in vivo studies, although few of them have been performed with a purified glycoconjugate fraction. Li, Peng, and Wang (2007) evaluated the antioxidative capacity of LBP by in vitro methods including superoxide radical–scavenging activity, reducing power, β-carotene linoleate model, and inhibition of mice erythrocyte hemolysis mediated by peroxyl radicals. They concluded that the data clearly established the antioxidative potency of LBPs. Studies with rats and mice have demonstrated that LBP can lessen oxidative stress and DNA damage (Li 2007; Li and Zhou 2007; Wu et al. 2006), inhibit lipid oxidation induced by free radicals in a high-fat diet (Ma et al. 2009), and protect against oxidative damage in skeletal muscle caused by exhaustive exercise (Niu et al. 2008). Zhao et al. (2005) reported that LbGp5 promotes the survival of human fibroblasts cultured in suboptimal conditions and, therefore, may have skin-protective properties. Additional claims on the benefits of LBP include antifatigue (Luo, Yan, and Zhang 1999) and cholesterol-lowering activities (Luo, Li, and Zhang 1997; Yu, Wu, and Niu 2009).

It must be kept in mind that wolfberry is rich in a number of potent antioxidants that are not polysaccharides, such as zeaxanthin and polyphenols. The phenolic derivatives in particular are likely to interact with and bind to the polysaccharides and proteins during the extraction of LBP, and they could subsequently copurify with the LBPs during alcohol precipitation, as for tea polysaccharides of an AGP nature (Zhou et al. 2009). It is a definite possibility that some of the reported antioxidant effects of wolfberry polysaccharides are in part the result of contaminating low-molecular phenolic compounds.

In summary, polysaccharide extracts from the fruit of *L. barbarum* L. demonstrate a broad spectrum of bioactive properties, many of which are attributed to the presence of AGPs or glycoconjugates, although it is noted that antioxidant activity may be due to the contamination of polysaccharides fractions with phenolic compounds. Polysaccharides from wolfberry appear to possess several of the properties already claimed for AGPs and AGs from other plant sources. Whether LBPs possess additional benefits or possess more potent bioactivity than AGPs from other plants remains to be proved. Ongoing research to refine the isolation and purification of the several polysaccharide types of *L. barbarum* will clarify the specific role played by glycoconjugates and the other polysaccharides in these benefits.

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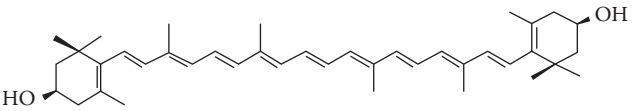


FIGURE 14.4 Structure of zeaxanthin.

TABLE 14.2
Referenced Data on Estimated Daily Intake of Zeaxanthin

Zeaxanthin (mg/day)	Zeaxanthin + Lutein (mg/day)	Country	Reference
0.3		United States	Yeum et al. 1996
	2.5	Holland	Goldbohm et al. 1998
	2.7–3.1	United States	Tucker et al. 1999
	2.4–2.9	Costa Rica	El-Sohemy et al. 2002
0.4–5.2		Global	Hartmann et al. 2004

14.3.2 Zeaxanthin

Zeaxanthin is a xanthophyll, an oxygenated carotenoid (Molecular weight (MW) 568.85 $gmol^{-1}$) with antioxidant and blue light–absorbing properties (Figure 14.4). Zeaxanthin is found in many vegetables and fruits at low levels, with wolfberry being its best-known natural source (Weller and Breithaupt 2003). Earlier published works referred to β-carotene as the main carotenoid of wolfberry fruit; however, in 1999, two groups (Lam and But 1999; Zhou et al. 1999; Li et al. 1999) independently reported that the main carotenoid of wolfberry is zeaxanthin dipalmitate. The finding of high zeaxanthin content in wolfberry was substantiated by further studies, and Weller and Breithaupt (2003) confirmed that 80% of the total carotenoids in wolfberry were in the form of zeaxanthin dipalmitate. More detailed analysis revealed that zeaxanthin dipalmitate (1.14 mg/g dry weight) was present in the largest amount (88% of total carotenoids), followed by β-cryptoxanthin monopalmitate and its two isomers (2–4%), zeaxanthin monopalmitate and its two isomers (1–5%), all-trans-β-carotene (2%), and all-trans-zeaxanthin (0.1%) (Inbaraj et al. 2008).

14.3.2.1 Estimated Zeaxanthin Intake: Potential Contribution from Wolfberry

Sommerburg et al. (1998) investigated the presence of zeaxanthin in 33 vegetables and fruits. Orange pepper (37% of total carotenoids) was found to be the vegetable with the highest amount of zeaxanthin. They found that dark-green leafy vegetables, previously recommended for a higher intake of lutein and zeaxanthin, had very low amounts of zeaxanthin (0–3% of total carotenoids). By far, the richest source known for zeaxanthin is wolfberry fruit, a quasi "natural pill," enriched in a component that appears essential for eyesight (Landrum and Bone 2001; Cheng et al. 2005). The zeaxanthin content of wolfberry depends on the stage of maturity, season, and the drying process (Ma et al. 2008), and in our studies we found that it varies from 1.18–2.41 mg/g dried fruit.

Data on estimated zeaxanthin intake and bioavailability are scarce, because before the year 2000 the two carotenoids lutein and zeaxanthin were not measured separately by high-performance liquid chromatography (HPLC), and much of the data available are expressed as combined intake of both lutein and zeaxanthin (Table 14.2). It appears that estimated zeaxanthin intake is generally <1 mg/day. From the fact that wolfberry zeaxanthin content is approximately 0.8 mg/g, the richness of wolfberry as a source of zeaxanthin is clear.

Eating a few grams of wolfberry each day would certainly boost plasma levels of zeaxanthin, as was reported for synthetic zeaxanthin in a human supplementation study with 1 or 10 mg/day for 42 days (Hartmann et al. 2004).

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14.3.2.2 Mechanism of Intestinal Zeaxanthin Absorption

In the stomach, carotenoids and lipid molecules separate from the rest of the chyme and form oil droplets, which enter the small intestine and are mixed with pancreatic juice and bile salts (Tyssandier et al. 2001). The oil droplets get covered by bile salts, which creates a negative surface charge that permits the binding of colipase to the lipid/aqueous interphase. The colipase then binds to the pancreatic lipases, which hydrolyzes the bond between zeaxanthin and fatty acids, for instance, palmitic acid for wolfberry (Borel et al. 1996; Chitchumroonchokchai and Failla 2006). The liberated zeaxanthin is absorbed by the intestine (Perez-Galvez et al. 2004). It is unknown whether a part of zeaxanthin is re-esterified during the absorption process, but some esterified zeaxanthin has been detected in plasma and tissues (Granado et al. 1998). The micellar solubilization of zeaxanthin into lipids is mandatory for their absorption. There is increasing evidence of the existence of a facilitated, protein-carrier-mediated transport of carotenoids (Reboul et al. 2005; During, Dawson, and Harrison 2005). Once absorbed into the enterocyte, zeaxanthin is packed into new oil-droplet structures called prechylomicrons, which are transported into the extracellular space by exocytosis before entering the lymphatic system and general circulation.

14.3.3 The Vitamin C Precursor 2-*O*-(β-D-Glucopyranosyl) Ascorbic Acid

Plants and, in particular, their fruits are generally a good source of ascorbic acid (vitamin C). Earlier compositional data about wolfberry lacked mention of any significant amounts of ascorbic acid. The mystery was lifted only when researchers from Suntory, Ltd., Japan, isolated, identified, and synthesized an ascorbic analog as 2-*O*-(β-D-glucopyranosyl) ascorbic acid (Maeda, Nakao, and Fukami 2003; Toyoda-Ono et al. 2004). The levels of acid found in wolfberry (0.5%) were similar to those known for lemons, making wolfberry one of the richest sources of ascorbic acid. Glucoside was found in animal experiments to be hydrolyzed into ascorbic acid and glucose, which makes it a promising source of natural vitamin C for food and beverage applications where thermal and acid treatments are needed. Some of the physical features of this molecule can be extrapolated from a synthetic and commercially available isomer named 2-*O*-(α-D-glucopyranosyl) ascorbic acid (Hayashibara, Co., Okayama, Japan). Our tests with an acidified sugar-containing beverage at a pH of 3.5 revealed remarkable stability of the vitamin C precursor when compared with a similar beverage made with ascorbic acid (Figure 14.5). Although the fate and potential physiological benefits of this vitamin C precursor have not yet been reported, it cannot be denied that the presence of this precursor in TCM preparations will have a significant impact on the health status of patients or weak persons. Regularly taken, such as prescribed in TCM formulas (15–30 g/day), the precursor

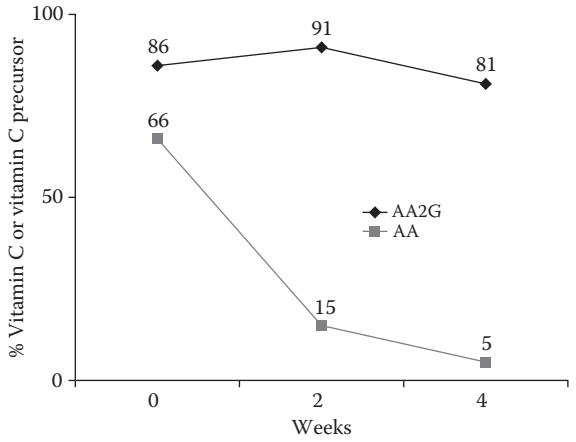


FIGURE 14.5 Stability of vitamin C precursor (AA2G) versus ascorbic acid (AA) in a beverage at 30°C.

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would add 45–90 mg of ascorbic acid equivalents to the daily intake, which is within the current recommended daily intake limit.

14.4 BIOAVAILABILITY OF WOLFBERRY BIOACTIVES AND EFFECTS OF PROCESSING

Wolfberry is a rich source of bioactives that may act synergistically. As the fruit contains both water-soluble (e.g., LBP, vitamin C precursor) and fat-soluble (e.g., zeaxanthin) bioactives, the bioavailability and nutritional properties of wolfberry and its products are likely to be affected by the method of extraction and incorporation into a product. Conventional extraction techniques extract only water-soluble compounds of wolfberry, as described, for instance, for commercial LBP extraction (Yin and Dang 2008). Hot water extraction is a standard practice of TCM. For wolfberry, the use of hot water allows the extraction of hydrophilic compounds, but most of the lipophilic components such as zeaxanthin dipalmitates, lipophilic vitamins, and other lipids are lost. Food is an integral delivery system. Eating diets rich in fruits and vegetables is known to be beneficial to health. But the benefits of eating fruits and vegetables may not be reproduced by consuming purified extracts of phytonutrients or supplements with vitamins (Sesso et al. 2008). Evidence suggests that it is the complementary and/or synergistic effects of several of these phytonutrients that promotes health or produces protective effects against disease, and that isolating one or another of them is less beneficial for human nutrition than consuming the whole food in which a phytonutrient is found. Today, the consumer expects to get the benefits of these phytonutrients in efficient, convenient, and natural formulations. Therefore, from natural bioactives to tasty foods, a new approach must preserve the integrity of the bioactive raw materials. That is, it must be possible to deliver all intrinsic benefits of a fruit, vegetable, or other plant-based materials, including TCM herbs, in their natural, stable, and bioavailable form. To meet such an expectation, innovative ways of delivering the healthy properties of plant extracts are needed, in particular of identifying the most suitable extraction process, analyzing the chemical stability and bioavailability of the extracts, and verifying eventual interactions to the specific food matrix.

14.4.1 Lacto-Wolfberry: The Generation of a Natural, Stable, and Bioavailable Formulation

Although wolfberry is commonly used in TCM, it is challenging to include this fruit in commercial food and beverage products. The Nestlé Research Center (Lausanne, Switzerland) has developed a process that makes it possible to deliver the full benefits of the whole fruit in a simple, natural, and tasty formulation called Lacto-Wolfberry. In this process, milk is used as both an extracting agent and a carrier for the retention of water- and oil-soluble bioactives of the whole fruit (Wang et al. 2005). The process consists of the following three key steps: (1) milling the fruit in milk or in milk protein–containing solution, (2) separating insoluble fibers to obtain an aqueous suspension, and (3) optionally drying the suspension to obtain a powder (Figures 14.6 and 14.7). Such a process is particularly suitable for converting lipophilic bioactives of raw materials into water-soluble formulations. The Lacto-Wolfberry formulation has a profile advantageously close to that of the essential active components of the wolfberry fruit, and has good stability, miscibility, and dispersibility in aqueous systems. In addition, Lacto-Wolfberry was found to have enhanced nutritional value, in the form of significantly better zeaxanthin bioavailability and stability. It was demonstrated in a clinical study (Benzie et al. 2006) that the bioavailability of zeaxanthin from Lacto-Wolfberry is significantly (three times) higher than that from wolfberry powder (Figure 14.8).

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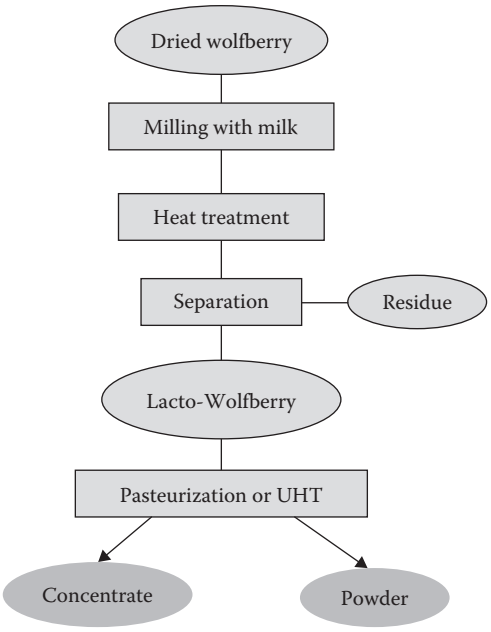


FIGURE 14.6 Flow diagram of the Nestlé Lacto-Wolfberry process.

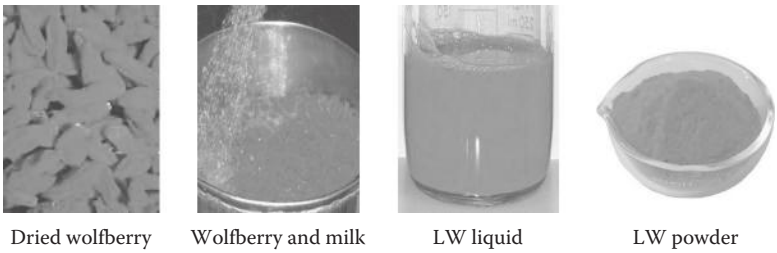


FIGURE 14.7 Different stages of the Nestlé Lacto-Wolfberry process.

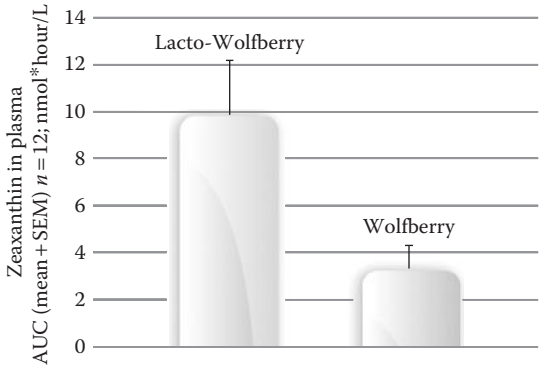


FIGURE 14.8 Mean zeaxanthin uptake in terms of area under the curve (AUC) 0–10 hrs (area under the curve in 12 healthy adults) from Lacto-Wolfberry versus a powdered wolfberry preparation, each containing 15 mg of zeaxanthin. (Adapted from Benzie, I. F. F., W. Y. Chung, J. Wang, M. Richelle, and P. Bucheli. 2006. *Brit J Nut* 96:154–60.)

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14.4.2 Bioavailability of Zeaxanthin from Wolfberry

Zeaxanthin is concentrated within the central macula of the human retina (Landrum and Bone 2001). Because of the richness of wolfberry in zeaxanthin and its perceived benefits for eyesight, it was speculated that the consumption of wolfberry will affect health positively. One of the first studies that tested this hypothesis was done with rhesus monkeys (Leung et al. 2001) that were fed for 6 weeks a diet containing a carotenoid fraction extracted from wolfberry fruit (2.2 mg zeaxanthin/day). The authors of this study observed that the serum levels and macular density of zeaxanthin were raised in these monkeys when compared with monkeys fed a control diet without carotenoids. The serum and tissue levels of zeaxanthin and lutein were studied. After feeding wolfberry extracts, serum levels and macular density of zeaxanthin were found to be increased. This observation was followed by several human intervention studies that revealed absorption of zeaxanthin. Breithaupt et al. (2004) reported the first human intervention study that used a fat-soluble fraction of wolfberry and compared it to the absorption of synthetic zeaxanthin (DSM Nutritional Products, Kaiseraugst, Switzerland). The blood plasma levels of zeaxanthin increased for both zeaxanthin supplements, showing a maximum between 9 and 24 hours after ingestion. Although the results were not statistically significant, with a high variability in the uptake of the wolfberry sample, they indicated that zeaxanthin from wolfberry was better absorbed than that from synthetic nonesterified zeaxanthin. Cheng et al. (2005) found in a 28-day supplementation trial that ingestion of 15 g of wolfberry (whole berries) per day markedly increased plasma levels of zeaxanthin (2.5-fold increase). A study by Hartmann et al. (2004) indicated that supplementation with 10 mg of synthetic zeaxanthin increased the plasma concentrations of zeaxanthin by a factor of 20.

14.5 IMMUNOMODULATING ACTIVITY

Wolfberry has been prized in China for many years for its immune system-boosting property; however, clinical data demonstrating the effects of dietary intake of wolfberry on immune responses are lacking, and there is little international scientific literature on its effects and the mechanisms by which it exerts its reputed immunological benefit. Most of the reported data about the immunomodulation effects of wolfberry come from animal, ex vivo, and in vitro studies, and are mostly attributed to its polysaccharide fraction (LBP). Nevertheless, one cannot exclude the possibility that compounds such as antioxidants and vitamins present in wolfberry could also be involved in immunomodulation. Immune cells are known to be particularly sensitive to oxidative stress because their plasma membranes contain a high percentage of polyunsaturated fatty acids. Therefore, wolfberry could exert an immune-enhancing effect through, for example, the presence of zeaxanthin, a potent lipophilic antioxidant.

Human studies of immunomodulation by wolfberry are rare. In a rodent model, subcutaneous injection of wolfberry juice resulted in an increase in the size of the thymus and the spleen, as well as an increase in T lymphocytes number and rate of T cell maturation (Wang, Xing, and Zhou 1990). Healthy mice receiving an intraperitoneal injection of wolfberry juice combined with blueberry and raspberry juices showed an increase in spleen size and in the number of splenic macrophages compared to mice treated with a saline control (Chao et al. 2004). An increase in the number of splenic macrophages in a healthy animal would indicate an increased ability to fight disease since macrophages are the main phagocytic cells in the spleen, which provide an essential defense by filtering infectious agents from the blood. But, notably, no effect was observed with injection of pure wolfberry juice.

14.5.1 Role of *Lycium barbarum* Polysaccharides

The immunomodulation effect of wolfberry has been mainly attributed to the presence of LBPs in the fruit. In vitro experiments show that LBPs exhibit immune-enhancing properties by increasing the proliferation of mouse spleen cells, induced or not by a T cell mitogen (i.e., ConA or PHA;

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Geng et al. 1989; Duan et al. 2001; Huang et al. 2001; Peng, Huang et al. 2001; Peng, Qi et al. 2001; Peng, Wang, and Tian 2001; Qi, Zhang et al. 2001; Du, Liu, and Fang 2004; Chen, Kwong Huat Tan, and Chan 2008) or a B cell mitogen (i.e., lipopolysaccharide [LPS]; Peng, Huang et al. 2001). Consistent with the in vitro data, it has been reported that LBP given either intraperitoneally or orally to mice resulted in a stronger proliferation of spleen cells after in vitro culture in the presence or absence of mitogen (Peng, Wang, and Tian 2001; Chen, Kwong Huat Tan, and Chan 2008). In addition, mice receiving intragastric administration of LBP (LBP-X at 5–20 mg/kg/day for 6 days) showed increased SRBC-specific humoral response (SRBC is a T cell–dependent antigen) and delayed-type hypersensitivity (DTH) response (Luo, Yan, and Zhang 1999), indicating that LBPs selectively strengthen T cell–mediated immune responses.

Mitogen-induced lymphocyte proliferation and IL-2 production significantly decrease with aging and are surrogate biomarkers of aging. IL-2 acts mainly on mitogen- or antigen-activated T cells to promote their survival and proliferation in vitro and to potentiate the function of cytotoxic T cells. It has been demonstrated that LBP enhances in vitro proliferation of lymphocytes from normal adult mice, aged mice (Geng et al. 1989), and senescence-accelerated mice (Huang et al. 2001). Interestingly, LBP was shown to raise the level of IL-2 produced by cells from aged mice to the same level as that produced by cells from adult mice (Geng et al. 1989). Furthermore, intragastric administration of crude LBP (100 mg/kg daily for 8 weeks) in a D-galactose-induced mouse model of aging resulted in increased proliferation of spleen cells and expression of IL-2 (Deng et al. 2003). An increased superoxide dismutase (SOD) activity of erythrocytes and a decreased level of serum advanced glycation end products (AGE) were also observed, indicating that LBP can prevent the decline of immune function observed with aging by inhibiting the production of AGE (Deng et al. 2003). Taken together, these studies support the antiaging effect of wolfberry by strengthening T cell–mediated immune responses.

The immunomodulatory effects of LBP on dendritic cells (DC), one of the most potent antigen-presenting cells that plays a pivotal role in the initiation of immune response (Banchereau and Steinman 1998; Lanzavecchia and Sallusto 2001), has been evaluated (Zhu, Zhao, and Chen 2006; Zhu et al. 2007). It was found that LBP stimulated the cell surface coexpression of MHC class II molecules and CD11c and the secretion of IL-12 p40 by bone marrow–derived DCs (BMDCs) in vitro. In addition, LBP inhibited mannose-receptor-mediated endocytosis by DCs and augmented their capacity to promote the proliferation of naïve allogenic T cells (Zhu, Zhao, and Chen 2006; Zhu et al. 2007). Together, these results suggest that LBPs are capable of promoting the phenotypic and functional maturation of DCs, making them ready for T cell–mediated immune responses. Further, it was demonstrated that LBPs have immunomodulatory effects on macrophages (Peng, Wang, and Tian 2001; Li et al. 2005; Li, Ma, and Liu 2007). It was found that LBPs increased the phagocytic activity of macrophages and nitric oxide (NO), and IL-1β and TNF-α production in vitro (Li et al. 2005). Gastric administration of LBP (200–500 mg/kg for 30 days) enhanced phagocytic index and activity in a senescence-accelerated mouse model (Li, Ma, and Liu 2007). In this context, it is interesting to note that LBPs were able to activate microglial cells, the brain macrophages, as reported by Chang and So (2008). A few studies demonstrate that LBP can upregulate humoral immune responses. It has been found that LBPs enhance in vitro antibody production by splenocytes from normal mice and senescence-accelerated mice (Qi, Zhang et al. 2001). In addition, LBP given orally to mice resulted in an increase in antigen (SRBC)-specific humoral response (Luo, Yan, and Zhang 1999).

14.5.2 Influence of Glycan Composition and Protein Content of *Lycium barbarum* Polysaccharide

Preparations and fractions of LBP isolated from *L. barbarum* were different for different groups, and this might explain the differences in the immunostimulatory effects observed. The format of linkage between different carbohydrates or among carbohydrates and proteins, and the protein content of LBP seem to determine the biological activity of LBPs. Four homogeneous preparations of LBP—LBP 1a-1, LBP 1a-2, LBP 3a-1, and LBP 3a-2—enhanced the proliferation of mouse

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spleen cells induced by ConA (Duan et al. 2001). The LBPs with a main chain of α-(1→4)-D-polygalacturonans (i.e., LBP 3a-1 and LBP 3a-2) demonstrated a stronger immunomodulation activity than the LBPs with α-(1→6)-D-glycans (i.e., LBP 1a-1 and LBP 1a-2).

The role of the carbohydrate was further investigated by Peng, Huang et al. (2001). Five polysaccharide components (LBP1–LBP5) were purified from the crude LBP and from some of them (i.e., LBP1, LBP3, LBP4, and LBP5) the glycoconjugates (i.e., LbGp1, LbGp3, LbGp4, and LbGp5B) and their glycan chains (i.e., LbGp1-OL, LbGp3-OL, and LbGp4-OL) were obtained. The glycoconjugate LbGp5B was found to enhance mouse spleen cell proliferation whether ConA or LPS was used or not (Peng, Qi et al. 2001). The glycoconjugates LbGp1, LbGp3, and LbGp4 and their glycan chains LbGp1-OL, LbGp3-OL, and LbGp4-OL were found to enhance mouse spleen cell proliferation (Qi, Huang et al. 2001). The effects of glycan chains were stronger than those of respective conjugates, suggesting that the glycan chains may be the important active structure of carbohydrate conjugates. The homogeneous glycoconjugate LbGp4 and its purified glycan LbGp4-OL were both found to enhance the proliferation of mouse spleen cells in the absence of mitogen (Huang et al. 2001; Peng, Huang et al. 2001). Interestingly, it has been demonstrated that although they induced in vitro the proliferation of B lymphocytes whether LPS was used or not, they had no effect on the proliferation of T lymphocytes in the presence or absence of ConA. From binding assays, it was suggested that a receptor-binding site exists on B cells for those arabinogalactans. In addition, it was demonstrated that the immunostimulatory effect of LbGp4 was associated with activation of the expression of the nuclear factor κB (NF-κB) and the activator protein-I (AP-I; Peng, Huang et al. 2001).

Among other preparations of homogeneous fractions of LBP, fractions LBPF4 and LBPF5 were reported to increase the proliferation of mouse spleen T cells, but not B cells (Chen, Kwong Huat Tan, and Chan 2008). The other three fractions, LBPF1, LBPF2, and LBPF3, were unable to activate the T cells. It was suggested that this could be due to the higher protein content of LBPF4 and LBPF5 compared to LBPF1, LBPF2, and LBPF3. This was supported by the finding that the T cell stimulatory effect of LBPF4 and LBPF5 was significantly compromised when the proteins were digested (Chen, Kwong Huat Tan, and Chan 2008). T cell activation induced by LBP was further investigated, and LBPF4 and LBPF5, as well as crude LBP, were shown to (1) activate two transcription factors NFAT and AP-1, which play important roles in T cell activation; (2) enhance the expression of the T cell activation marker CD25; and (3) induce cytokine gene transcription and protein secretion of IL-2 (an essential cytokine for T cell growth) and interferon-γ (IFN-γ, a cytokine promoting the differentiation of Th-1 cells and, therefore, a predominantly cell-based immune response; Chen, Kwong Huat Tan, and Chan 2008). Consistent with this last observation, exposure of aged T cells to the glycopeptide LbGp3 resulted in increased IFN-γ expression and decreased IL-10 expression (Yuan et al. 2008). It has been reported that LBP_{3p} dose-dependently increased the expression of IL-2 and TNF-α at the levels of mRNA and protein in cultured human peripheral blood mononuclear cells (PBMCs; Gan et al. 2003). These LBP-induced changes in IL-2, IFN-γ, TNF-α, and IL-10 cytokine expression suggest a transition from Th-2 cells (producing IL-4, IL-5, and IL-10) to Th-1 cells (producing IL-2, IFN-γ, and TNF-α). Since during aging there is a shift toward a more pronounced Th-2-type immune response (Rink, Cakman, and Kirchner 1998), these effects of LBP on cytokine production (Gan et al. 2003; Chen, Kwong Huat Tan, and Chan 2008; Yuan et al. 2008) support an antiaging property of wolfberry.

14.5.3 Indirect Immune Benefits of *Lycium barbarum* Polysaccharide

The immunomodulatory effects of wolfberry can also be evaluated through its antitumor property. Indeed, the antitumor capacity of wolfberry can be attributed to the stimulatory effects of LBP on immune cells. In a clinical trial, patients suffering from advanced cancer treated with lymphocyte-activated killer (LAK) cells and IL-2 combined with oral administration of LBP (1.7 mg/kg/day) showed a significantly higher regression of cancer and a more marked increase in natural killer cell

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activity than patients treated with LAK/IL-2 alone (Cao, Yang, and Du 1994), suggesting that LBP may be useful as an adjuvant for the treatment of cancer.

The antitumor effect of LBP and the mechanisms involved have been further examined in different preclinical models. In a brain G422 tumor-bearing mouse model, it was reported that LBP combined with irradiation and 1,3 bis-(2-chloroethyl)-1-nitrosourea (BCNU) not only increased the life span of mice but also improved cellular immune function (Sun 1994). Oral administration of LBP (LBP_{3p} at 10 mg/kg for 10 days) in S180-bearing mice was found to significantly inhibit the growth of the transplantable sarcoma S180 and to improve macrophage phagocytosis, spleen cell proliferation, the activity of cytotoxic T lymphocytes, and the expression of IL-2 mRNA (Gan et al. 2004). In H22 hepatoma-bearing mice, it has been suggested that the antitumor effect of LBP given orally could be mediated by increased $CD4^+$ and $CD8^+$ T cell numbers in tumor-infiltrating lymphocytes (He et al. 2005). In rodent models of irradiation- and chemotherapy-induced myelosuppression, subcutaneous injection of LBP (50–200 mg/kg) promoted the recovery of PBMCs (Gong et al. 2005). Since it was reported that LBP stimulated the production of granulocyte-macrophage colony-stimulating factor (GM-CSF, a major factor that regulates hematopoiesis) by human PBMCs in vitro, it was suggested that the therapeutic effects of LBP in those models resulted from the stimulation of PBMCs to produce GM-CSF (Gong et al. 2005).

14.6 ANTIDIABETIC ACTIVITY OF WOLFBERRY

A review of the official list of TCM herbs used for treating diabetes in China (Jia, Gao, and Tang 2003) does not specially mention wolfberry; nevertheless, its use for preventing and alleviating diabetes can be traced back to the sixteenth century. In ancient times, TCM doctors called patients showing symptoms related today to diabetes *xiao ke* (消渴), which literally means "leanness and thirst." It is known today that many xiao ke patients have abnormal blood and urine sugar levels. In the TCM treatise *Compendium of Materia Medica* (本草纲目), wolfberry has been recorded for the treatment of xiao ke, indicating its potential application in treating diabetes. With this historical background, the traditional benefits of wolfberry alone or in combination with other herbs in relation to diabetes have been investigated in scientific and clinical studies in China (Bai 1998), and there are numerous reports of studies, largely using animal models, that have demonstrated positive effects of wolfberry in relation to diabetes. For example, therapeutic effects on diabetes and related complications, such as improved hemorrheology markers (e.g., plasma viscosity, hematocrit, and equation k value of erythrocyte sedimentation rate) of diabetes mellitus and diabetic nephropathy, were reported on patients (Li, Pen, and Zhang 1999; Liu et al. 1995; Li, Ma, and Liu 2007).

A water extract of wolfberry showed a significant hypoglycemic effect in adrenalin-induced and alloxan-induced diabetic mouse models and improved sugar tolerance (Tan 2008). In another study, such an extract increased blood insulin levels and helped to improve the function and recovery of impaired β-cells of pancreatic islets (Tian and Wang 2005). Wolfberry was also found to reduce serum total cholesterol (TC) and triglyceride (TG) concentrations and, at the same time, to markedly increase high-density lipoprotein cholesterol (HDL-C) levels in hyperlipidemic or diabetic rabbits (Luo et al. 2004). LBPs have been identified as one of the most active ingredients related to wolfberry's biological activities. For instance, LBPs remarkably lowered blood glucose in alloxan-induced diabetic rabbit and mouse models, apparently due to the cytoprotective effect of LBPs on β-cells of pancreatic islets (Luo et al. 1997; Wang et al. 1999). In the streptozotocin-induced diabetic rat model, LBPs were found to improve abnormal oxidative indices, protecting liver and kidney tissue (Li 2007). Treatment with LBP significantly lowered the levels of fasting blood glucose, NO, malondialdehyde (MDA), and NO synthase (NOS) activity in a streptozotocin-induced rat diabetes model group, whereas the level of fasting insulin, functional index of β cells, and SOD activity were significantly increased in the LBP group, suggesting that the effect of LBP on blood glucose and β cells might be related to an increase in pancreatic islet SOD activity and a decrease in NOS activity (Huang et al. 2006). LBP was also found to decrease cellular DNA damage in

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peripheral lymphocytes of non-insulin-dependent diabetes mellitus (NIDDM) rats, possibly via a decrease in oxidative stress levels (Wu, Guo, and Zhao 2006). LBP can alleviate insulin resistance, which is associated with increasing cell-surface level of glucose transporter 4 (GLUT 4) trafficking and intracellular insulin signaling in NIDDM rats (Zhao, Li, and Xiao 2005).

These findings indicate that wolfberry may have therapeutic effects for treating diabetes comparable to chemical drugs without causing significant side effects during short- and long-term treatment of diabetic complications. However, comparison data supporting this hypothesis have not been reported so far.

14.7 CARDIOVASCULAR BENEFITS OF WOLFBERRY

Over the last decade, some research investigations have demonstrated the cardiovascular protective potential of wolfberry or its extracts. Among other mechanisms, effects on oxidative stress may underlie the therapeutic promise for the cardiovascular benefit of wolfberry, as seen for in vitro and in vivo experimental settings. Such effects are likely related to the modulation of both neuronal- and endothelial-dependent NO pathways by wolfberry.

The NO pathway (particularly, the classical endothelial-dependent pathway) is one of the most important mechanisms for maintaining the functional and structural integrity of the vasculature and the heart (Figure 14.9). Inhibition of the pathway, especially by endogenous NOS inhibitors and reactive oxygen species (ROS; e.g., superoxide anions and hydrogen peroxide), has physiological and pathological consequences, including blood pressure elevation, thrombosis, and atherosclerosis. NO is constitutively produced by both neuronal and endothelial NOSs (i.e., nNOs and eNOs) during the conversion of the NOS amino acid substrate L-arginine to L-citrulline. Centrally, one putative notion

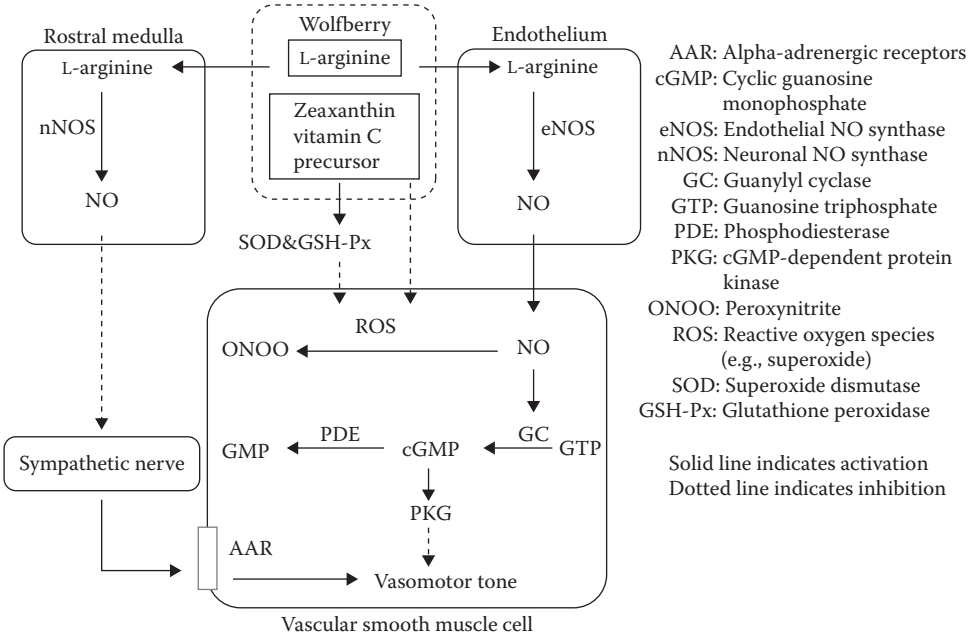


FIGURE 14.9 The NO pathway (particularly the classical endothelial-dependent pathway) plays a very important role in maintaining the functional and structural integrity of the vasculature and the heart. Wolfberry may promote cardiovascular health by inhibiting reactive oxygen species (i.e., ROS) and by providing substrate (i.e., L-arginine) for NO synthase, thereby enhancing both the synthesis and bioavailability of NO. (Modified from Augustyniak, R. A., G. D. Thomas, R. G. Victor, and W. Zhang. 2005. *Curr Pharm Des* 11:3307–15; Thomas, G. D., W. Zhang, and R. G. Victor. 2001. *JAMA* 285:2055–7.)

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is that neuronal NO produced in the cardiovascular control center of the autonomic nervous system is part of a signal transduction pathway that tonically restrains sympathetic outflow generated from the rostral brainstem, leading to decreased adrenergic tone in the arteries and the heart. Peripherally, endothelial-dependent NO production and its vascular dilatory action in the adjacent vascular smooth muscle cells have been established over the last two decades to be the predominant mechanisms in modulating vascular function (Thomas, Zhang, and Victor 2001; Augustyniak et al. 2005). It is reported that wolfberry contains L-arginine (Gross, Zhang, and Zhang 2006), which is a substrate for NOS (Figure 14.9). More importantly, and as noted in Section 14.3.3, wolfberry is also rich in a precursor of ascorbic acid, 2-*O*-(β-D-glucopyranosyl) ascorbic acid, which is very stable and detectable in the bloodstream of both artery and portal vein after oral administration (Toyoda-Ono et al. 2004) and in zeaxanthin (Breithaupt et al. 2004; Cheng et al. 2005; Benzie et al. 2006). Both the vitamin C precursor and zeaxanthin scavenge and inactivate ROS. Moreover, wolfberry is reported to increase serum levels of the antioxidant enzyme SOD and glutathione peroxidase (GPx; Amagase, Sun, and Borek 2009). Working together, these compounds can increase both the synthesis and the bioavailability of constitutive NO in the cardiovascular system directly and decrease sympathetic discharge to the peripheral organs. Because NO deficiency is involved in the pathogenesis of disorders such as hypertension and atherosclerosis, enhancing the activity of the NO pathway or increasing NO bioavailability constitutes a way of promoting cardiovascular health or lowering cardiovascular risk. In one of the rare human studies on wolfberry, 25 independently living subjects aged 64–80 years who took 50 g/day of wolfberry fruit for 10 days showed significantly increased blood SOD and hemoglobin (by 48% and 12%, respectively), and a 65% decrease in lipid peroxides (Li et al. 1991).

In rats that underwent two-kidney, one-clip surgery (a classical model of activated renin angiotensin system with ROS overexpression; Zhao et al. 2006), high blood pressure developed after 20–25 days. Treatment with 10% LBP (at a dose of 0.5 mL/100 g body weight) from wolfberry that was started two days postsurgery prevented the development of hypertension. Aortic rings from rats treated with LBP showed a significantly lower vasoconstrictive response in response to phenylephrine (PE) compared to aortic rings from control animals, whereas the vasodilatory response to acetylcholine (ACh), which represents the function of endothelium, was significantly increased in rats treated with LBP compared to control animals (Jia et al. 1998). In cultured cardiomyocytes, blocking L-type calcium channels glycopeptides from wolfberry lowered intracellular free calcium concentrations stimulated by hypoxia and KCl (Xu, Huang, and Tian 2005). However, in a randomized, double-blind, placebo-controlled clinical trial of 16 healthy subjects who took 120 mL of wolfberry juice for 14 days, no changes in cardiovascular parameters, that is, blood pressure and heart rate, were seen, although these subjects reported increased ratings for energy level, athletic performance, quality of sleep, ease of awakening, ability to focus on activities, mental acuity, calmness, feelings of health, and reduced fatigue and stress (Amagase and Nance 2008). Clinical investigations of wolfberry in patients with cardiovascular disorders are scant, and further study is warranted.

The protective effects of wolfberry have been seen also in other models of oxidative stress to the cardiovascular system. For example, during chemotherapy with the anticancer drug doxorubicin, ROS are generated by the drug and damage cardiomyocytes, causing myofibrillar loss and cytoplasmic vacuolization. This oxidative stress related to doxorubicin cardiotoxicity is accompanied by prolonged QT and ST intervals and an elevated ST segment, leading to arrhythmia and mortality. In a rodent study, treatment with wolfberry extract (at 25 mg/kg, per oral administration) effectively improved myocardial lesions and cardiac function and lowered mortality by 13% in doxorubicin-treated animals, without affecting the anticancer property of doxorubicin (Xin et al. 2007). In rabbits fed a high-fat diet for 8 weeks, which induces aortic atherosclerosis accompanied by increased plasma cholesterol and triglycerides, decreased HDL-C, and increased oxidative stress and levels of inflammatory cytokines, treatment with an oil extract of wolfberry seeds was found to upregulate SOD and GPx and downregulate NF-κB, and TNF-α levels. The potency of the antiatherosclerotic effect of this wolfberry extract was reported to be comparable to that of lovastatin (Jiang et al. 2007).

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14.8 IMPROVEMENT OF SEXUAL FUNCTION BY WOLFBERRY

Wolfberry has been used traditionally to enhance sexual function, and there is some scientific evidence for such a claim. In cultured seminiferous epithelium, ultraviolet light–induced lipid peroxidation was inhibited by LBP (Wang et al. 2002). This could indicate an antioxidative ability of LBP; however, ultraviolet exposure does not represent a direct causal factor for declined sex function or fertility. To this effect, hyperthermia is an important risk factor, as seminiferous epithelium is very sensitive to heat. A finding of interest is that hyperthermia-induced structural and functional damage was largely prevented by LBP (Wang et al. 2002; Luo et al. 2006) in vivo. Moreover, free radical–induced cytochrome *c* reduction and H_2O_2-induced DNA oxidative damage in seminiferous epithelial cells were also inhibited by polysaccharides derived from wolfberry (Wang et al. 2002; Luo et al. 2006). In vivo experiments repeated the effect of LBP on heat-induced functional and structural damage to the testis (Luo et al. 2006). Furthermore, in rats with unilateral castration, LBP improved copulatory performance and reproductive function, such as shortened penis erection latency and mount latency, increased sexual hormone levels, augmented accessory sexual organ weights, and improved sperm quantity and quality (Luo et al. 2006).

As far as the mechanism is concerned, improvement in sexual function by wolfberry is largely linked to its effect on the NO-cGMP axis by providing more substrate for NO synthesis and scavenging ROS (Figure 14.9). In a different experiment, an herbal formulation containing wolfberry seeds enhanced intracavernous pressure and NO-cGMP activity in the penile tissues of male rats (Sohn et al. 2008). Thus, the mechanism of sexual function improvement is unlike that of other erectile enhancement drugs such as sidlenafil, which are potent and selective inhibitors of cGMP-specific phosphodiesterase (PDE) type 5 (PDE5). Wolfberry is suggested to independently augment NO-cGMP bioavailability via PDE.

14.9 COGNITIVE BENEFIT OF WOLFBERRY

Traditionally, wolfberry was not particularly considered for preventing loss of cognitive function in an aged population; but this may be due to fact that our average life span did not exceed 50 years until the early twentieth century (Zhang and Zhang 2009). Amyloid-beta (Aβ or Abeta) is a peptide of 39–43 amino acids that, by increasing the activity of caspase-3 and lactate dehydrogenase (Anfuso, Lupo, and Alberghina 1999; Ho et al. 2007; Yu et al. 2007), causes neuronal apoptosis (neurotoxicity) and is suggested to be the main component of amyloid plaques in cerebral vasculature and in Alzheimer's disease associated with aging. Extract of wolfberry prevented double-stranded RNA-dependent protein kinase (PKR) from being phosphorylated by Abeta, thereby inhibiting caspase-3 and lactate dehydrogenase (Yu et al. 2005, 2007).

A recent investigation also demonstrated the beneficial effects of a milk-based wolfberry preparation (WP) on cognitive dysfunction in a rat model of prenatal stress (Feng et al. 2010). It was found that physical restraint induced mental stress in pregnant rats, which caused a significant decrease in cognitive function in the female offspring. When the pregnant mothers were pretreated with WP, the prenatal stress–induced cognitive dysfunction in their offspring was significantly decreased. Mechanistic experiments showed that WP scavenged hydroxyl and superoxide radicals (determined by an electron spin resonance spectrometric assay) in neuronal tissue. Furthermore, $FeCl_2$/ascorbic acid–induced dysfunction in brain mitochondria as characterized by increases in ROS and lipid peroxidation and decreases in the activities of complexes I and II, as well as decreases in glutamate cysteine ligase in vitro, were inhibited by WP (Feng et al. 2010).

These promising in vitro and animal findings raise the possibility of formulating a wolfberry-based therapy for preventing neurodegenerative disease in the elderly and for protecting early brain development in the neonate.

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14.10 VISION BENEFITS OF WOLFBERRY

The consumption of wolfberry has been related for centuries by Chinese doctors and consumers to a perceived benefit to vision (Cheng et al. 2004; Potterat 2010). Despite a strong belief in the benefits of wolfberry on eyesight, very few human studies of wolfberry supplementation on visual parameters have been reported.

A 15-day regimen of wolfberry juice supplementation had no effect on visual acuity in healthy young adults (Amagase and Nance 2008). Age-related macular degeneration (AMD) is a primary cause of vision loss in the elderly, negatively impacts quality of life, and raises the risk of clinical depression, hip fractures, and placement in nursing homes (Mangione et al. 1999; Wysong, Lee, and Sloan 2009). The prevalence of AMD ranges from 1.5% in people over 40 years to over 15% in women over 80 years (Friedman et al. 2004). Unfortunately, there is no cure for AMD, and current treatment options have limited effectiveness and introduce significant patient risk (Kaufman 2009). Therefore, AMD prevention strategies should be identified and implemented.

Wolfberry is the richest natural source known of zeaxanthin, and the uptake and increase of plasma zeaxanthin concentration upon supplementation has been demonstrated (Cheng et al. 2005). However, there is little direct evidence to support the idea that consumption of wolfberry-derived zeaxanthin can subsequently increase macular pigment optical density, that is, concentrations of macular lutein and zeaxanthin, and ultimately lower AMD risk (Beatty et al. 2001; Carpentier, Knaus, and Suh 2009). It was reported for AMD subjects that lutein and zeaxanthin concentrations are lower by about 32% in three concentric regions centered in the fovea (Bernstein et al. 2002), and a positive association was found between serum concentrations of lutein and zeaxanthin and the macular pigment density (Bone et al. 2000; Gale et al. 2003). The only supplementation study with wolfberry zeaxanthin that showed such efficacy was conducted with rhesus monkeys (Leung et al. 2001). The serum and tissue levels of zeaxanthin and lutein were studied after feeding the animals an extract of wolfberry for 6 weeks as a daily supplement (equivalent of 2.2 mg zeaxanthin/day). The serum levels and macular density of zeaxanthin increased over the duration of the study, and were found to be significantly higher than those of the control monkeys. A similarly clear increase was observed in a very small human supplementation study (with two subjects and administration of 30 mg of synthetic zeaxanthin per day for 4 months; Bone et al. 2003).

Regular consumption of wolfberry may play a role in the prevention and/or stabilization of AMD and maintain macular pigment optical density (Bone et al. 2003). However, long-term clinical studies with a minimum of 4 months of supplementation will be needed to ascertain if wolfberry has a direct or indirect effect on vision in the elderly.

14.11 CONCLUSION

Wolfberry fruit has a remarkable history and a positive image in Chinese medicine and culture. Modern science indicates that its benefits, long known to the Chinese, are indeed the result of the presence and combination of several biologically active molecules. First, there is the widely studied LBP, a group of complex arabinogalactan proteins. Our insight indicates that the biological relevance of LBP may be limited as there is doubt that the identified structure and content of wolfberry LBPs are very different from those known from other plants. The preparation and analysis of LBPs are very complex; thus, it remains to be seen whether the potential benefits and efficacy of LBPs can be demonstrated in human trials. The unusual vitamin C precursor named 2-*O*-(β-D-glucopyranosyl) ascorbic acid and the carotenoid zeaxanthin, an essential component of the human macula, were discovered and subjected to more study recently. Their potential benefits are partially known, but only long-term supplementation studies with defined, clear outcomes will tell us more about their true benefits and mode of action. Having developed a holistic approach, similar to that used in TCM, by using the emulsifying properties of skim milk, we were able to develop a new

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formulation of wolfberry fruit that combines for the first time in one preparation all the bioactives from wolfberry, not only the water-soluble bioactives (as in TCM) but also the equally important fat-soluble ones such as zeaxanthin.

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15 Botanical Phenolics and Neurodegeneration

Albert Y. Sun, Qun Wang, Agnes Simonyi, and Grace Y. Sun

CONTENTS

15.1 Introduction 315
15.2 Oxidative Stress and Neurodegenerative Disorders 316
15.2.1 Alzheimer's Disease 316
15.2.1.1 Resveratrol and Grape Polyphenols 317
15.2.1.2 Curcumin (Diferuloylmethane) 318
15.2.1.3 Apocynin 319
15.2.1.4 Epigallocatechin-3-gallate (EGCG) 319
15.2.2 Parkinson's Disease 320
15.2.3 Stroke 322
15.3 Integrated Signaling Mechanisms of Botanical Phenolics in Neurodegenerative Disorders 324
Acknowledgments 325
References 325

15.1 INTRODUCTION

There is ample evidence indicating that different reactive oxygen species (ROS), for example, superoxide, hydrogen peroxide, and hydroxyl and peroxyl radicals, are produced in cells under normal and pathological conditions (Sun et al. 2008). When the rate of ROS generation exceeds the capacity of antioxidant defense, there is consequential oxidative damage to DNA, proteins, and lipids. In the central nervous system (CNS), oxidative stress is implicated in mechanisms leading to neuronal cell injury in various pathological states. Recently, the term "nitrosative stress" has been used to indicate cellular damage elicited by reactive nitrogen species (RNS), which include nitric oxide (NO) and its congeners such as peroxynitrite and nitroxyl anion. Together, oxidative and nitrosative stresses are implicated in the pathology of many neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and stroke. The brain is particularly vulnerable to oxidative damage because it utilizes a large amount of oxygen for energy and has relatively low antioxidant defense enzymes, especially during aging. In addition, membranes in brain cells contain abnormally high proportions of polyunsaturated fatty acids (PUFAs). Of the different types of cells in the brain, neurons are especially vulnerable to insults by toxic compounds, and are sensitive to damage by ischemia/stroke, seizure, and other excitotoxic injury. Oxidative damage to lipids (lipid peroxidation) is associated with a progressive loss of membrane integrity, reduction of mitochondrial membrane potential, and increase in plasma membrane permeability to Ca^{2+}. Oxidative damage to proteins leads to the formation of carbonyl and nitrosylated derivatives. Further, ROS damage to DNA results in nuclear condensation and altered gene expression. Therefore, oxidative stress is an important risk factor for

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neurodegeneration. In recent years, extensive effort has been devoted to developing novel strategies to overcome different types of insults in the brain (Sun et al. 2008; Farooqui and Farooqui 2009).

Many vegetables, fruits, grains, roots, flowers, and seeds are rich in polyphenolic compounds, and they offer beneficial effects in protecting against diseases involving oxidative stress, such as cancers and cardiovascular and neurodegenerative diseases. Although the mechanisms through which these compounds exert beneficial effects are not well understood, there is a general consensus that they possess antioxidant and anti-inflammatory properties, and are capable of chelating metal ions (Rice-Evans and Miller 1997; Martin et al. 2002; Ndiaye et al. 2005; Sun et al. 2008). Recent studies further reveal that some compounds may contribute specific biochemical effects that are beyond their antioxidant and radical-scavenging properties, for example, involvement in alterations of members of the "vitagene" system, such as heme oxygenase-1 (HO-1), heat shock protein (Hsp) 70, thioredoxin, and sirtuins. These effects may have an impact on the onset and progression of neurodegenerative diseases and aging. The understanding of these metabolic and signaling effects of polyphenols has paved the way for novel nutritional interventions (Calabrese et al. 2008, 2009). In this chapter, we review recent studies on four botanical phenolic compounds: resveratrol from grapes, curcumin from turmeric, apocynin from *Picrorhiza kurroa*, and epigallocatechin (EGC)-gallate from green tea. We discuss their potential beneficial effects in the prevention and treatment of neurodegenerative diseases, with an emphasis on AD, PD, and stroke.

15.2 OXIDATIVE STRESS AND NEURODEGENERATIVE DISORDERS

15.2.1 Alzheimer's Disease

Alzheimer's disease is the most common form of dementia and is a progressive, age-dependent neurodegenerative disorder affecting specific regions of brain that control memory and cognitive functions. The pathogenesis of AD is characterized by the accumulation of amyloid plaques and the presence of neurofibrillary tangles in neurons (Terry et al. 1991; Selkoe and Podlisny 2002; McKeel et al. 2004). Many studies have demonstrated that oxidative stress is an early event in the development of AD (Akama and Van Eldik 2000; Butterfield 2002; Butterfield et al. 2002a,b; Perry et al. 2002; Mattson 2004). Although the underlying mechanisms are still unclear, there is evidence that the soluble oligomeric form of amyloid-β peptides (Abeta) may be a key cytotoxic compound that impairs synaptic plasticity; long before Aβ is incorporated to form the amyloid plaques (Small 2001; Small, Mok, and Bornstein 2001; Selkoe and Podlisny 2002; Mattson 2004; Takahashi et al. 2004). Studies with transgenic mice and with cultured cells demonstrated that cytotoxic Aβ may cause neuronal cell death through generation of ROS (Zerbinatti et al. 2004; Ashe 2005; Barghorn et al. 2005; Smith et al. 2005). There is evidence that Aβ induces oxidative stress and causes neuronal damage by targeting excitatory ionotropic glutamate receptors, especially the *N*-methyl-D-aspartic acid (NMDA) subtype. Studies conducted in our laboratories demonstrated the ability of oligomeric Aβ and NMDA to stimulate cortical neurons and trigger signaling pathways, leading to the activation of mitogen-activated protein kinases (MAPKs) and phospholipase A2 (PLA2; Shelat et al. 2008). Our study and those conducted by others further demonstrate that these excitatory pathways involve ROS production by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Zhu et al. 2005; Shelat et al. 2008; Zhu et al. 2009). Involvement of Aβ and NMDA in neuronal excitotoxicity is in line with recent developments on memantine, an NMDA receptor antagonist used for the treatment of AD. Memantine is prescribed to treat moderate to severe AD patients, and is the most recently approved AD medication in the United States.

Besides changes in glutamatergic neurotransmission, loss of cholinergic neurons is also an important abnormality in AD pathology. The cholinergic hypothesis assumes that aberrant cholinergic transmission in the basal forebrain, some cortical regions, and the hippocampus is associated with memory impairment in advanced stages of AD (Contestabile, Ciani, and Contestabile 2008). Measurement of choline acetyltransferase activity, the key enzyme for acetylcholine synthesis, has

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been used for many years as a reliable marker for damage of the cholinergic pathways. Stereologic counting of the basal forebrain cholinergic neurons has also been used to assess neurodegenerative changes in the forebrain cholinergic system. Acetylcholine esterase inhibitors to slow the hydrolysis of acetylcholine at the synaptic terminals are symptomatic drugs for the treatment of AD (Takada-Takatori et al. 2009). Drugs such as donepezil, rivastigmine, and galantamine are currently prescribed to treat mild to moderate AD patients. Donepezil was also recently approved to treat severe AD. In general, these drugs are helpful in maintaining the abilities of individuals to carry out their daily living activities and in maintaining thinking, memory, or speaking skills. However, these drugs can offer only transient help (over a few months) and cannot reverse the progression of the disease.

Another mechanism by which Aβ induces neurodegeneration may involve inflammatory processes, which are triggered by oxidative changes (Butterfield 2002; Butterfield et al. 2002a,b). Currently, there are several ongoing, large-scale AD-prevention trials aiming at using antioxidants to decrease oxidative and inflammatory damage and for prevention and treatment of AD. One such ongoing trial, "Prevention of Alzheimer Disease with Vitamin E and Selenium (PREADVISE)," is sponsored by the National Institute for Aging and the National Cancer Institute, and aims at assessing whether taking selenium and/or vitamin E supplements can help prevent memory loss and dementia in AD (Kryscio et al. 2004, 2006).

Due to the diverse functional effects offered by many natural botanical compounds, there is widespread interest in considering them as possible therapeutics for treating AD patients (Howes and Houghton 2003; Howes, Perry, and Houghton 2003; Bastianetto and Quirion 2004; Anekonda and Reddy 2005). Plant-derived compounds are generally safer to use as compared with synthetic drugs (Raskin et al. 2002). Since neuroinflammation and oxidative damage are observed in the brains of transgenic rodent models of AD, a number of studies have used these animal models to test the neuroprotective effects of botanical compounds (Ringman et al. 2005; Anekonda 2006; Anekonda and Reddy 2006; Chauhan and Sandoval 2007; Mancuso et al. 2007; Sun et al. 2008). A brief description of these studies is included in Sections 15.2.1.1 through 15.2.1.4.

15.2.1.1 Resveratrol and Grape Polyphenols

Resveratrol (3,4′,5-trihydroxystilbene) is a polyphenolic compound found in purple grapes, red wine, peanuts, and several other plants (Baur et al. 2006; Baur and Sinclair 2006). Our earlier studies indicated that a dietary supplement of polyphenols extracted from grape skin and seeds could offer protection against oxidative damage to brain synaptic membranes (Sun and Cheng 1999; Sun et al. 1999). Studies with rat pheochromocytoma (PC-12) cells further showed that resveratrol was more effective in protecting oxidative damage than vitamins E and C combined (Chanvitayapongs, Draczynska-Lusiak, and Sun 1997). In an animal model for aging, resveratrol was shown to protect against neuronal damage and excitotoxicity induced by the administration of kainic acid in rats (Wang et al. 2004; Wang et al. 2005c). A time-course study of bioavailability of resveratrol in rats indicated that this compound is readily transported to the blood, liver, and brain shortly after intraperitonial (i.p.) injection, and that most resveratrol was converted to a glycoconjugate form (Wang et al. 2002). Over the years, many other studies in vitro and in vivo have attempted to elucidate the underlying mechanisms of the neuroprotective effects of resveratrol (Gao et al. 2006; Lu et al. 2006; Raval, Dave, and Perez-Pinzon 2006; Choi et al. 2007; Tsai et al. 2007; Sun et al. 2008).

Besides alleviating stroke damage, resveratrol can offer multiple protective effects for other neurodegenerative diseases. This compound appears to mimic the effects of dietary calorie restriction, which has been shown to trigger the activation of sirtuin proteins (Howitz et al. 2003; Lamming, Wood, and Sinclair 2004; Wood et al. 2004; Sinclair 2005). Indeed, resveratrol has been found to increase the life span of a number of lower organisms, including yeasts, nematodes, and fruit flies (Howitz et al. 2003), and this effect is attributed to the activation of sirtuins, which are evolutionarily conserved nicotinamide adenine dinucleotide (NAD)-dependent histone deacetylases that are known to participate in the pathomechanisms of numerous age-related disorders (Tissenbaum and Guarente

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2001; Cohen et al. 2004; Parker et al. 2005; You and Mak 2005). Although the exact mechanism of resveratrol in activating sirtuin 1 (SIRT1) and prolonging life span in organisms remains unclear, there is evidence that activation of SIRT1 is associated with the triggering of downstream proteins, such as peroxisome proliferator–activated receptor coactivator-1α (PGC-1α), the forkhead transcription factor (FOXO) family, Akt (protein kinase B), and nuclear factor κB (NF-κB; Pallas et al. 2009). Several studies with cell and animal models suggest that resveratrol can exert neuroprotective effects through its ability to activate SIRT1 and other vitagenes (Rasouri, Lagouge, and Auwerx 2007). In a study with cultured embryonic mouse neurons, activation of the SIRT1/PGC-1 pathway was shown to protect against axonal degeneration of neurons and to decrease accumulation of amyloid peptides.

Studies demonstrating the ability of resveratrol to activate SIRT1 and other vitagenes make resveratrol a promising candidate as a therapeutic agent for treating AD (Anekonda 2006; Mancuso et al. 2007). Two recently conducted studies show that the deleterious effects of high-fat and high-calorie diets in mice can be mitigated by dietary supplementation with resveratrol. In one study, resveratrol reversed the shortened life span resulted the high-fat diet, and in the second study, resveratrol increased SIRT1 activation, PGC-1α deacetylation, and mitochondrial biogenesis in muscle (Calabrese et al. 2009). Interestingly, a synergistic protection can be achieved when resveratrol is administered in combination with catechin (C), another plant phenolic compound (Conte, Pellegrini, and Tagliazucchi 2003). In a rat model of sporadic AD, administration of resveratrol prevented cognitive impairment and oxidative stress induced by intracerebroventricular streptozotocin (Sharma and Gupta 2002). In another animal model of AD and tauopathy, resveratrol decreased neurodegeneration in the hippocampus and prevented learning impairment (Kim et al. 2007). A study conducted by Wang et al. (2006) showed that consumption of red wine by Tg2576 mice can attenuate deterioration of spatial memory function and Aβ neuropathology.

It is worth noting that besides causing in vivo effects, resveratrol can also inhibit the formation of Aβ fibrils and destabilize fibrilized Aβ (Ono et al. 2006a; Ono, Naiki, and Yamada 2006b). Resveratrol was reported to decrease amyloid-β secretion from different cell lines (Marambaud, Zhao, and Davies 2005). In other studies, resveratrol suppressed neuroinflammation by inhibiting NADPH oxidase activity and attenuating NF-κB-induced expression of inducible NO synthase (iNOS) and cyclooxygenase-2 (COX-2; Bi et al. 2005; Kim et al. 2006). Taken together, these studies indicate that besides its antioxidant property, resveratrol may also exert neuroprotective effects through activation of sirtuin and vitagenes.

15.2.1.2 Curcumin (Diferuloylmethane)

Curcumin is derived from turmeric, the powdered rhizome of the medicinal plant *Curcuma longa* Linn., which is widely used as a spice in Southeast Asian and Middle Eastern cooking (see also Chapter 13 on turmeric). Turmeric is thought to have many medicinal properties; it is used as an antiseptic for cuts, burns, and bruises and used as an antibacterial agent. In Asian countries, curcumin can also help with stomach problems and other ailments. Besides being a strong antioxidant and anti-inflammatory agent (Ono et al. 2004), curcumin was also found to bind amyloid directly and inhibit Aβ aggregation as well as fibril and oligomer formation in vivo (Yang et al. 2005). Curcumin was found to inhibit the formation and extension of Aβ fibrils and to destabilize fibrilized Aβ (Ono et al. 2006a; Ono, Naiki, and Yamada 2006b).

There is a great need for well-designed studies to assess whether dietary curcumin is efficient in treating AD. In a study conducted on Tg mice, conventional nonsteroidal anti-inflammatory drug (NSAID), ibuprofen, and curcumin were compared for their ability to protect against Aβ-induced damage in mice (Frautschy et al. 2001). Dietary curcumin (2000 ppm), but not ibuprofen, suppressed oxidative damage and synaptophysin loss. Dietary curcumin also decreased Aβ deposits, prevented Aβ-induced spatial memory deficits in the Morris water maze test, and prevented postsynaptic density loss in Tg mice (Frautschy et al. 2001). Both low and high doses of curcumin significantly lowered oxidized proteins and interleukin 1β, a proinflammatory cytokine that was elevated in the brains of the AD mice (Lim et al. 2001). Besides its antiamyloidogenic, antioxidant,

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and anti-inflammatory abilities (Ringman et al. 2005), curcumin can also alter signaling molecules and pathways in cells (Begum et al. 2008). Clearly, more clinical trials are needed to assess the therapeutic use of curcumin for the treatment of AD (Ringman et al. 2005; Fiala et al. 2007).

15.2.1.3 Apocynin

Apocynin (4-hydroxy-3-methoxy-acetophenone) is isolated from the root of *Picrorhiza kurroa*, a creeping plant native to the mountains of India, Nepal, Tibet, and Pakistan. Apocynin may also be obtained from other sources, for example the rhizome of Canadian hemp (*Apocynum cannabinum*), and other *Apocynum* species (e.g., *A. androsaemifolium*). *P. kurroa* has long been used as an herbal medicine for the treatment of liver and heart problems, jaundice, and asthma. The anti-inflammatory property of this herb is attributed to its ability to prevent ROS and peroxide formation in the body.

Apocynin has been shown to specifically inhibit NADPH oxidase by blocking the assembling of cytosolic NADPH oxidase subunits with the membrane subunits. The NADPH oxidase is a superoxide-producing enzyme and is increasingly recognized for its dual-edged role in health and disease and in mediating cell-signaling pathways (Sun et al. 2008). The prototypic NADPH oxidase comprises of a membrane-associated cytochrome b558 complex containing a p22 phox and a gp91 phox subunit and several regulatory cytosolic subunits, for example, p47 phox, p40 phox, and p67 phox. In addition, the small G protein Rac1 or Rac2 can also associate with the gp91 phox subunit to confer full activity. The NADPH oxidase is expressed in all brain cells, and is especially high in activated microglial cells. Recent studies have identified several isoforms of gp91 phox in different cell types, although their specific roles in mediating pathological pathways have not yet been fully elucidated (Block 2008; Chen, Song, and Chan 2009; Sorce and Krause 2009).

There is accumulating evidence suggesting that aberrant neuron–glia interactions may play an important role in the progression of neurodegenerative diseases. Astrocytes are the most abundant cell type in the human brain, and they are important in supporting neurons in a number of functions, including synapse formation and plasticity, energetics, and redox metabolism. Both astrocytes and microglia are immunologically active cells in the CNS and are involved in the inflammatory responses to injury and disease. In the AD-affected brain, glia-mediated inflammatory response has been linked to the enhancement of the deposition of amyloid plaques and neuronal damage (Salmina 2009). In particular, microglial cells are activated by cytotoxic Aβ and by different forms of neuronal injury, and these cells become a chronic source of neurotoxic cytokines and ROS that leads to impairment of neuronal function. In activated microglial cells, ROS produced by NADPH oxidase may cause neurotoxicity by directly interacting with nearby neurons. Alternatively, intracellular ROS may stimulate signaling pathways in microglia and amplify the production of proinflammatory and neurotoxic cytokines (Block 2008). In a coculture model, addition of macrophage cell lines deficient in NADPH oxidase gp91phox subunit failed to kill neurons. This study demonstrated that ROS generation by NADPH oxidase in microglial cells can play a crucial role in neuronal killing (Qin et al. 2006). Along this line, apocynin, the NADPH oxidase inhibitor, is considered a therapeutic compound to combat Aβ-induced microglial proliferation and neuronal death (Jekabsone et al. 2006; Mander, Jekabsone, and Brown 2006).

15.2.1.4 Epigallocatechin-3-gallate (EGCG)

Human epidemiological and experimental data on animals suggest health benefits of tea drinking (see also Chapter 12 on tea). Tea consumption is inversely correlated with the incidence of dementia, AD, and PD (Mandel et al. 2008b). Analysis of individual compounds in tea by high-performance liquid chromatography (HPLC) revealed high levels of C, epicatechin (EC), EC-3-gallate (ECG), EGC, EGC-3-gallate (EGCG), and gallic acid. Among these compounds, EGCG has been found to be particularly effective in offering neuroprotection in studies with cellular and animal models. There is evidence that in addition to the known antioxidant and anti-inflammatory properties of this compound, EGCG may protect neurons through modulation of signal transduction pathways, cell survival/death genes, and mitochondrial function (Mandel et al. 2008b). In BV-2 microglial

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cells, EGCG pretreatment effectively ameliorated Aβ-induced cytotoxicity and manifestation of proapoptotic signals. Furthermore, Aβ induces iNOS and production of NO in BV-2 cells, and EGCG effectively suppressed the Aβ-mediated NO in these cells (Kim et al. 2009). The EGCG (or curcumin) could also attenuate Aβ-induced ROS production and β-sheet structure formation (Shimmyo et al. 2008). In Swedish Tg mice overexpressing mutant human amyloid precursor protein (APP), EGCG administered orally in drinking water (50 mg/kg) decreased Aβ deposition. Both i.p. and oral administration of EGCG to the APP Tg mice also suppressed phosphorylation of tau isoforms and improved cognitive function. Interestingly, animals treated intraperitoneally with EGCG appeared to show a more pronounced benefit than those fed orally (Rezai-Zadeh et al. 2008). In a study with human neuronal cell line MC65, EGCG was found to enhance APP processing by the nonamyloidogenic proteolytic enzymes and decreased Aβ levels. In this study, EGCG was also found to decrease nuclear translocation of c-Abl and to block APP-C99-dependent GSK3 β activation (Lin et al. 2009). Another study also demonstrated the neuroprotective effects of EGCG by suppressing Aβ-induced β-secretase (BACE)-1 upregulation in Tg2576 AD mice (Rezai-Zadeh et al. 2005). Taken together, these observations lend strong support to the neuroprotective effects of green-tea polyphenols in retarding the progression of neurodegenerative disorders such as those exhibited in AD and PD.

15.2.2 Parkinson's Disease

Parkinson's disease is a chronic and progressive degenerative disease associated with impaired motor control, speech, and other functions. The disease is named after an English physician named James Parkinson, who gave a detailed description of the disease in an 1817 work entitled *An Essay on the Shaking Palsy.* This disease belongs to pathological conditions of movement disorders and is characterized by muscle rigidity, resting tremor, slowing of movement (bradykinesia) and, in extreme cases, a nearly complete loss of movement (akinesia). Secondary symptoms may include cognitive dysfunction, subtle language problems, and depression. These symptoms are caused by loss of dopaminergic neurons in the substantia nigra. Parkinson's disease affects approximately 1% of the population over the age of 50 years. Despite numerous hypotheses and continued speculations, the etiology of PD remains unclear. The discovery of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), an environmental toxin that can selectively damage the substantia nigra and subsequently result in Parkinsonian syndromes in animals and humans, has accelerated the search for other neurotoxins as the possible cause of PD (Langston 1987; Tipton and Singer 1993). Although a number of studies have used the MPTP model for studying degeneration of dopamine (DA) neurons and related pathophysiology of PD (Adams and Odunze 1991; Schapira 1996), other environmental toxins such as manganese (Sun, Yang, and Kim 1993), dimethoxyphenyl-ethylamine (DMPEA; Koshimura et al. 1997), and paraquat (Miller, Sun, and Sun 2007; Miller et al. 2009) have also been found to kill DA neurons. These studies provide information indicating that oxidative damage, mitochondrial and proteasomal dysfunction, and inflammation are underlying factors for degeneration of dopaminergic neurons in PD.

Dopamine is metabolized by monoamine oxidase (MAO) or through auto-oxidation with the generation of superoxide, hydrogen peroxide, and hydroxyl radicals. These oxidative events are considered the underlying causes for damage of dopaminergic neurons. NO, which may be released by inflammation-induced microglia (Castano et al. 1998) or generated by excitotoxic insults (Gonzalez-Hernandez, Perez de la Cruz, and Mantolan-Sarmiento 1996; Abekawa, Ohmori, and Koyama 1997), may also play a role in the pathogenesis of PD. The production of ROS and RNS together is an important cause for damage of DA neurons in PD.

The most widely used form of treatment for PD is L-dopa, a compound that can be transformed to DA in the dopaminergic neurons by L-aromatic amino acid decarboxylase (often known as dopa-decarboxylase). However, only 1–5% of L-dopa can enter the dopaminergic neurons. The remaining L-dopa can be metabolized to DA elsewhere, and it causes a wide variety of side effects.

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Carbidopa and benserazide are dopa decarboxylase inhibitors. These compounds help to prevent the metabolism of L-dopa before it reaches the dopaminergic neurons and are generally given in combination with L-dopa. The DA agonists such as bromocriptine, pergolide, pramipexole, ropinirole, cabergoline, apomorphine, and lisuride are only moderately effective and frequently produce side effects including somnolence, hallucinations, and/or insomnia. Dopamine agonists may act by stimulating the DA receptors. However, these compounds may cause the DA receptors to become progressively less sensitive, thereby eventually exaggerating the symptoms. The MAO-B inhibitors selegiline and rasagiline can decrease the symptoms somewhat by inhibiting MAO-B, thereby inhibiting the breakdown of DA in the dopaminergic neurons. Metabolites of selegiline include levoamphetamine and levomethamphetamine, both of which are adrenergic drugs and cause side effects (Aminoff 2007).

An increasing number of studies demonstrate that plant polyphenols, especially flavonoids, are useful in protecting against brain damage in PD. These studies, including ours, have used either single compounds such as resveratrol, curcumin, EGCG, or complex mixtures/extracts such as grape, blueberry, and green tea (Weinreb et al. 2004; Lau, Shukitt-Hale, and Joseph 2005; Mercer et al. 2005; Chen, Jin, and Li 2007; Sun et al. 2008). Their neuroprotective effects may involve, at least in part, their ROS-scavenging and iron/metal-chelating activities, as well as their anti-inflammatory properties. Resveratrol administration was found to protect mice from MPTP-induced motor coordination impairment, hydroxyl radical overloading, and neuronal loss (Lu et al. 2008). Resveratrol has also been tested to produce beneficial effects in the 6-hydroxydopamine (6-OHDA)-induced PD rat model. This model involves chronic inflammation, mitochondrial dysfunction, and oxidative stress, and loss of dopaminergic neurons in the substantia nigra. Resveratrol treatment significantly decreased the levels of COX-2, tumor necrosis factor (TNF)-α messenger ribonucleic acid (mRNA), and COX-2 protein expression in the substantia nigra (Jin et al. 2008).

Curcumin is known to exert neuroprotective effects and ameliorate PD symptoms, due mainly to its antioxidant and anti-inflammatory properties (Zbarsky et al. 2005; Chen et al. 2006; Rajeswari 2006; Lee et al. 2007; Jagatha et al. 2008). Similar to its ability to dissociate aggregated Aβ, curcumin can also inhibit aggregation of α-synuclein, the presynaptic protein associated with the formation of neuronal inclusions (Pandey et al. 2008). Since curcumin can target multiple reactions and proteins in cells, including transcription factors, growth factors, antioxidant enzymes, cell-survival kinases, and signaling molecules, there is increasing interest in considering the potential use of this compound as a therapeutic to combat neurodegenerative diseases (Ramassamy 2006; Salvioli et al. 2007; Goel, Kunnumakkara, and Aggarwal 2008).

NADPH oxidase is regarded an important source of ROS in 1-methyl-4-phenylpyridinum (MPP+)-induced apoptotic neuronal death (Zhang et al. 2008). Treatment with NADPH oxidase inhibitors, such as diphenyleneiodonium chloride (DPI), apocynin, and superoxide dismutase (SOD) mimetics, could block the MPP+-induced ROS production in these cells (Anantharam et al. 2007; Miller, Sun, and Sun 2007; Miller et al. 2009). The environmental toxin paraquat, when used together with iron, could activate microglial cells. Apocynin could attenuate the release of superoxide from activated microglial cells and suppress MPP+-induced cytotoxic cell death (Anantharam et al. 2007; Peng et al. 2009). Thus, specific inhibition of NADPH oxidase–targeting dopaminergic neurons may prove beneficial against the progression of PD.

A prominent pathological feature of PD is the abnormal accumulation of iron associated with neuromelanin in the melanin-containing DA neurons. Lewy bodies, which are the morphological hallmark of PD, are comprised of lipids, redox-active iron, and aggregated α-synuclein, and are associated with ubiquitinated, hyperphosphorylated neurofilaments. The EGCG has been found to protect against neurodegeneration induced by neurotoxins in mice and rats and prevent the accumulation of iron and α-synuclein in the substantia nigra pars compacta (SNpc; Youdim 2003). It can also inhibit ROS production, suppress the cytotoxicity of rotenone in human neuroblastoma SH-SY5Y cells (Chung, Miranda, and Maier 2007), and protect against MPTP-induced damage in mice (Choi et al. 2002; Mandel et al. 2008b). Since EGCG can exhibit antioxidant effects and chelate

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iron, a combination of iron chelation and antioxidant therapy may provide additional neuroprotective effects against PD and other neurodegenerative diseases (Mandel, Maor, and Youdim 2004).

15.2.3 Stroke

Stroke is the rapidly developing loss of brain functions caused by disturbance in blood supply to the brain, and is the third leading cause of death and the first leading cause of long-lasting disability in aging adults. Stroke can be attributed to ischemia (lack of blood supply) caused by thrombosis or embolism, or to hemorrhages caused by disruption of blood vessels. As a result, the affected area of the brain is unable to function, leading to the inability to move, understand, or formulate speech. Ischemic stroke is caused by a thrombus (blood clot) occluding blood flow in an artery in the brain. Definitive therapy is aimed at removing the blockage by breaking up the clot by thrombolysis, or by removing the clot mechanically through a thrombectomy. Stroke is occasionally treated with thrombolytic drugs. Oral anticoagulants such as warfarin are the mainstay of stroke prevention in high-risk subjects. Aspirin and antiplatelet drugs are effective in secondary prevention after a stroke or a transient ischemic attack. Tissue plasminogen activator (tPA) is a thrombolytic drug used to dissolve the clot and unblock the artery. However, the use of tPA is limited to 3 hours after the onset of stroke. In patients with intracerebral hemorrhage, anticoagulants and antithrombotics can sometimes worsen the bleeding. Effective medication or drug therapy for hemorrhagic stroke treatment is limited. Stroke prevention strategies include controlling hypertension and diet, performing regular exercise, and quitting smoking and alcohol use. In order to study the pathoetiology and possible prevention of stroke, many animal models have been developed in which blood flow is focally or globally, permanently or transiently, or completely or incompletely interrupted. Focal cerebral ischemia animal model is usually produced by occlusion of the middle cerebral artery (MCAO), since it reflects the predominant form of clinical stroke. Since oxidative stress is an important underlying cause of neuron cell death in ischemia/reperfusion (I/R) injury, a number of studies have been carried out to test the beneficial effects of dietary antioxidants, including plant polyphenolics (Bravo 1998; Youdim and Joseph 2001; Deschamps et al. 2001; Voko et al. 2003). These studies provide strong evidence for the protective effects of botanical antioxidants, which can act at multiple levels to influence both the early and late phases of stroke (Simonyi et al. 2005; Curin, Ritz, and Andriantsitohaina 2006).

Earlier studies from our laboratory indicated protective effects of resveratrol against delayed neuronal cell death (DND) induced by global cerebral ischemia in gerbils (Wang et al. 2002). We also demonstrated that dietary supplementation with grape powder for 2 months could protect the brain from global ischemic injury (Wang et al. 2005a). Results from our studies are in agreement with others showing that grape seed extract is neuroprotective against ischemic injury (Hwang et al. 2004; Feng et al. 2007).

There are in vitro and in vivo studies indicating the ability of curcumin to exert protective effects against ischemia-induced neuronal injury. In our previous study, administration of curcumin to gerbils was found to decrease global ischemia-induced lipid peroxidation, mitochondrial dysfunction, and apoptotic indices (Wang et al. 2005b). In addition, curcumin also ameliorated the increase in locomotor activity observed at 48 hours after ischemic insult (Wang et al. 2005b). In another study by Jiang et al. (2007), curcumin was protective even when administered after focal ischemia. The authors attributed the protective effects to preservation of blood–brain barrier integrity (Jiang et al. 2007). Consistent with other bioavailability studies, our study (Wang et al. 2005b) also showed a rapid increase in curcumin in plasma after i.p. injection, which reached the brain within 1 hour after treatment (Ringman et al. 2005; Goel, Kunnumakkara, and Aggarwal 2008).

Immunohistochemical studies conducted in our laboratory demonstrated an increase in NADPH oxidase subunit expression after transient focal cerebral ischemia, and the increase was attributed to activated microglia (data not shown). Apocynin was shown to inhibit ischemic damage in different animal models (Wang et al. 2006; Tang et al. 2007) and to ameliorate blood–brain barrier damage

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in experimental stroke (Kahles et al. 2007). In our study using the gerbil global cerebral ischemia model, apocynin was shown to inhibit I/R-induced increase in lipid peroxidation, oxidative DNA damage, and glial cell activation in the hippocampus (Wang et al. 2006). Pretreatment (but not post-treatment) with apocynin was found to decrease ischemia-induced superoxide levels and lower brain damage (Jackman et al. 2009). Several studies have demonstrated that EGCG administration before or after ischemia also significantly decreased neuronal damage (see Table 15.1).

TABLE 15.1
Studies on Botanical Compounds Used in AD, PD, and Stroke Models

Compounds	Model	Effects	References
		AD models	
Resveratrol	i.c.v. streptozotocin	+	Sharma and Gupta 2002
Curcumin	Aβ infusion	+	Frautschy et al. 2001
	Tg2576	+	Lim et al. 2001; Yang et al. 2005
EGCG	Aβ infusion, performance status	+	Lee et al. 2009
	Aβ infusion	+	Rasoolijazi et al. 2007
	Tg 2576	+	Rezai-Zadeh et al. 2005, 2008
		PD models	
Resveratrol	6-OHDA	+	Jin et al. 2008
	MPTP	+	Lu et al. 2008
Curcumin	6-OHDA	+	Zbarsky et al. 2005
	MPTP	+	Rajeswari and Sabesan 2008
EGCG	6-OHDA	–	Leaver et al. 2009
	MPTP	+	Levites et al. 2001; Choi et al. 2002; Mandel and Youdim 2004
Apocynin	Paraquat	+	Cristovao et al. 2009
		Stroke models	
Resveratrol	MCAO	+	Sinha, Chaudhary, and Gupta 2002; Inoue et al. 2003; Tsai et al. 2007; Yousof et al. 2009
	MCAO and CCAO	+	Huang et al. 2001
	CCAO	+	Wang et al. 2002
Curcumin	MCAO	+	Thiyagarajan and Sharma 2004; Jiang et al. 2007; Dohare et al. 2008; Shukla et al. 2008; Yang et al. 2009; Zhao et al. 2008; 2010
	CCAO	+	Ghoneim et al. 2002; Al-Omar et al. 2006; Wang et al. 2005b
EGCG	MCAO	+	Choi et al. 2004
		±	Rahman et al. 2005b
	CCAO	+	Lee, Suh, and Kim 2000; Lee et al. 2003; Lee, Bae, and Lee 2004; Park et al. 2009
Apocynin	CCAO and hypoxia	+	Sutherland et al. 2005
	CCAO (2X)	–	Pu et al. 2007
		+	Kahles et al. 2007; Tang et al. 2007; Chen, Song, and Chan 2009
Apocynin	CCAO	+	Wang et al. 2006; Suh et al. 2008
	Neonatal H/I	–	Doverhag et al. 2008

Notes: CCAO: common carotid artery occlusion; H/I: hypoxia/ischemia; i.c.v.: intracerebral ventricular; "+" indicates improvement, and "–" indicates no effect or worse outcome.

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15.3 INTEGRATED SIGNALING MECHANISMS OF BOTANICAL PHENOLICS IN NEURODEGENERATIVE DISORDERS

Many plant extracts and identified plant-derived compounds have been found useful for treatment and prevention of neurodegenerative disorders. However, their underlying molecular mechanisms and therapeutic value are still largely unknown (Howes and Houghton 2003; Howes, Perry, and Houghton 2003; Anekonda and Reddy 2005). Investigation of the health benefits of these natural compounds poses substantial challenges to modern medicine. Polyphenols are divided into different groups, depending on the number of hydroxyl groups and derivatives to the benzene rings. Flavonoids make up the largest and the most important group of polyphenols, which can be divided into subgroups such as flavanols (C, EC), flavonols (quercetin, myricetin, kaempferol), flavanons (hesperetin, naringenin), flavons (apigenin, luteolin), isoflavonoids (genistein, daidzein), and anthocyanins (cyanidin, malvidin). Depending on their molecular structure, the positions of their hydroxyl groups, and the presence of conjugated dienes, these flavonoids may have different antioxidant properties and ROS-scavenging activities.

The pathophysiological mechanisms underlying neurodegenerative disorders are complex and diverse, and range from oxidative stress to inflammatory responses and apoptosis (Figure 15.1). The complexity of cell-signaling pathways may explain the difficulties encountered in finding effective treatments. Although much progress has been made in understanding the pathogenesis of AD, current therapeutic approaches merely address symptoms. Novel therapeutic approaches using natural botanical antioxidants may be suggested to ameliorate neurotoxicity and chelate transition metals (e.g., iron and copper). Both experimental and epidemiological evidence demonstrate that flavonoid

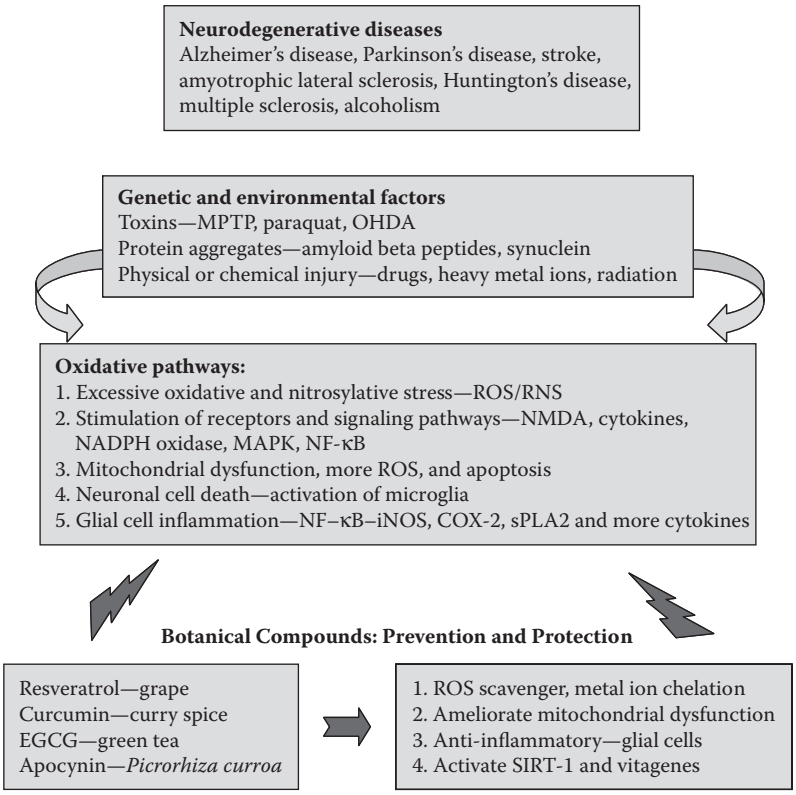


FIGURE 15.1 Oxidative pathway involved in neurodegenerative diseases: Preventive and protective mechanisms exerted by botanical phenolics.

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polyphenols improve age-related cognitive decline and are neuroprotective in models of PD, AD, and cerebral I/R injuries (Mandel et al. 2008a; Sun et al. 2008).

Many plant polyphenols have been suggested as excellent candidates for development as therapeutic agents for treatment of neurodegenerative diseases (Figure 15.1). In particular, there is increasing interest in using resveratrol for treatment of progressive neurodegenerative maladies such as AD and PD. It is recognized that some botanical compounds may selectively target a single pathway, whereas others may act globally on multiple pathways. For example, apocynin, a known inhibitor of NADPH oxidase, has been used mainly to block ROS production by NADPH oxidase, whereas resveratrol, curcumin, and EGCG can target multiple pathways. Besides its ROS-scavenging activity, resveratrol can activate SIRT1, which is related to histone acetylation and deacetylation and alteration of proteins involved in the suppression of apoptotic pathways (Rahman, Biswas, and Kirkham 2006). Resveratrol can also affect Nrf2/Keap 1 pathway, which is linked with the activation of antioxidant proteins (Rubiolo, Mithieux, and Vega 2008). Inhibition of the NF-κB pathway by resveratrol and EGCG can decrease the production of inflammatory factors, for example, iNOS, COX-2, and secretory phospholipase A2 (sPLA2), which, in turn, can suppress the vicious cycle of cell death caused by oxidative stress. The multiple effects associated with resveratrol may offer an effective therapeutic remedy to restore neuronal homeostasis (Saiko et al. 2008). Future studies should be directed to investigate whether other phytochemicals also exhibit multiple functions (Calabrese et al. 2008). Questions about bioavailability, biotransformation, synergism with other dietary factors, and their ability to cross the blood–brain barrier also need to be addressed prior to application of botanical compounds on humans. Table 15.1 summarizes recent studies in which different botanical compounds in different paradigms were used to test neuroprotective effects on AD, PD, and stroke models.

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16 Cardiovascular Disease

Richard Walden and Brian Tomlinson

CONTENTS

16.1 Introduction 334
16.2 Essential Micronutrients and Cardiovascular Disease 334
16.2.1 Vitamin A 336
16.2.2 Carotenoids 336
16.2.3 Lycopene 336
16.2.4 B Vitamins 336
16.2.5 Homocysteine and B Vitamins 337
16.2.6 Niacin 338
16.2.7 Vitamin C 338
16.2.8 Vitamin D 339
16.2.9 Vitamin E 339
16.2.10 Flavonoids 340
16.2.11 Flavanol-Rich Cocoa 341
16.2.12 Vitamin K 341
16.2.13 Magnesium 341
16.2.14 Iron 342
16.2.15 Trace Minerals 342
16.2.16 L-Carnitine 343
16.2.17 Omega-3 Polyunsaturated Fatty Acids 343
16.2.18 Coenzyme Q10 344
16.2.19 Summary 344
16.3 Herbal Medicines and Cardiovascular Disease 344
16.3.1 Hawthorn (*Crataegus* Species) 345
16.3.2 Garlic (*Allium sativum*) 345
16.3.3 Danshen (*Salvia miltiorrhiza*) 346
16.3.4 Lingzhi (*Ganoderma lucidum*) 347
16.3.5 Maidenhair Tree (*Ginkgo biloba*) 347
16.3.6 Foxglove (*Digitalis purpurea/lanata*) 347
16.3.7 Ginseng (*Panax* Species) 348
16.3.8 Other Herbal Medicines 348
16.3.9 Summary 349
16.4 Herbal Medicine and Drug Interactions 349
16.5 Research Needs 350
16.6 Conclusions 350
References 351

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16.1 INTRODUCTION

The cardiovascular diseases (CVDs) considered in this chapter have been the major cause of morbidity and mortality in developed countries over the last several decades, and developing countries are rapidly catching up with this epidemic. The underlying pathology is atheromatous vascular disease, resulting in coronary artery disease (CAD), cerebrovascular disease, and peripheral vascular disease, and the subsequent development of heart failure and cardiac arrhythmias. The major risk factors for these disorders were recognized over many years, and they include high levels of low-density lipoprotein (LDL) cholesterol, smoking, hypertension, diabetes, abdominal obesity, psychosocial factors, insufficient consumption of fruits and vegetables, excess consumption of alcohol, and lack of regular physical activity. There has been continued research to help define more precisely the cardiovascular risk of an individual with respect to genetic factors, more complex lipid traits, and inflammatory markers, but it was reconfirmed in the INTERHEART study that the conventional risk factors accounted for over 90% of the population attributable risk for myocardial infarction (MI; Yusuf et al. 2004). There is extensive evidence to show that drug treatment of conventional risk factors is effective in reducing cardiovascular events. Many large clinical trials with the HMG CoA reductase inhibitors (statins) have showed that lowering of LDL cholesterol with these agents decreases coronary and cerebrovascular events (Baigent et al. 2005), and that the target for LDL cholesterol becomes lower with each new set of guidelines and the availability of more potent drugs (Anderson et al. 2007). Likewise, more effective treatment of hypertension with various classes of antihypertensive drugs has been associated with greater benefits (Turnbull et al. 2008), but some recent studies suggest we may be reaching the optimal level of treated blood pressure in some patient groups (ACCORD Study Group 2010). Apart from the treatment of cardiovascular risk factors with pharmacological agents and the use of antithrombotic drugs, there is growing awareness of the role of dietary factors and herbal medicines in the prevention of CVD and the possibility of their use in treatment. Much of this interest centers on the use of antioxidant vitamins and the antioxidant properties of herbal materials, although some herbal materials may also improve conventional cardiovascular risk factors or have antithrombotic effects. In this chapter, we focus mainly on the results from large clinical trials and meta-analyses rather than from mechanistic studies, and we start by considering the use of antioxidant vitamins and other essential micronutrients in Section 16.2 before moving to a discussion of individual herbs in Section 16.3.

16.2 ESSENTIAL MICRONUTRIENTS AND CARDIOVASCULAR DISEASE

The use of supplements of essential micronutrients (EMNs) in orthodox medical practice remains controversial, although adequate amounts of these substances are known to be necessary for the maintenance of health. Although it has long been proved that vitamin D, ascorbic acid, and vitamin B12 are the key to treating rickets, scurvy, and pernicious anemia, respectively, it is less accepted that subclinical deficiency states exist for these and other essential substances that may escape recognition in chronic illness, including CVDs. The estimated average requirement (EAR) to prevent deficiency states for common EMNs have been formulated and modified over the years. Recommendations from the United Kingdom Department of Health guidelines according to age and sex are given in Table 16.1. However, the use of "supraphysiological" or pharmacological doses of these or other EMNs in CVD remains a controversial issue and has the potential to be harmful.

The value of pharmacological doses is questionable for most EMNs, with the possible exceptions of folic acid, niacin, and magnesium, although caution must be taken with their use. Only when a deficiency state exists does the administration of the appropriate supplement achieve the therapeutically desired result for the treatment or prevention of the disease in question. Supplements taken in deficiency states should not generally be taken indefinitely, unless the problem is related to

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TABLE 16.1
Vitamin and Nutraceutical Supplements That Have Been Used for Prevention or Treatment of CVD

Nutraceutical	Estimated Average Requirement (Adults)	Doses Used in Clinical Studies
Vitamin E	Men: >4 mg/day; women >3 mg/day[g]	100–600 mg/day
Vitamin C	25 mg/day	250–500 mg/day
Thiamin	0.8–1.0 mg/day[f]	
Vitamin B12	1.25 μg/day	
Vitamin D	10 μg/day after 65 years[b]	
Vitamin K	1 μg/kg/day[c]	
Folic acid	150 μg/day	
Pyridoxine (vitamin B6)	Men: 1.4 mg/day; women: 1.2 mg/day[e]	
Niacin (nicotinic acid)	Men: 16 mg/day; women: 12 mg/day[f]	1000–2000 mg single dose
Carotenoids	—[d]	β-Carotene 20–50 mg/day
Flavonoids	—[d]	200–1000 mg/day
Alcohol		
Magnesium	Men: 250 mg/day; women: 200 mg/day	800–1000 mg/day
Zinc	Men: 7.3 mg/day; women: 5.5 mg/day	No study
Manganese	1.4 mg/day[a]	
Selenium	Men: 75 μg/day; women: 60 μg/day	
Chromium	25 μg/day[a]	200 μg/day
Molybdenum	50–40 μg/day	No study
Calcium	525 mg/day	
Coenzyme Q10	—[d]	300 mg/day
L-carnitine	—[d]	2 g/day
Omega-3 fatty acids	—[d]	EPA 1500 mg/day

Note: Estimated average requirement: Department of Health, United Kingdom. (HMSO 1991)

[a] Considered adequate
[b] Also for those confined indoors
[c] Lack of adequate data—safe intake recommended
[d] No reliable data
[e] Varies with protein intake
[f] Varies with energy intake
[g] Varies with PUFA intake

some nonmodifiable underlying disease or environmental factor. Furthermore, some patient groups may have special needs for EMNs. For instance, smokers may require above-average intakes of vitamin C, as do diabetics and older persons. Moreover, even if the diet contains the theoretical EMN, the needs of an individual may not be met due to poor absorption, altered metabolism, or associated deficiencies and disease states. In most cases, supplementation should be given only until body stores are replenished, but sometimes, there may be a need to continue the dosage. Restoration of one EMN may result in improved absorption of another or, in some cases, may unmask a partial deficiency. When evaluating an EMN in clinical trials, the substance under study is often given alone for reasons of scientific purity; but deficiency of one EMN is frequently accompanied by others and, thus, an unnatural, even hazardous, situation (sometimes unrecognized) can result if a high dose of a single EMN is given. A classic example is the risk of adverse effects when folic acid

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is given alone. A minority of patients having cyancobalamin deficiency are put at risk of subacute combined degeneration of the spinal cord. It is safer to give folic acid and cyancobalamin together unless the B12 status is known to be normal.

16.2.1 Vitamin A

Included under this heading are several related fat-soluble compounds derived from animal tissue, including retinal and retinoic acid, and a water-soluble vitamin A–related compound β-carotene, which is found in vegetables. These substances have antioxidant activity as quenchers of reactive oxygen species, with the potential to protect against inflammatory disease and atherosclerosis. However, clinical studies with β-carotene failed to show significant efficacy in the prevention of CVD.

16.2.2 Carotenoids

Numerous natural carotenoids are present in fresh fruits and vegetables, and some have been studied extensively in the prevention of coronary heart disease (CHD). Carrots are a primary source of β-carotene. Elevated levels of serum β-carotene were associated with a lower risk of cancer and were found to reduce overall mortality rates (Greenberg et al. 1996); early observational studies reported an association between a high dietary intake of β-carotene and a lowered incidence of CVD (Gey and Puska 1989; Kardinaal et al. 1993). However, the Medical Research Council/British Heart Foundation (MRC/BHF) Heart Protection Study showed no benefit from β-carotene taken 20 mg daily (in combination with 600 mg of vitamin E and 250 mg of vitamin C) on morbidity or mortality in high-risk individuals (Heart Protection Study Collaborative Group 2002). Likewise, the Women's Antioxidant Cardiovascular Study (WACS) found no CVD risk reduction with β-carotene at 50 mg taken every other day, with ascorbic acid at 500 mg taken daily, or vitamin E at 600 IU taken every other day in women at high risk (Cook et al. 2007). The evaluation of the relation between vegetable intake and CHD risk in the Physicians' Health Study concluded that the consumption of vegetables rich in carotenoids was associated with a reduced risk of CHD (Liu et al. 2001), but after 12 years' follow-up there was no impact of supplementation of β-carotene 50 mg alternate days on CVD, cancer, or overall mortality among primarily nonsmokers (Hennekens et al. 1996).

16.2.3 Lycopene

Lycopene is an oxygenated carotenoid with twice the antioxidant activity of β-carotene. Tomatoes are the best source of lycopene, which is the focus of research as a precursor to vitamin A. Epidemiological studies and supplementation clinical trials suggest a decrease in CVD risk, but not all studies confirm this (Sesso et al. 2005), and controlled clinical studies conducted recently with lycopene and well-defined subject populations could find no definite evidence for CVD prevention (Riccioni et al. 2008). Current research is focusing more on the role of lycopene for prevention and treatment of prostate cancer.

16.2.4 B Vitamins

These EMNs are water soluble and consequently readily excreted. Depletion can occur as a result of the use of high-dose diuretics in the treatment of congestive heart failure (CHF). Thiamine supplementation is recommended for patients with refractory CHF who are unresponsive to diuretics, although its role in heart failure not related to proven thiamine deficiency remains controversial (Sica 2007). There may be a role for thiamine supplementation in preventing the cardiomyopathy sometimes seen with diabetes. A study conducted in the streptozotocin-induced diabetic rat model showed diabetic cardiomyopathy could be prevented by high-dose thiamine (Kohda et al. 2008). Nocturnal cramps and other involuntary leg muscle contractions in patients taking diuretics may

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be related to B vitamin depletion or magnesium deficiency. A small study conducted on 28 elderly patients showed considerable improvement in nocturnal leg cramps when they were treated with vitamin B complex (Chan et al. 1998).

16.2.5 Homocysteine and B Vitamins

A lot of research interest on homocysteine has been aroused since McCully hypothesized that homocysteine could accelerate vascular disease in homocysteinuria, a rare autosomal-recessive deficiency of cystathione β-synthetase (McCully 1969). A connection between high plasma homocysteine levels and occlusive vascular disease, CHD, and CHF has since been confirmed (Selhub et al. 1995). Women may be especially vulnerable to this condition. Homocysteine levels have been found to be higher in CVD patients compared to controls, although the effects were attenuated after adjusting for other risk factors and not all studies have found an association (Ridker et al. 1999; Zee et al. 2007; Pradhan et al. 2008). High homocysteine levels were reported in association with hypertension, resulting in a 25 times higher incidence of stroke in a study of postmenopausal women (Ridker et al. 1999). Supplements of B vitamins decreased homocysteine plasma levels significantly, and this decrease was dose dependent. Doses greater than the EAR doses have been given to patients with hyperhomocysteinemia and documented CHD (Hankey and Eikelboom 1999).

Plasma homocysteine levels are partly genetically determined by a common polymorphism in the enzyme 5,10-methylene tetrahydrofolate reductase (MTHFR), which is involved in the metabolism of folate. About 10% of people are homozygous for a C677T polymorphism in the MTHFR gene that increases homocysteine levels by about 20%. A large meta-analysis found the odds ratio for stroke to be 1.26 (95%; confidence intervals [CI]: 1.14–1.40) for TT versus CC homozygotes, which is in proportion to the difference in homocysteine that can be attributed to the polymorphism (Casas et al. 2005). A study in healthy Japanese men reported that subjects with the TT genotype MTHFR C677T showed the greatest decrease in plasma homocysteine levels with folic acid supplementation, suggesting that a pharmacogenetic preventative approach for atherosclerosis is possible (Miyaki et al. 2005).

High-dose supplementation with a combination of folic acid, vitamin B12, and vitamin B6 slowed the progression of early-stage subclinical atherosclerosis measured by carotid artery intima media thickness (CIMT) compared with placebo ($p = .02$) in well-nourished, healthy, B vitamin–"replete" individuals at low risk for CVD and with a fasting plasma homocysteine ≥ 9.1 μmol/L, but there was no significant treatment effect in subjects with baseline fasting plasma homocysteine < 9.1 μmol/L (Hackam, Peterson, and Spence 2000; Hodis et al. 2009). Similar findings have been reported from other studies. Concomitant treatment with antioxidant vitamins did not improve the response to folic acid in some studies (Title et al. 2000). Folic acid and cyancobalamin together were more efficacious than vitamin B6 in lowering plasma homocysteine (Lee et al. 2004). A decreased rate compared with placebo of exercise electrocardiogram (ECG) tests showing myocardial ischemia (odds ratio: 0.40 [0.17–0.93]; $p = .035$) was found with folic acid and vitamin B6 supplementation for 2 years in high-risk subjects (Vermeulen et al. 2000). The effect of folic acid in improving endothelial function in CHD patients may be independent of decreases in plasma homocysteine (Doshi et al. 2002) and was suggested to be dose dependant (Moat et al. 2006). However, formal clinical trials have not confirmed encouraging early results in the prevention of CVD. There is so far no clear evidence of benefit from B vitamin supplementation on CVD risk reduction (Lonn 2008), including the Vitamins Intervention for Stroke Prevention trial, Heart Outcomes Prevention Evaluation (HOPE) trial, the Cambridge Heart Antioxidant study, the Norwegian Vitamin trial, and the more recent Western Norway B Vitamin Intervention Trial (Toole et al. 2004; Bonaa et al. 2006; Lonn et al. 2006; Ebbing et al. 2008). In HOPE 2, lowering of homocysteine with folic acid and vitamins B6 and B12 did decrease the overall risk for stroke, but not stroke severity or disability (Saposnik et al. 2009). There are a number of trials that are still in progress; until positive results become available, B vitamin supplements at the EAR for adults (folic acid: 400 μg; B6: 2 mg; B12: 6 μg) are considered safe in CVD prevention but not routinely recommended (Lonn 2008).

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16.2.6 Niacin

High-dose niacin (nicotinic acid, vitamin B3) is used in the treatment of hyperlipidemia and hypercholesterolemia to improve the plasma lipid profile and prevent atherosclerosis. Recent studies suggest that niacin additionally improves the vascular endothelial cell redox state, by inhibiting vascular inflammatory genes, oxidative stress, and key cytokines that are involved in atherosclerosis (Ganji et al. 2009). Other studies suggest that high-density lipoprotein (HDL)-cholesterol directly improves endothelial function. Nicotinic acid dose-dependently raises serum HDL cholesterol, lowers triglycerides, and, with higher doses, lowers LDL cholesterol, resulting in a decreased cardiovascular risk score (Poldermans et al. 2008). Extended-release (ER) niacin improved endothelial function in patients with CHD who had low baseline HDL cholesterol but not those with normal HDL cholesterol (Warnholtz et al. 2009). In addition to improving serum lipid concentrations and lipid particle characteristics, treatment with niacin has also been found to improve inflammatory markers in some studies (Kuvin et al. 2006).

Although niacin may increase insulin resistance and blood glucose levels, its beneficial effects on plasma lipids, lipoproteins, and other factors appear to outweigh these potential disadvantages. In a subgroup analysis from the HDL-Atherosclerosis Treatment Study (HATS), similar decreases in the rate of progression of coronary stenosis and in primary clinical events were seen in patients with metabolic syndrome and insulin resistance compared to the overall patient group. In another study, patients with dysglycemia showed less benefit than those with normal glucose levels from treatment with a combination of niacin and a statin for 3 years compared to placebo (Vittone et al. 2007). It is therefore important to monitor glucose levels in patients with diabetes or the metabolic syndrome treated with high doses of niacin. In the Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol-6-HDL and LDL Treatment Strategies in Atherosclerosis (ARBITER 6-HALTS) study, patients with CHD or a CHD risk equivalent on long-term statin therapy were randomized to additional treatment with ER niacin (target dose: 2000 mg/day) or ezetimibe (10 mg/day). The primary end point was the change from baseline in CIMT. The change in CIMT was significantly decreased with niacin but not with ezetimibe (Taylor et al. 2009). The incidence of major cardiovascular events was also lower in the niacin group than in the ezetimibe group (1% vs. 5%; $p = .04$), suggesting niacin may be useful in addition to statins in some patient groups.

16.2.7 Vitamin C

The role of vitamin C supplementation in CVD remains unproved. The antioxidant properties of vitamin C are thought to act synergistically with those of vitamin E, decreasing the formation of peroxyl radicals and blocking lipid peroxidation. Vitamin C also has an action on endothelial vasodilator function in heart failure by increasing available nitric oxide (NO; Frishman 1999), but high doses of vitamin C have also been associated with decreased levels of NO production by endothelial cells (Mikirova, Ichim, and Riordan 2008). One clinical trial reported that vitamin C slowed progression of atherosclerosis in men and women over 55 years of age (Kritchevsky et al. 1995). The elderly and other groups in the population, including males, smokers, diabetics and hypertensives, who are at increased risk of CHD, have been found to have lower-than-average vitamin C blood levels. Women taking oral estrogen contraceptives may also have below-average vitamin C levels. In the Nurse's Health Study, vitamin C supplements were associated with a lower risk of CHD in women (Osganian et al. 2003), and a British study found vitamin C blood levels at entry to be inversely related to death during extended follow-up from all causes, including ischemic heart disease in both sexes (Khaw et al. 2001). The authors concluded that the equivalent of one extra portion of vitamin C–rich food per day as fruit and vegetables could lower the risk of death by 20%. However, in that study, intake of other essential nutrients may have influenced the results. Furthermore, the MRC/BHF Heart Protection Study showed no benefit from vitamin C supplementation (250 mg daily) on morbidity and mortality in high-risk patients with CVD (Heart Protection

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Study Collaborative Group 2002). The WACS also showed no overall effect of ascorbic acid on cardiovascular events among women considered at high risk for CVD (Cook et al. 2007). A similar conclusion came from the Physicians' Health Study II, which showed no benefit from taking 500 mg of vitamin C daily to prevent CVD events (Sesso et al. 2008). The value of vitamin C in established CVD remains unknown.

16.2.8 Vitamin D

Vitamin D receptors are found in vascular smooth muscle (Somjen et al. 2005), the endothelium (Merke et al. 1989), and cardiomyocytes (Holick 2006). There is evidence to suggest an association between low levels of 25-hydoxyvitamin-D and the accelerated development of CVD (Moats and Rimm 2007; Dobnig et al. 2008; Giovannucci et al. 2008; Wallis, Penckofer, and Sizemore 2008). Studies show an inverse relationship between vitamin D levels and plasma renin activity, hypertension, and coronary artery calcification (Resnick, Muller, and Laragh 1986; Zittermann, Schleithoff, and Koerfer 2007). Recent results from the Framingham offspring study suggest a direct association between vitamin D deficiency and the subsequent incidence of CVD (Wang et al. 2008). Low levels of vitamin D have also been linked with the occurrence of fatal strokes (Pilz, Dobnig et al. 2008), heart failure (Moats and Rimm 2007), sudden cardiac death (Pilz, Marz et al. 2008), and calcific aortic stenosis.

The prevalence of CHD was noted to increase with increasing distance from the equator, suggesting that a deficiency of sunlight, and therefore vitamin D, could cause CHD (Fleck 1989). Recent studies also suggest that disproportionately low vitamin D levels may largely explain the higher cardiovascular death rates among black Americans (Fiscella and Franks 2010). Despite these findings, it is not clear what dosage of vitamin D is necessary for the prevention of CVD (Zittermann and Koerfer 2008). Prospective studies need to be performed to test whether vitamin D supplementation can actually prevent CVD.

16.2.9 Vitamin E

A great deal of work has been done in studying tocopherols, the principal lipid-soluble antioxidants found in tissue and plasma. Oxidation of unsaturated fatty acids in LDL particles is widely recognized as a key factor in atherogenesis. Vitamin E blocks the lipid peroxidation chain reaction in the LDL particle (Pryor 2000). Supplements can decrease lipid peroxidation by as much as 40%. Stabilizing plaque, reducing inflammation, platelet aggregation, expression of adhesion molecules on the arterial wall, and enhancing vasodilation are the potential mechanisms of cardioprotection with vitamin E (Pryor 2000). The antioxidant and anticoagulant properties of vitamin E are believed to protect against MI and thrombotic stroke. The value of vitamin E in the prevention of atherosclerosis is the subject of an extensive review article (Pryor 2000).

Prospective controlled clinical trials give a confusing picture. Statistical reanalysis of all data from previous major clinical trials of supplementation suggests that treating patients with preexisting CVD with vitamin E is ineffective (Stephens et al. 1996; Rapola et al. 1997; GISSI-Prevenzione Investigators 1999; Yusuf et al. 2000; Jialal, Traber, and Devaraj 2001; Vivekananthan et al. 2003). The results of the MRC/BHF Heart Protection Study (Heart Protection Study Collaborative Group 2002) on 20,536 high-risk individuals showed no benefit from vitamin E supplementation (600 mg daily) on morbidity and mortality. However, 100 and 200 mg of vitamin E caused a marked improvement in arterial compliance (Rasool et al. 2008), and a recent report from the Women's Health Study showed that women receiving supplements of vitamin E had a lower risk of venous thromboembolic disease (Glynn et al. 2007). The WACS tested the effects of ascorbic acid (500 mg/day), vitamin E (600 IU every other day), and β-carotene (50 mg every other day) on the combined outcome of MI, stroke, coronary revascularization, and CVD death among 8171 female health professionals and found no significant overall benefits (Cook et al. 2007). In addition, the

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Physicians' Health Study II revealed no cardiovascular benefits from supplements of vitamin E at doses of 400 IU on alternative days when administered to 14,641 male physicians over 50 years of age (Sesso et al. 2008). The National Centre for Complementary and Alternative Medicine (NCCAM) is currently studying the effect of α-tocopherol supplements (1200 IU/day) on the progression of carotid atherosclerosis in patients with CHD (stable angina pectoris or previous MI) in a placebo-controlled, randomized, double-blind trial over 2 years. Despite the large number of studies conducted with vitamin E, proof of its value in healthy individuals and in those with CVD has not been obtained as yet.

An explanation for the disappointing clinical results with vitamin E may come from a recent study showing that the α isomer (found in supplements) suppresses the gamma isomer (found in dietary foods). A standardized preparation of the gamma isomer, when and if available, merits clinical study (Gutierrez et al. 2009).

16.2.10 Flavonoids

The traditional French diet is high in saturated fats, but residents of France have a lower incidence of CAD than Americans. This is the so-called French paradox. The typical French diet includes regular intake of fresh fruit and vegetables. These contain phytonutrients that may lower peroxidative tendencies and retard atherogenesis and thrombosis. Consumption of red wine could be another factor. Ethyl alcohol in small quantities may help to prevent cardiovascular or cerebrovascular disease (Frishman et al. 2003). In healthy men, both cava (sparkling wine) and gin, although to a lesser extent, have been shown to decrease inflammatory markers of atherosclerosis (Vazquez-Agell et al. 2007). However, the rich polyphenolic content of red wine (and green tea) has made both of them popular choices for possible cancer and CVD prevention (Ullah and Khan 2008). A study of the antioxidant activity of red wine in volunteers showed that two glasses of red wine taken before food increase serum antioxidant activity for at least 4 hours (Maxwell, Cruickshank, and Thorpe 1994). Red wine increases antioxidant activity through a flavonoid–polyphenol effect. A small study performed in the Netherlands (Hertog et al. 1993) studied the use of the dietary bioflavonoids, phenolic acids, and quercetin. There was a decrease in the incidence of heart attack and sudden death over 5 years in relation to increasing tertiles of flavonoid (quercetin, kaempferol, myricetin, apigenin, and luteolin) intake assessed by dietary history. Quercetin-rich black tea and apples and onions were considered the most suitable foods studied, since they contain polyphenols in amounts similar to those found in red grapes. Short- and long-term consumption of black tea was found to reverse endothelial vasomotor dysfunction but not to decrease ex vivo platelet aggregation in patients with CHD (Duffy, Keaney et al. 2001; Duffy Vita et al. 2001).

Resveratrol, which is found in grape skin and seeds, activates platelet NO synthase and inhibits production of reactive oxygen species and platelet activation. This may explain the beneficial effects of moderate wine intake on ischemic CVD (Gresele et al. 2008). The potential beneficial effects of resveratrol were reviewed recently (Bertelli and Das 2009). It has been suggested that resveratrol acts as an antiaging compound, induces the expression of several longevity genes, and prevents aging-related decline in cardiovascular function (Das, Mukherjee, and Ray 2010), but this has not been confirmed by intervention trials.

Oligomeric proanthocyanidins are free-radical scavengers that inhibit lipid peroxidation and have anti-inflammatory and antiallergenic properties. Like carotenoids, they are found predominantly in brightly colored fruits and vegetables. These foods represent a safe source of polyphenols and quercetin, which is believed to be particularly active in preventing LDL oxidation. In a placebo-controlled crossover study of supplementation with quercetin at 150 mg daily for 6 weeks in overweight or obese subjects with metabolic syndrome traits, there was a decrease in systolic blood pressure by 2.6 mmHg ($p < .01$) and a decrease in plasma concentrations of atherogenic oxidized LDL; there was also a small but significant decrease in serum HDL cholesterol concentrations ($p < .001$), although the ratio LDL:HDL cholesterol was unchanged (Egert et al. 2009).

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The optimal amount and form of flavonoids in the diet is not known, and some of these flavonoids have rather low bioavailability. Despite the uncertainties, many individual flavonoids are available as food supplements in doses as high as 500 and 1000 mg, and amount to 10–20 times the daily intake of a typical vegetarian diet. The Physician's Health Study did not show any association between intake of flavonoids and all CAD events (Rimm et al. 1996). The Kuopio Ischemic Heart Disease Risk Factor Study concluded that a high intake of flavonols and the mean CIMT were negatively associated (Mursu et al. 2007). Other studies are in progress (Frishman, Beravol, and Carosella 2009). Until the results of prospective controlled studies are available, patients and those at risk of CVD may be encouraged to include tea, apples, and onions in their diet, but current research does not support the use of supplemental flavonoids.

16.2.11 Flavanol-Rich Cocoa

There is evidence from short-term in vitro and in vivo studies of a cardioprotective effect of cocoa and chocolate (Hollenberg and Fisher 2007; Mehrinfar and Frishman 2008). They are suggested to have antioxidant properties, anti-inflammatory properties, antiplatelet aggregation, and antihypertensive effects (Cohen and Townsend 2007; Flammer et al. 2007), and to improve vascular function. Evidence for the cardiovascular benefits of cocoa flavanols comes largely from short-term and uncontrolled studies, and additional well-designed long-term clinical trials of cocoa are required (Mehrinfar and Frishman 2008). A recent study from Germany with follow-up of 19,357 participants over 8 years, as part of the European Prospective Investigation into Cancer and Nutrition, found a significant decrease in the risk of the combined outcome of first MI or stroke in people in the top quartile compared to those in the bottom quartile of chocolate consumption (Buijsse et al. 2010). This appeared to be in part due to lower blood pressure in those with higher chocolate consumption.

16.2.12 Vitamin K

Insufficient vitamin K in the diet has been associated with an increased risk of soft-tissue calcification and atherosclerosis (Erkkila and Booth 2008). In animal models, the multiple forms of vitamin K have been found to reverse the arterial calcification caused by vitamin K antagonists, and it has also been reported that matrix Gla protein (MGP) may act as a vitamin K–dependent calcification inhibitor. A study of phylloquinone (vitamin K1) 500-μg daily supplementation compared with a multivitamin in older men and women showed in a subgroup analysis of participants with good adherence to therapy (≥85% adherent) that there was less progression of coronary artery calcification in the phylloquinone group than in the control group ($p = .03$; Shea et al. 2009). These data should be considered only as hypothesis-generating data, and further studies are warranted. Vitamin K supplementation might prove useful in preventing bone and vascular disease in patients with chronic kidney disease or end-stage renal disease (Krueger et al. 2009).

16.2.13 Magnesium

A high intake of magnesium, potassium, and calcium from an increased consumption of fruit and vegetables may lower blood pressure and decrease CAD and stroke (Houston and Harper 2008). The epidemic-like increase in diabetes and associated CVD in Pacific Islanders was found to coincide with a change in diet from traditional fish and fruit to a Western-style diet with high fat, high carbohydrate, and low magnesium intake, among other things (Ringrose and Zimmet 1979). Magnesium deficiency has been found to induce vasoconstriction and enhance vascular endothelial injury, thereby promoting the development and progression of atherosclerosis. A relationship between low magnesium concentration in serum at 48 hours after onset of ischemic stroke and the intensity of the resulting neurological deficit has been reported (Cojocaru et al. 2007). For

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angina resulting from coronary artery spasm, treatment with magnesium has been found efficacious (McLean 1994; Shechter et al. 2000). Magnesium status is better measured by mononuclear white blood cell magnesium levels than blood levels. Low levels have been found to predispose patients with acute MI to excess mortality and morbidity.

Studies show an association between intravenously administered magnesium supplementation during the first hour of admission for MI and decreases in morbidity and mortality. The multiple physiological and cardioprotective activities of magnesium include antiarrhythmic effects, calcium channel–blocking effects, improvement in NO release from coronary endothelium, and inhibition of blood coagulation (Shechter et al. 1999). Intravenously administered magnesium has been reported to be effective in preventing atrial fibrillation and ventricular arrhythmias following cardiac and thoracic surgery; in decreasing ventricular response in acute-onset atrial fibrillation (including in patients with Wolff–Parkinson–White syndrome); and in the treatment of digoxin-induced supraventricular and ventricular arrhythmias, multifocal atrial tachycardia, and polymorphic ventricular tachycardia or ventricular fibrillation resulting from drug overdoses. Although intravenously administered magnesium is not of value in monomorphic ventricular tachycardia and electroshock-resistant ventricular fibrillation, it may be useful as an adjunct to digoxin in controlling ventricular response in new-onset atrial fibrillation. (Ho, Sheridan, and Paterson 2007; Ho 2008).

Studies show other benefits of magnesium in various cardiovascular conditions, including antiplatelet effects (Shechter et al. 1999), ability to relieve symptoms of mitral valve prolapse (Lichodziejewska et al. 1997), and ability to improve left ventricular function in patients with stable CHD (Pokan et al. 2006). Magnesium sulfate is commonly used for prevention of eclamptic seizures. The specific mechanisms of action remain unclear but may include cardiovascular effects of peripheral and cerebral vasodilation, as well as blood–brain barrier protection and anticonvulsant effects (Euser and Cipolla 2009). However, supplemental magnesium and potassium should be avoided in renal insufficiency. Additional studies are needed to improve our understanding of a link between magnesium intake, indicators of magnesium status, and heart disease.

16.2.14 Iron

The importance of iron to health is well known, and there is increasing interest in identifying and treating iron deficiency in cardiac failure. Debate has been going on regarding whether erythropoietin (EPO) is needed in addition to iron to improve symptoms and exercise capacity in anemic heart failure patients. In one study of anemic patients (Hb ≤ 12 g/dL) with stable CHF, intravenous injections of iron sucrose alone over 12 days increased hemoglobin, decreased symptoms, and improved exercise capacity (Bolger et al. 2006). In patients with heart failure of New York Heart Association (NYHA) functional class II or III decreased left ventricular ejection fraction (≤40%) and iron deficiency, treatment with intravenous iron (ferric carboxymaltose) resulted in great improvements in self-reported symptoms and objective assessments of heart failure in the ferric carboxymaltose–treated group (Anker et al. 2009). Results were similar in patients with or without anemia, and mortality, adverse events, and serious adverse events were similar in the active treatment and placebo groups. This trial had several limitations, such as a high dropout rate, and it is not known if oral iron replacement might provide similar benefits or if long-term benefits may be seen (Dec 2009). Nonetheless, it does seem appropriate to assess iron status in patients with CHF and to give some effective form of iron replacement in those with iron deficiency. Conversely, as in patients with thalassemia, excessive iron deposition in the heart can cause cardiomyopathy, heart failure, and cardiac arrhythmias.

16.2.15 Trace Minerals

Trace amounts of cobalt are essential to health and are involved in the formation of vitamin B12 (hydroxocobalamin). Excessive intake can result in thyroid insufficiency, and in 1966 the syndrome

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"beer drinker's cardiomyopathy" appeared in Quebec City, Canada, characterized by pericardial effusion, elevated hemoglobin levels, and CHF (Barceloux 1999).

Chromium is important in glucose and lipid metabolism, and it may help in regression of cholesterol-induced atherosclerosis. In a study of 40 hypercholesterolemic patients, a combination of 200 μg of chromium polynicotinate and grape-seed extract (proanthocyanidin) dosed at 100 mg twice daily resulted in decreases in LDL and total cholesterol (Preuss et al. 2000). There was no significant change in either HDL cholesterol or triglyceride levels in the treatment or placebo group. Since insulin resistance may be a major factor in disturbed lipid metabolism, chromium's favorable action on glucose/insulin metabolism may be a key factor in improving the lipid profile; however, a review of published studies concluded there are benefits to glucose and insulin metabolism only in diabetic patients and there are no overall effects on lipids (Balk et al. 2007). Although no significant adverse reactions have been noted from chromium polynicotinate doses of 400 μg/day, a high dose of chromium picolinate has been associated with renal failure (Wasser, Feldman, and D'Agati 1997).

Selenium is an essential mineral and an antioxidant with immune-enhancing and anticancer properties. It is a cofactor of the enzyme glutathione peroxidase, an antioxidant found in platelets and the arterial wall. Soil deficiencies of selenium can lead to Keshan disease, a multifocal myocardial necrosis, with cardiomyopathy, CHF, and cardiac arrhythmias. A subsidiary report from the Physician's Health Study showed no relationship between selenium blood levels and the risk of MI in well-nourished subjects (Salvini et al. 1995). The reliability of plasma selenium level measurements has been questioned, and other more accurate methods have been proposed (Kok et al. 1989). Primary prevention trials and most secondary prevention studies suggest that supplementation does not result in any significant decrease in ischemic CVD events (Hercberg et al. 2004). Current evidence is insufficient to support a beneficial role for selenium in cardiovascular prevention, but it is considered safe at doses below 200 μg, although people living in regions with high selenium intake may be at risk of diabetes and hypercholesterolemia from taking selenium supplements (Navas-Acien, Bleys, and Guallar 2008). Excessive selenium can also result in alopecia, abnormal nails, emotional lability, lassitude, and a garlic odor to the breath (Kendler 1997).

16.2.16 L-Carnitine

In one study, L-carnitine was used as an adjunct therapy to percutaneous coronary intervention (PCI) for non-ST elevation acute coronary syndrome (NSTEMI). This resulted in decreased levels of cardiac markers, suggesting some prevention of cardiac damage, although this result needs to be confirmed (Xue et al. 2007). In a combination with α-lipoic acid, acetyl-L-carnitine was suggested to lower blood pressure (McMackin et al. 2007), but at present, there is no definitive evidence for cardiovascular benefits.

16.2.17 Omega-3 Polyunsaturated Fatty Acids

Ever since a very low incidence of CHD in Greenland Eskimos was reported (Bang, Dyerberg, and Nielsen 1971), which was believed to be related to the high intake of seafood containing long-chain omega (n)-3 polyunsaturated fatty acids (n-3 PUFAs), many studies in other populations have supported the theory that marine n-3 PUFAs protect against thrombosis, atherosclerosis, and CHD. Some prospective cohort studies and randomized control trials (RCTs) in secondary prevention have indicated that consuming fish or fish oil containing the n-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is associated with decreased cardiovascular death rate, whereas consumption of the vegetable oil–derived n-3 fatty acid a-linolenic acid is not as effective (Breslow 2006); however, the results of studies are not all consistent. One systematic analysis concluded that long-chain and short-chain omega-3 fats do not have a clear effect on total mortality, combined cardiovascular events, or cancer (Hooper et al. 2006). Various studies show that doses

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>3 g/day of EPA plus DHA can improve many CVD risk factors, including lowering of plasma triglycerides, blood pressure, platelet aggregation, and inflammation, and improvement of vascular reactivity, but it has been considered that the therapeutic effects are more likely due to suppression of fatal arrhythmias than due to stabilization of atherosclerotic plaques (Breslow 2006).

A recent systematic review and meta-analysis on the effects of fish oil DHA and EPA on mortality and arrhythmias, which examined 12 studies totaling 32,779 patients, concluded that fish oil supplementation was associated with a significant decrease in deaths from cardiac causes, although it had no effect on arrhythmias or all-cause mortality (Leon et al. 2008). The optimal formulations for EPA and DHA remain unclear (Leon et al. 2008). Most recently, a large study from Denmark that followed 57,053 middle-aged men and women for 7.6 years found that a modest intake of fatty fish was associated with a lower risk of acute coronary syndrome (ACS) with benefits seen for intakes >6 g of fatty fish per day; but no obvious additional benefit for higher intakes and no benefit from intake of lean fish were seen (Bjerregaard et al. 2010). There were fewer cases of ACS in women and no consistent associations with fish intake were observed. Current recommendations from the American Heart Association are that everyone should eat oily fish twice a week for primary prevention and that people with established CHD should take 1 g/day of EPA and DHA from oily fish or supplements (Pearson et al. 2002; Smith et al. 2006).

16.2.18 Coenzyme Q10

Coenzyme Q10 (ubiquinone) has a number of important physiological functions and has been used in oral forms to treat various cardiovascular disorders including angina pectoris, hypertension, and CHF; but the evidence to support beneficial effects of this coenzyme is still not available (Greenberg and Frishman 1990). It may have a role in the myalgia or myopathy associated with statin treatment or in cardiomyopathy with or without statins. However, this assumption remains to be confirmed (Silver et al. 2003; Wolinsky 2007; Chatzizisis, Vaklavas, and Giannoglou 2008; Joy and Hegele 2009).

16.2.19 Summary

Despite the extensive literature suggesting the benefits of antioxidant vitamins in observational studies, the results of interventional studies have largely been disappointing. There may still be a role for supplementation with folic acid, vitamin B12, and vitamin B6 to decrease homocysteine levels in stroke prevention, and recent data with vitamin D and CVD provides new opportunities for further research. Niacin clearly has beneficial effects on plasma lipids when given in pharmacological doses, and although n-3 PUFAs are suggested to have various benefits, their exact role in prevention and treatment of CVD still needs to be defined more clearly. Flavonoids such as resveratrol and flavanol-rich cocoa and chocolate appear to have beneficial effects that may be through specific mechanisms rather than a general antioxidant activity, and these merit further investigations. Other herbal medicines also have ingredients with specific pharmacologic effects that influence CVD, and these are discussed in Sections 16.3.1 through 16.3.9.

16.3 HERBAL MEDICINES AND CARDIOVASCULAR DISEASE

A wide variety of plant extracts have been used in traditional medicine over the centuries and some, such as digoxin, have been adopted in conventional medicine. In this section, we concentrate on those plants and herbs for which there is some evidence, if not final proof, supporting their value in the prevention or treatment of CVD. More detailed reviews can be found elsewhere in the literature (Mashour, Lin, and Frishman 1998; Frishman, Beravol, and Carosella 2009).

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16.3.1 Hawthorn (*Crataegus* Species)

Crataegus encompasses many species believed to be valuable in treating CVDs, particularly angina, heart failure, and hyperlipidemia (Chang et al. 2002). The leaves, flowers, and fruits of *Crataegus* species contain varying amounts of a number of biologically active substances, such as oligomeric procyanins, flavonoids, and catechins. The extract is suggested to have antioxidant properties, and inhibits the formation of thromboxane (Bahorun et al. 1994; Vibes et al. 1994). In traditional Chinese medicine (TCM), the fruit of the hawthorn (usually *Crataegus pinnatifida*; known as *shanzha*) is widely used for many indications, including digestive disorders and for lowering cholesterol and blood pressure (Chang et al. 2002). *C. oxyacantha* berry extract antagonized dietary-induced increases in cholesterol, triglycerides, and phospholipid levels in LDL fractions and very-low-density lipoprotein fractions in rats (Shanthi et al. 1994). Thus, it could inhibit the progression of atherosclerosis. This hypocholesterolemic action may be due to an upregulation of hepatic LDL receptors resulting in a greater influx of plasma cholesterol into the liver. A similar extract also increased degradation of cholesterol to bile acids and suppressed cholesterol biosynthesis (Rajendran et al. 1996). Extracts of *Crataegus* have been reported to have cardioprotective effects without affecting coronary blood flow in isolated perfused hearts; they have also been reported to have simultaneous positive cardiac inotropic and vasodilator actions (Blesken 1992; Nasa et al. 1993). A multicenter, placebo-controlled, double-blind study of *Crataegus* special extract WS 1442 was found to improve cardiac performance determined by heart rate product (systolic blood pressure × heart rate) in patients with NYHA class II heart failure (Weikl et al. 1996). One possible mechanism of action of flavonoids from *Crataegus* is the inhibition of 3′,5′-cyclic adenosine monophosphate phosphodiesterase (Schussler, Holzl, and Fricke 1995).

Hawthorn is relatively free from adverse effects (Daniele et al. 2006). In comparison with other inotropic agents such as epinephrine, amrinone, milrinone, and digoxin, *Crataegus* has the potential to lower arrhythmogenic risk because it prolongs the effective refractory period (Joseph, Zhao, and Klaus 1995; Popping et al. 1995). The concomitant use of hawthorn with cardiac glycosides can markedly enhance their activity; therefore, care must be taken (Mashour, Lin, and Frishman 1998). The use of *Crataegus* in CVD may seem promising, but more studies are needed to confirm the efficacy and safety of hawthorn extracts. However, some recent studies performed on the effects of *C. oxycantha* special extract WS 1442 in patients with heart failure, including the Survival and Prognosis: Investigation of *Crataegus* Extract WS 1442 in CHF (SPICE) trial, showed no significant benefits (Holubarsch et al. 2008; Zick, Gillespie, and Aaronson 2008).

16.3.2 Garlic (*Allium sativum*)

For centuries, garlic has been valued for its medicinal properties. As an herbal medicine, it has been more closely examined than many other herbs. Research focuses on garlic for preventing atherosclerosis. Multiple beneficial cardiovascular effects have been found, including lowering of blood pressure, inhibition of platelet aggregation, enhancement of fibrinolytic activity, lowering of cholesterol and triglyceride levels, and protection of the elastic properties of the aorta (Rahman and Lowe 2006).

The intact cells of garlic bulbs contain an odorless sulfur-containing amino acid, allinin. When garlic is crushed, allinin comes into contact with allinase, which converts allinin to allicin. This has potent antibacterial properties and is highly odoriferous and unstable. Ajoenes are the self-condensation products of allicin and are suggested to be responsible for garlic's antithrombotic action. Most authorities now agree that allicin and its derivatives are the bioactive constituents of garlic. Fresh garlic releases allicin in the mouth while chewing. Dried garlic preparations lack allicin but contain both allinin and allinase. Since allinase is inactivated in the stomach, dried garlic preparations should have an enteric coating so that they pass unaltered through the stomach to

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the small intestine, where allinin is enzymatically converted to allicin. Only a few commercially available garlic preparations are standardized for their yield of allicin based on the allinin content (Mashour, Lin, and Frishman 1998).

The consumption of large quantities of fresh garlic (0.25–1.0 g/kg or about 5–20 average-sized 4 g cloves) has been found to produce the aforementioned beneficial effects (Kleijnen, Knipschild, and ter Riet 1989). In support of this, a double-blind, crossover study of moderately hypercholesterolemic men, which compared the effects of 7.2 g of aged garlic extract with placebo on blood lipid levels, found a maximal decrease of 6.1% in total serum cholesterol levels and 4.6% in LDL cholesterol levels with garlic (Steiner et al. 1996). However, despite the positive evidence from a number of trials, full endorsement of garlic for CVD prevention is not currently possible. Many published studies have methodological shortcomings (Kendler 1987; Kleijnen, Knipschild, and ter Riet 1989; Jain et al. 1993; Silagy and Neil 1994b; Neil et al. 1996; Isaacsohn et al. 1998). Trials were small, lacked statistical power, had inappropriate methods of randomization, lacked dietary run-in periods, were of short duration, or failed to undertake intention-to-treat analysis. This has led to a cautious approach to previous meta-analyses (Neil et al. 1996). One more recent meta-analysis concludes that garlic decreases total cholesterol to a modest extent, an effect driven mostly by the modest decreases in triglycerides, with no appreciable effect on LDL or HDL cholesterol (Reinhart et al. 2009).

Garlic has also been studied in hypertension with no conclusive result (Simons, Wollersheim, and Thien 2009). A meta-analysis of eight trials suggested some clinical value in patients with mild hypertension, but the evidence was insufficient to recommend garlic for routine clinical therapy (Silagy and Neil 1994a). Garlic has been reported to show antiplatelet stickiness activity. This has been documented in vitro (Bordia, Verma, and Srivastava 1996), and another study examined the effect of consuming a clove of fresh garlic on platelet thromboxane production. After 26 weeks, serum thromboxane levels were lowered by about 80% (Ali and Thomson 1995). Thus, garlic may prove to be of benefit in the prevention of thrombosis. Another trial showed that long-term intake of standardized garlic powder at 300 mg daily for more than 2 years improved the elastic properties of the aorta (Breithaupt-Grogler et al. 1997). In these ways, garlic is beneficial to cardiovascular health, and these effects need further study. Moderate garlic consumption causes few adverse effects other than bad odor. However, with consumption of more than five cloves daily, heartburn, flatulence, and other gastrointestinal disturbances have been reported. Allergic contact dermatitis was also reported, and patch testing is available when garlic allergy is suspected (Delaney and Donnelly 1996). Due to its antithrombotic activity, garlic should be taken with caution by people on oral anticoagulants (Rose et al. 1990).

16.3.3 Danshen (*Salvia miltiorrhiza*)

The dried root of *S. miltiorrhiza*, known as *danshen* in TCM, is widely used in China for the treatment of angina pectoris, hyperlipidemia, and acute ischemic stroke (Zhou, Zuo, and Chow 2005). It has a range of potentially beneficial effects, including improving microcirculation, causing coronary vasodilatation, suppressing the formation of thromboxane, inhibiting platelet adhesion and aggregation, and protecting against myocardial ischemia. Danshen is widely used either alone or in combination with other herbal ingredients for patients with CAD and other CVDs (Cheng 2007). Clinical studies in ischemic stroke have various methodological problems and, therefore, reliable conclusions cannot be drawn from them (Wu, Liu, and Zhang 2007). Further high-quality randomized controlled trials should be performed. A more recent review of randomized controlled trials of danshen in ischemic vascular disease published in mainland China identified 150 trials from 1998 to 2007, but concluded that the quality of these trials has not improved significantly over recent years and the overall quality is still poor (Yu et al. 2009). Thus, although the mechanistic studies look promising, better clinical trials are needed to assess the efficacy and safety of this herb.

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It has been demonstrated that there is an interaction between *S. miltiorrhiza* and warfarin in rats (Chan et al. 1995), and there are several case reports of increased anticoagulation or hemorrhage (Chan 2001). The interaction seems to be mainly due to an inhibitory effect of danshen on warfarin metabolism through cytochrome P450 (CYP) 2C9 (Tomlinson et al. 2000), but there may also be a pharmacodynamic interaction.

16.3.4 Lingzhi (*Ganoderma lucidum*)

G. lucidum, or lingzhi, is a woody mushroom and a popular medicinal herb that is widely used in China and many other Asian countries to promote good health and longevity (Yuen and Gohel 2005; see also Chapter 9 on lingzhi). Many potential beneficial effects, including immunomodulation and anticancer activity, have been attributed to lingzhi (Boh et al. 2007). The active constituents include polysaccharides and oxygenated triterpenoids, which have a broad range of biological activities and pharmacological functions (Shiao 2003). Although it is not typically used for treating CVD, it does appear to have some benefits on the cardiovascular system. In vitro studies with certain extracts have reported effects including inhibition of cholesterol synthesis (Komoda et al. 1989), lowering of blood pressure by reducing sympathetic outflow from the central nervous system (Lee and Rhee 1990), and antioxidant effects (Zhu et al. 1999; Lee et al. 2001; Lai et al. 2006). Animal studies also suggest it may have hypoglycemic effects (Hikino et al. 1989; Zhang and Lin 2004; Seto et al. 2009).

16.3.5 Maidenhair Tree (*Ginkgo biloba*)

The *Ginkgo* species of tree has existed on Earth for over 200 million years. The root and kernels of *G. biloba* are widely used in TCM. A concentrated extract of *G. biloba* leaves was developed in the West in the 1960s. *G. biloba* extract (GBE) contains flavonoids that decrease capillary permeability and fragility and are free-radical scavengers, and terpenes (i.e., ginkgolides) that inhibit platelet-activating factors and decrease vascular resistance, thereby improving circulatory flow without appreciably affecting blood pressure (McKenna, Jones, and Hughes 2001; Koch 2005). There has been some support for the use of GBE in treating cerebral insufficiency and its symptoms of vertigo, tinnitus, memory loss, and mood disorder, but a recent placebo-controlled study of GBE administered at 120 mg twice daily found no effect on cognitive decline in older adults with normal cognition or with mild cognitive impairment (Snitz et al. 2009). Peripheral vascular disease and diabetic retinopathy are also potential conditions for treatment, but most recent reviews suggest the evidence for potential benefits is inconclusive (Birks and Grimley Evans 2009; Nicolai et al. 2009; Perez 2009; Snitz et al. 2009). Some early studies suggest beneficial effects in intermittent claudication for the standardized extract of *G. biloba*, EGb 761, (Mouren, Caillard, and Schwartz 1994), but the most recent analysis of the Cochrane database of systematic reviews found no evidence that *G. biloba* has a clinically significant benefit for patients with peripheral arterial disease (Nicolai et al. 2009).

The bioequivalence of various GBE products has not been established. Adverse effects with GBE are rare but have included gastrointestinal disturbances, headache, and allergic skin rash (Mahadevan and Park 2008). Although there is some concern that GBE may potentiate the effects of anticoagulant or antiplatelet drugs, there is very little evidence to support this assumption (Bone 2008).

16.3.6 Foxglove (*Digitalis purpurea/lanata*)

Potent cardioactive glycosides (digitalis, digitoxin, and digoxin) have been used in CHF for many years. Their treatment value is limited by a low therapeutic index, that is, the dose needing careful adjustment for each patient. Standardization of powdered digitalis, digitoxin, or digoxin is essential for safe and effective use. There are many other plant sources of cardiac glycosides, including

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Convallaria majalis (lily of the valley, convallaria), *Helleborus niger* (black hellebore), *Nerium oleander* (oleander), *Plumeria rubra* (frangipani), *Strophanthus hispidus* and *S. kombe* (strophanus), *Thevetia peruviana* (yellow oleander), and *Urginea maritima* (squill), to name but a few, and the venom of the cane toad (*Bufo marinus*) also contains cardiac glycosides (Mashour, Lin, and Frishman 1998). The skin and venom glands of the Chinese toads *Bufo gargarizans* and *B. melanostictus* is used in a TCM called *chansu* and a proprietary Chinese medicine called Lu Shen Wan. These contain bufotoxins, which have a digoxin-like effect and may cause toxicity when taken in excessive doses (Tomlinson et al. 2000). Some other herbal remedies such as Siberian ginseng (*Eleutherococcus senticosus*) may cause apparent increases in digoxin levels (McRae 1996). Reports of accidental poisonings and even suicide attempts with cardiac glycosides are frequent, and oleander species are often involved (Safadi et al. 1995; Eddleston and Warrell 1999; Davies and Mayne 2001; Fonseka et al. 2002; Rajapakse 2009). The use of digoxin in heart failure has gradually declined since the Digitalis Investigation Group study showed that digoxin did not reduce overall mortality in heart failure patients (The Digitalis Investigation Group 1997). However, it is still commonly used to control the heart rate in atrial fibrillation.

16.3.7 Ginseng (*Panax* Species)

Ginseng has been used medicinally in East Asian countries for thousands of years as an adaptogen and a tonic (see also Chapter 8 on ginseng). The two species that have been the most extensively researched are *Panax ginseng* (Asian ginseng) and *P. quinquefolius* (American ginseng). The name *Panax* is derived from the Latin word "panacea," which illustrates the usage of this herb for a wide range of conditions. *P. ginseng* and *P. notoginseng* are used in TCM for hemostasis and the treatment of patients with angina and CAD (Mashour, Lin, and Frishman 1998). The mode of action here may be as a calcium ion channel antagonist in vascular tissues, which may result in a lowering of blood pressure (Kwan 1995). In vitro studies of *P. notoginseng* show an enhancement of blood fibrinolytic parameters (Zhang, Wojta, and Binder 1994). Inhibition of atherogenesis by *P. notoginseng* saponins through decreased proliferation of smooth muscle cells (Lin et al. 1993), and dilatation of coronary arteries in rabbit tissue in vivo (Han 1992) suggest its possible use in angina. However, a systematic review of ginseng treatment in CVD concluded that there is insufficient evidence for this herb's efficacy (Buettner et al. 2006). The evidence for its benefits in treating diabetes is more convincing (Vuksan and Sievenpiper 2005; Xie, McHendale, and Yuan 2005; Ma et al. 2008; Vuksan et al. 2008). American ginseng (*P. quinquefolius*) appears to attenuate hyperglycemia by a variety of mechanisms that are not yet fully understood (Luo and Luo 2009).

16.3.8 Other Herbal Medicines

Many other herbal materials have been used for treating cardiovascular conditions. They have not been studied to the same extent as the ones listed here, although some did show demonstrable effects. For hyperlipidemia, the herbal extract from the resin of the *Commiphora mukul* or mukul myrrh tree, known as guggul, is widely used in Asia based on Indian Ayurvedic medicine. The presumed bioactive compounds, guggulsterones, are suggested to antagonize the farnesoid X receptor (FXR) involved in controlling cholesterol metabolism (Deng 2007). A short-term safety and efficacy study of a standardized guggul extract (guggulipid, containing 2.5% guggulsterones) in healthy adults with hyperlipidemia showed no improvement of serum lipids, and there was a dermatologic hypersensitivity reaction in some patients (Szapary et al. 2003). More promising effects were seen in rats with diabetes induced by a high-fat diet (Sharma et al. 2009).

Extracts of Chinese red yeast rice (*Monascus purpureus*) contain several active ingredients, including lovastatin, which can lower LDL cholesterol (Lin, Li, and Lai 2005; Huang et al. 2007; Gheith et al. 2009). These preparations appear to be safe in moderate doses, but they may not be

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TABLE 16.2
Potential Applications for Therapy in Cardiovascular Conditions of Some Common Herbal Medicines

Herb	Possible Cardiovascular Indications
Hawthorn (*Crataegus* species)	Heart failure, angina, hyperlipidemia
Garlic (*Allium sativum*)	Hypertension, hyperlipidemia, antithrombotic
Danshen (*Salvia miltiorrhiza*)	Angina, ischemic stroke, hyperlipidemia, antithrombotic
Lingzhi (*Ganoderma lucidum*)	Hyperlipidemia, hypertension, diabetes
Ginkgo (*Ginkgo biloba*)	Cerebral insufficiency, peripheral vascular disease, antithrombotic
Foxglove (*Digitalis* species)	Heart failure, atrial fibrillation
Ginseng (*Panax* species)	Angina, hypertension, diabetes

standardized well and are likely to have the same side effects and drug interactions as lovastatin when taken in large amounts.

As suggested with ginkgo, extracts of rosemary may have benefits in attenuating cognitive decline from cerebral insufficiency, but this remains unproved (Kennedy and Scholey 2006). Extracts of rosemary (*Rosmarinus officinalis*) do appear to have antiproliferative, antioxidant, and anti-inflammatory properties in various cell line studies (Cheung and Tai 2007).

The component tetrandrine isolated from *Stephania tetrandra* has antihypertensive and antiarrhythmic effects that have been demonstrated in experimental hypertensive animals and in hypertensive patients (Qian 2002). These effects come to action mainly through a calcium antagonistic effect, but other pharmacological mechanisms may also be involved. Rauwolfia preparations and veratrum alkaloids are mainly of historical interest in hypertension treatment (Moser 1986).

Extracts of horse chestnut (*Aesculus hippocastanum*) have been used in the treatment of chronic venous insufficiency, and they were found to be safe and well tolerated, with some beneficial effects in one study (Dickson et al. 2004). Extracts from Butcher's broom rhizome (*Ruscus aculeatus*) have also been widely used for the treatment of chronic venous insufficiency with some favorable reports (Vanscheidt et al. 2002).

16.3.9 Summary

Overall, many of the herbal medicines discussed here do appear to have pharmacological effects in vitro and in animal studies, which may influence CVD (Table 16.2). However, the evidence from properly conducted clinical trials is generally insufficient to draw definitive conclusions. The problems with standardization of herbal preparations and performance of properly controlled clinical trials to acceptable international standards need to be addressed before the true clinical value of these herbs can be defined.

16.4 HERBAL MEDICINE AND DRUG INTERACTIONS

Herbal medicines are frequently used in combination with conventional drugs, and interactions are likely to be more common than those that manifest clinically. Herb–drug interactions have been extensively reviewed in the literature (Hu et al. 2005; Izzo 2005; Izzo and Ernst 2009), and some of the common ones are mentioned in Sections 16.3.1, 16.3.2, 16.3.3, 16.3.5. Pharmacokinetic interactions mediated by drug-metabolizing enzymes or transporters are involved in many herb–drug interactions. Polymorphisms in the genes for these enzymes and transporters may influence the interactions mediated through these pathways (Tomlinson, Hu, and Lee 2008).

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Herb–drug interactions are likely to be more serious with drugs having a narrow therapeutic index, such as warfarin or digoxin. Herbs can interact with warfarin in several different ways (Greenblatt and von Moltke 2005). The interaction with danshen is mentioned in Section 16.3.3 and probably occurs by inhibition of warfarin metabolism through CYP2C19. St. John's wort (*Hypericum perforatum*; see also Chapter 11 on St. John's wort) interacts with warfarin and a number of other drugs by inducing the expression of several CYP enzymes and the drug transporter P-glycoprotein (P-gp or ABCB1), resulting in decreased plasma concentrations of the drugs involved and lowered efficacy (Borrelli and Izzo 2009). Digoxin is a substrate for P-gp, and St. John's wort can lower digoxin levels by increasing the activity of this transporter (Izzo 2004).

The finding that grapefruit juice substantially increased the plasma concentrations of felodipine by decreasing presystemic metabolism through selective post-translational downregulation of CYP3A4 expression in the intestinal wall identified another potential mechanism for drug interactions with natural products (Bailey et al. 1998). This is important with other dihydropyridine calcium-channel blockers and drugs that undergo substantial presystemic metabolism mediated by CYP3A4, including lovastatin and simvastatin in the cardiovascular field. This interaction seems to be most prominent with grapefruit juice and has not been described to date with herbal medicines. Grapefruit juice may also have a small effect on digoxin phamacokinetics, possibly through inhibition of the organic anion transporting polypeptide (OATP) rather than P-gp (Bressler 2006).

16.5 RESEARCH NEEDS

The evidence from large clinical trials is generally not supportive of the cardiovascular benefits from supplements of antioxidant vitamins or other EMNs, and guidelines generally recommend increasing intake of foods that are rich in these materials rather than using specific supplements. Omega-3 PUFAs may be one exception, and niacin in pharmacological doses clearly has effects on plasma lipids. With the herbal medicines used in the prevention or treatment of CVD, the clinical trial evidence is mostly not sufficient to support any definitive recommendations. Many of the trials have been too small, and different trials have often used different herbal preparations with different standardizations, so the meta-analyses of herbal treatment effects may not always consider the same active compounds. Many herbal medicines do have ingredients with demonstrable pharmacological effects, but larger clinical trials with properly standardized materials are needed before any clear conclusions can be drawn. The potential antihyperglycemic effects of various ginseng preparations and possibly of lingzhi (*G. lucidum*) are areas for further investigation, and more clinical trials with garlic preparations for treating hyperlipidemia or hypertension are warranted. The role of danshen (*S. miltiorrhiza*) in treating CVDs can still be defined more clearly with appropriate clinical trials, and although some well-controlled studies with hawthorn extracts in heart failure have not shown significant benefits, there may still be a role for some hawthorn preparations in hyperlipidemia or other areas of CVD prevention.

16.6 CONCLUSIONS

The cardinal importance of a well-balanced diet that includes adequate fruit and vegetables has been rediscovered after some years of oversight during the era of great pharmaceutical and therapeutic advances. In times of plenty, it is important to control calorie intake and lower the consumption of animal fats and alcohol in association with taking adequate regular physical exercise and mental recreation for the maintenance of good health. A worldwide chronic disease epidemic of obesity, diabetes, and consequent CVDs is replacing the diminished burden of infectious diseases. It is evident that there is a place for the use of EMNs where these are deficient, but their value in treating established CVDs is unproved in most instances. Herbal remedies, although they have a long history of use in traditional medicine and show promising biological actions, remain clinically unproved and are as yet often insufficiently standardized to be recommended as therapy. This situation is

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likely to change with further research. The evidence to support the use of these alternative therapies from clinical trials is not yet secure, but custom and practice make it likely that they will continue to be used for the prevention or treatment of CVDs, among other indications.

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17 Herbs and Spices in Cancer Prevention and Treatment

Christine M. Kaefer and John A. Milner

CONTENTS

17.1 Introduction 361
17.2 Allspice 364
17.3 Basil 365
17.4 Caraway 367
17.5 Cardamom 368
17.6 Cinnamon 368
17.7 Clove 370
17.8 Coriander 371
17.9 Cumin 371
17.10 Dill 372
17.11 Garlic 372
17.12 Ginger 373
17.13 Rosemary 375
17.14 Saffron 375
17.15 Thyme 376
17.16 Conclusion 376
References 377

17.1 INTRODUCTION

Historically, spices have shaped many events throughout the world. Many voyagers, including the legendary Christopher Columbus, explored the seas in search of treasured spices. These valued commodities contribute not only flavors but also serve as colorants and preservatives in a wide variety of cultures. Today, spices are increasingly revered not only for their culinary properties but also for their potential health benefits. Although the health attributes associated with spice use may arise from their antioxidant properties, their biological effects may arise from their ability to induce changes in a number of cellular processes, including those involved with drug metabolism, cell division, apoptosis, differentiation, and immunocompetence.

The complexity of understanding the biological response to spices first surfaces in the criteria used to distinguish what constitutes a culinary spice and how they differ from culinary herbs. These terms are often used interchangeably in the scientific and lay literature. The U.S. Food and Drug Administration (FDA) defines a spice as an "aromatic vegetable substance, in the whole, broken, or ground form," whose significant function in food is "seasoning rather than nutrition" and from which "no portion of any volatile oil or other flavoring principle has been removed" (Food and Drug Administration 2007:205–208). While this is a viable definition, it does not consider the biological consequences of consuming these items and how they differ from herbs. The U.S. National Arboretum offers an alternative definition and describes spices as "flavorings (often of tropical origin) that are dried and culinary herbs that are fresh or dried leaves from plants which can be used

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for flavoring purposes in food preparation" (United States National Arboretum 2002). We must remember that the quantity of an item consumed does not dictate its importance. Thus, to avoid the health significance in any definition would appear flawed. In this chapter, we use the terms "herbs" and "spices" interchangeably and assume that both have properties that extend beyond simply providing flavor and color.

There is little doubt that nutrition and health are intimately linked (Kennedy 2008). For generations, people have alleged that foods provide greater benefits than simply supplying energy. Beliefs in the medicinal properties of foods have surfaced in many early writings of man. Hippocrates is frequently quoted as having said "Let food be thy medicine and medicine be thy food." Epidemiological, preclinical, and clinical studies continue to provide fundamental insights into the dynamic relationships between nutrients—defined here as any substance in the diet that brings about a physiological effect—and health. Today, claims about the ability of foods, including spices, to lower disease risk or to enhance the quality of life continue to captivate our lives (Kaefer and Milner 2008; Kochhar 2008; Krishnaswamy 2008; Iyer et al. 2009). Three types of biomarkers—exposure, effect, and susceptibility—are needed to evaluate the effects of spices in cancer prevention and therapy (Figure 17.1). Additional information about the amounts of specific spices required to bring about a response (effect) and the interactions of spices with other constituents of the diet, microbes in the gastrointestinal tract, environmental exposures, and human genetics (susceptibility factors) will be needed to unravel the true benefits of adding spices to the diet.

Spices may be a key to determining the balance between pro- and anticancer factors that regulate risk and tumor behavior (Figure 17.2). About 75% of U.S. households use dietary approaches to reduce their risk of diseases, including cancer (Sloan 2005). Americans between the ages of 36 and 55 are increasingly interested in adopting healthy eating behaviors and are gravitating toward ethnic cuisines based on perceived health benefits (Uhl 2000). Many of these ethnic foods are loaded with unique and flavorful spices; however, while dietary guidelines in several countries tend to support the incorporation of spices into diets, quantifiable recommendations for specific amounts have not yet been forthcoming (Tapsell et al. 2006).

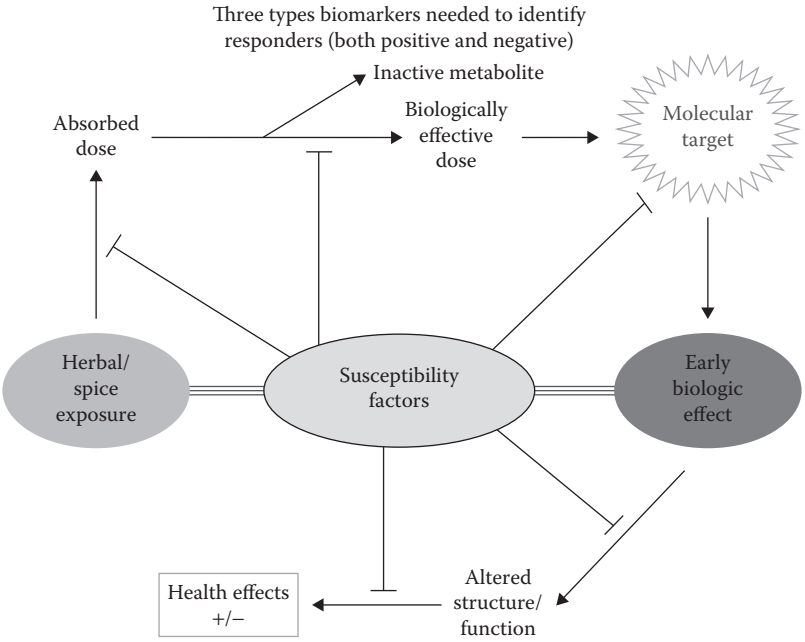


FIGURE 17.1 **(See color insert.)** Three types of biomarkers (exposure, effect, and susceptibility) are needed to assess the benefits or risk of spices.

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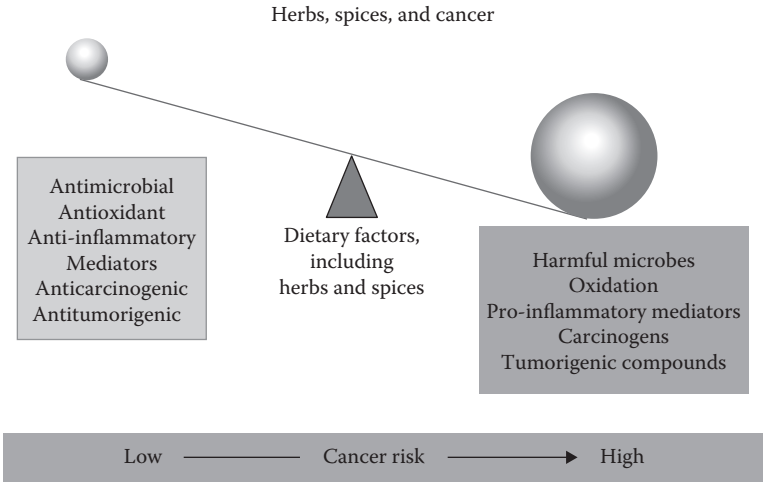


FIGURE 17.2 Multiple factors may influence the need for spices for reducing the risk of cancer or changing the biological behavior of cancerous cells.

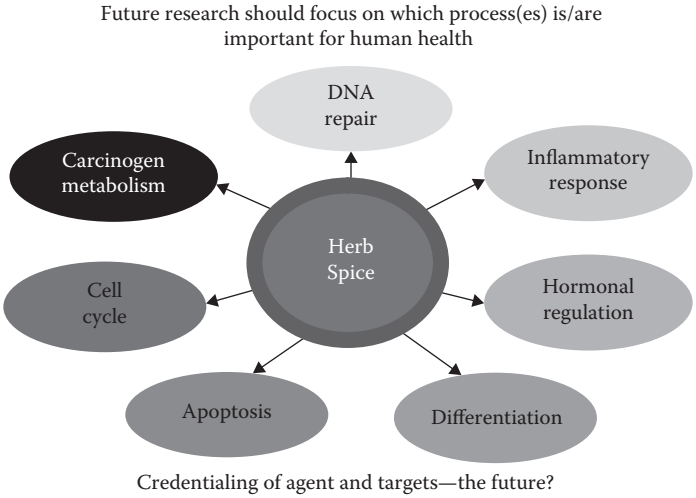


FIGURE 17.3 Multiple cancer-related processes may account for the ability of spices to inhibit experimentally induced cancers. While these processes are likely critical for determining the risk of cancer and tumor behavior in humans, only limited clinical evidence exists that spices in physiological relevant exposures can alter one or more of these process.

Between 1970 and 2005, the overall per capita consumption of spices in the United States doubled, increasing from about 1.6 to 3.3 pounds per year (United States Department of Agriculture Economic Research Service, 2007). As expected, the consumption of some spices increased far more than others; for example, garlic consumption increased more than sixfold. According to a report by Buzzanell (1995) from the United States Department of Agriculture's (USDA) Economic Research Service, the increasing domestic use of spices reflects a number of factors. Among these are the growing Hispanic and Asian populations within the United States, an increasing trend toward the use of culinary herbs and spices to compensate for less salt and lower fat foods and a general increase in the popularity of ethnic foods.

This chapter reviews culinary herbs and spices for their ability to modify several cellular processes that are linked to the risk of cancer and/or tumor behavior (Figure 17.3). The ability of spices

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to serve as inhibitors of carcinogen bioactivation, decrease free radical formation, suppress cell division and promote apoptosis in cancerous cells, suppress microbial growth, and regulate inflammation and immunocompetence will be discussed as plausible mechanisms by which selected spices may promote health and disease resistance. The low toxicity and wide acceptance of spices may make them particularly useful as a subtle personal dietary change that may decrease risk for several diseases. It is already appreciated that the addition of about 1 g/day of herbs to one's diet can significantly contribute to total antioxidant intake (>1 mmol) and offers a better source of antioxidants than many food items (Dragland et al. 2003; see also Chapter 2 on antioxidants in herbs and spices). Because several spices are effective antioxidants, they may be particularly important in decreasing oxidative damage due to environmental stress, including excess calorie intake.

More than 180 spice-derived compounds have been identified and explored for their health benefits (Aggarwal et al. 2008). It is beyond the scope of this chapter to deal with all herbs and spices that may influence the risk of cancer and tumor behavior. Therefore, a decision was made to review those with some of the more impressive biological responses reported in the literature, and a conscious effort was made to provide information about the amount of spices needed to bring about a response and thus their physiological relevance. When possible, recent reviews are included to provide readers with additional insights into the biological response(s) to specific spices and to prevent duplication of the scientific literature. Because there is a separate chapter devoted to curcumin (a bioactive component in turmeric) in this book and there are also several excellent reviews published about curcumin (Patel and Majumdar 2009; Aggarwal 2010; Bar-Sela, Epelbaum, and Schaffer 2010; Epstein, Sanderson, and Macdonald 2010), turmeric is not discussed in this chapter.

17.2 ALLSPICE

The term "allspice" was coined in the 1600s by the English, who thought the herb combined the flavors of cinnamon, nutmeg, and cloves. Allspice is also referred to as "Jamaica pepper," "kurundu," "myrtle pepper," "pimenta," and "newspice." Ground allspice is not a mixture of spices as some still believe, but arises from the dried unripe berries of the tree *Pimenta dioica*. This tree is native to the Greater Antilles, southern Mexico, and Central America. Today, *P. dioica* is cultivated in many warm areas throughout the world. Allspice is also available commercially as an essential oil.

Allspice is claimed to possess antimicrobial, antioxidant, anti-inflammatory, analgesic, antipyretic, anticancer, and antitumorigenic properties (Rompelberg et al. 1996; Al-Rehaily et al. 2002; Kluth et al. 2007). It contains a multitude of potential bioactive agents that may contribute to health promotion, including flavonoids, phenolic acids, catechins, and several phenylpropanoids (Al-Rehaily et al. 2002). Berries contain about 2–5% essential oils that include the following bioactive compounds: eugenol (60–75%), eugenol methyl ether, cineole (eucalyptol), phellandrene, and caryophyllenes (Kluth et al. 2007). The antioxidant and antimicrobial activities of allspice may be associated with eugenol (Rompelberg et al. 1996; Kluth et al. 2007).

Billing and Sherman (1998) reported that allspice was as effective as garlic and onions in suppressing microbial growth. The significance of its antimicrobial properties was recently highlighted by evidence that allspice and eugenol were effective in lowering the virulence of *Escherichia coli* O157:H7 (Takemasa et al. 2009). Nevertheless, there are concerns that allspice oil can be toxic and promote inflammation, nausea, and vomiting when consumed in excess.

The anticancer properties of allspice may be in part due to its ability to influence cytochrome P450 (CYP) activity and thereby influence carcinogen bioactivation. Kluth et al. (2007) cultured human liver carcinoma cells and human colon adenocarcinoma cells and studied the ability of the spice extract to activate mechanisms related to phase I detoxification enzymes. The allspice extract (3 mg/mL in dimethyl sulfoxide) did not activate pregnane X receptor (PXR) directly but did strongly activate the CYP3A4 promoter. Thus, the activation of transcription factors to bind to response elements seems like a plausible mechanism by which allspice, and potentially eugenol, function. There is specificity in the response to allspice and eugenol because gastrointestinal glutathione peroxidase

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(GPx), a phase II enzyme linked to removal of reactive oxygen species (ROS), was not influenced by allspice or eugenol (Kluth et al. 2007).

Inflammation is linked to increased risk of cancer (Dinarello 2010) and appears to be influenced by allspice consumption. Although controlled clinical interventions are not available, evidence in rodents suggests potency (Al-Rehaily et al. 2002). Providing an oral allspice suspension (500 mg/kg body weight) significantly inhibited carrageenan-induced paw edema and cotton pellet granuloma in rats. It also suppressed acetic acid-induced writhing and tail flick reaction time and decreased yeast-induced hyperpyrexia in mice. Interestingly, the suspension also appeared to have antiulcer and cytoprotective activity in rats by protecting gastric mucosa against indomethacin and various necrotizing agents, including 80% ethanol, 0.2 M sodium hydroxide (NaOH), and 25% sodium chloride (NaCl), suggesting that it might also have an impact on cyclooxygenase (COX) activity. It remains unclear what molecular target alteration(s) account for this response.

Evidence exists that allspice can alter the proliferation of several cultured cancerous cells. While cell viability was reduced about 50% when allspice extract was added to prostate cancer cells (LNCaP cells), it did not influence the viability of cultured human prostate cancer cell lines (DU145) or cervical epithelial carcinoma (HeLa) cells (Lee et al. 2007). The mechanism by which allspice leads to cellular growth depression remains largely unresolved. However, recent studies by Lee et al. (2007) suggest that epigenetics may be involved. A depression in histone acetyltransferase (HAT) activity may be involved. Androgen-induced HAT activity was decreased by 70% when allspice was provided at 100 μg/mL. Allspice also suppressed androgen receptor (AR) acetylation in LNCaP cells and significantly decreased histone H3 and H4 acetylation, indicating that a repression of AR-mediated transcription was induced due to shifts in histone and nonhistone acetylation. While these in vitro studies are intriguing, there is a need for controlled interventions in animal models before exploring allspice's potential benefit as a dietary antitumorigenic agent.

17.3 BASIL

Basil (*Ocimum basilicum*) is a culinary herb prominently featured in Italian and Southeast Asian cuisines. While many varieties of basil exist, sweet basil is one of the most predominant and most frequently examined herbs for its health benefits. Basil is originally native to Iran, India, and other tropical regions of Asia, but now it is widely available throughout the world. Basil's antioxidant, antimutagenic, antitumorigenic, antiviral, and antibacterial properties likely arise from a variety of components including linalool, 1,8-cineole, estragole, and eugenol (Muller et al. 1994; Chiang et al. 2005; Makri and Kintzios 2007). Similar to most culinary spices, far more information is needed about the variation in content of constituents as a function of plant varietal, growing conditions, and processing.

The essential oil of basil possesses antimicrobial properties (Wannissorn et al. 2005). Moghaddam, Karamoddin, and Ramezani (2009) investigated the effect of basil on *Helicobacter pylori* and found that methanol, butanol, and n-hexane fractions of basil demonstrated antagonistic activity against the bacteria (MIC = 39–117 μg/disk). While not as potent as amoxicillin, its effectiveness raises possibilities of using individual or multiple spices as potent antimicrobials, especially in areas where commercial antibiotics are in limited supply (Moghaddam, Karamoddin, and Ramezani 2009).

The effects of basil are not limited to its antimicrobial properties because evidence indicates that it also can lower oxidative damage in animal models (Dasgupta, Rao, and Yadava 2004). Feeding mice 200 and 400 mg/kg body weight with a hydroalcoholic extract of basil leaves for 15 days markedly increased GPx (1.22–1.4 fold), glutathione (GSH) reductase (1.16–1.28 fold), catalase (1.56–1.58 fold), and superoxide dismutase (1.1–1.4 fold; Dasgupta, Rao, and Yadava 2004). The change in activity in one or more of these enzymes may explain the decrease in lipid peroxidation caused by basil in studies by Dasgupta, Rao, and Yadava (2004). Drăgan et al. (2007) examined the effects of balsamic vinegar–enriched extracts from several herbs (rosemary, sage, and basil) in soups and salads on oxidative stress and quality of life measures in women with stage IIIB and IV

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breast cancer. While there was a decrease in oxidative stress, the complexity of the dietary intervention made it impossible to determine the component(s) that led to improvements.

Several studies provide evidence that basil is an antimutagenic spice (Kusamran, Tepsuwan, and Kupradinun 1998; Stajkovic et al. 2007). Stajkovic et al. (2007) studied the antimutagenic effects of basil on mutagenicity in *Salmonella typhimurium* TA98, TA100, and TA102 cells in the presence or absence of liver microsomal activation. The essential oil of basil, at concentrations ranging from 0.5 µL/plate to 2.0 µL/plate, inhibited mutations from ultraviolet irradiation (dose = 6 J/m^2) by 22–76%. Mutations caused by 4-nitroquinoline-N-oxide (0.15 µg/plate) were decreased by 23–52%, and those from 2-nitropropane (14.9 mg/plate) by 8–30%. These findings are consistent with studies by Jeurissen et al. (2008), who demonstrated that 50 µg/mL basil largely blocked DNA adduct formation caused by 1′-hydroxyestragole in the human hepatoma (HepG2) cell line, possibly by promoting phase II enzymes and thereby conjugation and elimination of this carcinogen. These findings likely explain the ability of basil to decrease the mutagenicity of aflatoxin B_1 (AFB_1) and benzo(*a*)pyrene (B(*a*)P) (Stajkovic et al. 2007). The mutagenicity of AFB_1 was inhibited by >30% by the presence of 1–2 mg/plate of a hexane-based basil extract and 0.5–1 mg/plate of chloroform- and methanol-based basil extracts. Because B(*a*)P mutagenicity was only inhibited by chloroform- and methanol-based basil extracts at doses of 2–5 mg/plate, multiple constituents might be responsible for basil's antimutagenic activities.

The anticancer properties of basil in preclinical studies are mixed. In studies with Sprague-Dawley rats fed with an AIN-76 diet with or without high concentrations of basil (6.25% and 12.5%), there was no clear indication of a decrease in 9,10-dimethyl-1,2-benzathracene (DMBA)-induced mammary cancer. It is unclear whether the quantity of the procarcinogen examined, the simultaneous induction of both phase I and II enzymes, or some other factors accounted for the lack of protection by adding basil to the animals' diet (Kusamran, Tepsuwan, and Kupradinun 1998). Nevertheless, there is evidence that basil can decrease DMBA-induced carcinogenesis. Providing Swiss mice with a diet containing 150 or 300 mg/kg body weight of basil extract decreased DMBA-induced skin tumors (12.5% reduction and 18.75% reduction for lower and higher doses, respectively), and lowered the tumor burden per mouse. Compared to the average number of tumors per mouse in the controls, the tumor burden was approximately 2.4 times lower ($p < .01$) in the low-dose basil group and 4.6 times lower ($p < .001$) in the high-dose basil group (Dasgupta, Rao, and Yadava 2004). It is unclear whether differences in the response between mice and rats reflect the species, the cancer site, or the dietary or procarcinogen exposures.

DNA methyltransferase (MGMT) is a critical repair protein in the cellular defense against alkylation damage. MGMT is highly expressed in human cancers and in tumors resistant to many anticancer alkylating agents. Niture, Rao, and Srivenugopal (2006) examined the ability of several medicinal plants to upregulate O^6-methylguanine adducts. Both ethanol and aqueous extracts of basil increased MGMT protein levels in HT29 human colon carcinoma cell lines 1.25-fold compared to controls after 72-hours incubation. Compared to the control, basil increased glutathione-S-transferase (GST) protein activity 1.33-fold after 12 hours of incubation; after 24 hours, GST activity increased 1.68-fold compared to the control, which declined to 1.47-fold after 72 hours incubation. Because MGMT is one of the body's first lines of defense against alkylation DNA damage, a small increase (two- to threefold) in this enzyme may protect against mutagenic lesions (Niture, Rao, and Srivenugopal 2006).

The anticancer properties of basil may also relate to its ability to influence viral infections. Individuals with hepatitis B are recognized to be at increased risk for hepatocellular carcinoma (Fung, Lai, and Yuen 2009; Ishikawa 2010). Chiang et al. (2005) evaluated the antiviral activities of basil extract and selected basil constituents in a human skin basal cell carcinoma cell line (BCC-1/KMC) and a cell line derived from hepatoblastoma HepG2 cells (2.2.15) against several viruses, including hepatitis B. Impressively, Chiang et al. (2005) found that the aqueous extract of basil, along with apigenin and ursolic acid, displayed greater anti-hepatitis B activity than two commercially available drugs, glycyrrhizin and lamivudine (3TC). Overall, these studies raise intriguing

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questions about the merits of using commercially available spices to retard viruses and potentially cancer. Undeniably, much more information is needed to clarify the amounts and durations needed to bring about a desired viral response and the mechanism by which a response occurs.

It should be noted that there are concerns about excess basil exposure. Estragole, a suspect procarcinogen/mutagenic found in basil, raises questions about the balance between benefits and risks with the use of this and other spices (Muller et al. 1994). Now, the majority of evidence points to the antimutagenic effects of basil outweighing the potential adverse effects associated with estragole-induced cell damage (Jeurissen et al. 2008).

17.4 CARAWAY

Caraway (*Carum carvi*), also known as "meridian fennel" or "Persian cumin," is native to western Asia, Europe, and northern Africa. The principal agents in caraway oil are believed to be carvone or *p*-mentha-1,8-dien-2-one and limonene or *p*-mentha-1,8-diene, the precursors of carvone and anethofuran (Zheng, Kenney, and Lam 1992). Although caraway appears to be a potent antioxidant in vitro, it has not been adequately examined in humans. Recently, Kapoor et al. (2010) showed that caraway essential oil and oleoresins were progressively effectively with dose as antioxidants and more effective than commercial butylated hydroxyanisole and butylated hydroxytoluene. Caraway oil and its ethanol oleoresin showed better reductive power than the other oleoresins. The scavenging and reducing power against the diphenylpicrylhydrazyl (DPPH) radicals that caraway oil and oleoresins provide may be associated with their ability to donate hydrogen and the presence of reductones.

Mazaki et al. (2006) examined the effect of caraway seed extract on mutagenesis induced by *N*-methyl-*N*-nitro-*N*′-nitrosoguanidine (MNNG) in *S. typhimurium* strains that are deficient in DNA MGMT. Their results indicated that caraway does not directly inactivate MNNG and O^6-methylguanine-DNA MGMT may be involved in the response. Animal models have also been used to explore the anticancer potential of caraway in sites ranging from colon to skin cancers. Schwaireb (1993) examined dietary caraway oil for its effects on skin tumors induced by DMBA and croton oil in female BALB/c mice. Mice fed with a diet containing 3% caraway oil for 23 weeks from the beginning of tumor promotion decreased the number of mice with papillomas ($p < .001$), the number of papillomas per mouse ($p < .0001$), and the average papilloma volume ($p < .0001$). The number of carcinomas in those animals provided with caraway oil were significantly less than in the controls (Schwaireb 1993). Deeptha et al. (2006) examined the effects of oral caraway (30, 60, and 90 mg/kg body weight per day for 15 weeks) on aberrant crypt foci in male Wistar rats treated with the carcinogen 1,2-dimethylhydrazine. Aberrant foci are early morphological events that represent an important step in colon cancer progression. Treatment of rats with 60 mg/kg body weight of caraway decreased carcinogen-induced aberrant crypt foci, indicators of oxidative stress, and fecal bacterial enzyme activity.

Induction of GST by anticarcinogenic compounds is an important mechanism by which several spices, including caraway, may promote carcinogen detoxification and thereby lower cancer risk. Zheng, Kenney, and Lam (1992) reported that activity of the detoxifying enzyme GST in the liver increased markedly after gavage treatment with 20 mg carvone or limonene in A/J mice. Carvone was also found to increase the GST activity in the forestomach by about 80% ($p < .05$), more than double the GST activity in the large intestinal mucosa ($p < .05$), and more than triple the GST activity in the small intestinal mucosa ($p < .005$). Carvone also increased glutathione (GSH) in the lung ($p < .005$) and in the small ($p < .05$) and large intestinal mucosa ($p < .05$).

Caraway may also influence carcinogen activation by its ability to modify carcinogen bioactivation. Polycyclic aromatic hydrocarbons and halogenated aromatic compounds such as 2,3,7,8-tetrodibenzo-*p*-dioxin (TCDD) are bioactivated by the xenobiotic-metabolizing CYP genes to form reactive metabolites that bind to DNA. Naderi-Kalali et al. (2005) reported that caraway extracts were effective in inhibiting the induction of CYP1A1 and CYP1A1-related RNA in rat

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hepatoma (H4IIE) cells. Caraway extracts >0.13 μM significantly inhibited CYP1A1 induction, as measured by the 2,3,7-ethoxyresorufin *O*-deethylase assay, with roughly a tenfold suppression in enzyme activity observed at concentrations of 1.3 and 13 μM, inhibiting TCDD-dependent induction by 50%–90%, depending on the solvent used (Naderi-Kalali et al. 2005). Overall, changes in both phase I and II enzymes are consistent with the ability of caraway and its active constituent to lower chemically induced cancers. The importance of caraway and its isolated components in drug detoxification mechanisms in humans remains largely unexplored.

17.5 CARDAMOM

Cardamom refers to herbs within the *Elettaria* (green) and *Amomum* (black) genera of the ginger family Zingiberaceae. Cardamom is a common ingredient used in Indian cooking and in various parts of Europe. As with many spices, cardamom has been demonstrated to have antioxidant properties. Kikuzaki, Kawai, and Nakatani (2001) examined extracts from black cardamom (*Amomum subulatum*) for their ability to scavenge radicals. The ethyl acetate-soluble fraction, containing several phenolic compounds (protocatechualdehyde, protocatechuic acid, 1,7-bis(3,4-dihydroxyphenyl)hepta-4E,6E-dien-3-one, and 2,3,7-trihydroxy-5-(3,4-dihydroxy-E-styryl)-6,7,8,9-tetrahydro-5H-benzocycloheptene), scavenged about 90% of DPPH radicals when provided at 100 μg/mL. Interestingly, at lower concentrations, its radical scavenging activity was comparable to that of α-tocopherol (Kikuzaki, Kawai, and Nakatani 2001). Feeding male albino Wistar rats with a high-fat diet supplemented with 10% black cardamom seed powder for 90 days was found to lower 2-thiobarbituric acid reactive substances (TBARS) by 28% ($p < .05$) in heart tissue (Dhuley 1999). In addition, cardamom caused significant increases ($p < .05$) in several antioxidant enzymes including catalase, superoxide dismutase, and GST in both liver and heart compared to controls (Dhuley 1999).

The ability of cardamom to inhibit chemical carcinogenesis was shown by Banerjee et al. (1994), who observed cardamom oil feeding (10 μL daily for 2 weeks) caused a significant decrease in liver CYP content in Swiss albino mice ($p < .05$). A 30% increase in GST activity ($p < .05$) and sulfhydryl levels ($p < .05$) in the liver also accompanied the cardamom oil treatment. These observations suggest that intake of cardamom oil affects the enzymes associated with xenobiotic metabolism and may therefore have benefits as a deterrent to cancer (Banerjee et al. 1994). Cardamom has also been demonstrated to decrease azoxymethane-induced colon carcinogenesis by virtue of its anti-inflammatory, antiproliferative, and proapoptotic activities. Providing aqueous cardamom suspensions can enhance detoxifying enzyme (GST activity) and decrease lipid peroxidation (Bhattacharjee, Rana, and Sengupta 2007).

Recently, cardamom aqueous extracts (1, 10, 50, and 100 mg/mL) were reported to significantly enhance splenocyte proliferation in a dose-dependent manner, especially when combined with black pepper (Majdalawieh and Carr 2010). While the effects of cardamom and black pepper were the opposite on T helper-1 and -2 cytokine release by splenocytes, the presence of both spices significantly enhanced the cytotoxic activity of natural killer cells against YAC-1 lymphoma cells. These findings provide evidence that cardamom may have anticancer benefits by modifying immunocompetence.

17.6 CINNAMON

Cinnamon is a spice obtained from the bark of an evergreen tree belonging to the Lauraceae family. Major constituents in cinnamon include cinnamaldehyde, eugenol, terpinene, α-pinene, carvacrol, linalool, safrole, benzyl benzoate, and coumarin (Tabak, Armon, and Neeman 1999). Cinnamon is widely used in traditional Chinese medicine. Several studies have examined its antioxidant properties. When inbred male albino Wistar rats were fed a high-fat diet with 10% cinnamon bark powder (*Cinnamomum verum*) for 90 days, oxidative stress was substantially decreased, as evident by a reduction in TBARS, a biomarker of free radical production (Dhuley 1999). Providing rats

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with cinnamon bark powder significantly increased several antioxidant-related enzymes, including catalase, superoxide dismutase, and GST in both liver and heart tissue, compared to controls. Glucose-6-phosphate dehydrogenase and GPx were also significantly increased ($p < .05$) in rats fed with cinnamon bark powder. These enzymes help maintain GSH levels, essential for cellular integrity and protection against oxidative damage from free radicals (Dhuley 1999).

The ability of cinnamon extracts to suppress the in vitro growth of *H. pylori*, a recognized risk factor for gastric cancer, gastric mucosa-associated lymphoid tissue lymphoma, and possibly pancreatic cancer, has stirred considerable interest in the potential use of this spice to suppress human cancers (Farinha and Gascoyne 2005; Eslick 2006). However, a pilot study involving 15 subjects given a cinnamon extract (80 mg/day) for 4 weeks was not effective in those infected with *H. pylori* (Nir et al. 2000). In this study, the colonization rate of *H. pylori* was measured by urea breath tests (UBTs). Although a decline in *H. pylori* counts was observed in six patients with exceptionally high counts according to their first UBT, the study did not demonstrate a decrease in colonization overall, and in some individuals increased colony counts occurred. The lack of success of cinnamon as a single treatment regimen against *H. pylori* is not terribly surprising given the consistent failure of single-agent antibiotic trials. Additional trials using higher cinnamon amounts and possibly in combination with other agents may be warranted to truly evaluate the effects of this spice (Nir et al. 2000). Tabak et al. (1996) examined several spices for their ability to inhibit *H. pylori*. Cinnamon and thyme were found to be the most potent inhibitors of *H. pylori* growth and urease activity. Tabak, Armon, and Neeman (1999) reported cinnamon's antibacterial activity against seven clinical isolates of *H. pylori* and the antiurease activity of two different cinnamon extracts (methylene chloride and ethanol) and their chemical constituents. They found that adding 100 μg cinnamon per disk produced an inhibition zone of approximately 80 mm wide, which was greater than the inhibition zones produced by several antibiotics (10 μg ampicillin, 30 μg tetracycline, 15 μg erythromycin, 30 μg nalidixic acid, and 25 μg co-trimoxazole). Although a concentration of 25 μg/mL completely inhibited four *H. pylori* strains, 50 μg/mL was the minimum inhibitory concentration for all seven strains. In a liquid medium, cinnamon extract began to inhibit *H. pylori* at a concentration >3 μg/mL and peaked at a concentration >12 μg/mL, and a similar pattern of inhibition was observed with urease. The efficiency of cinnamon extracts in inhibiting *H. pylori* in a liquid medium and its resistance to low pH may enhance its effects in an environment like the human stomach. The antibacterial effects of cinnamon extract may be due to cinnamaldehyde. Adding 200 μg cinnamaldehyde per disk produced an inhibition zone >90 mm, while eugenol (2000 μg/disk) produced an inhibition zone of 68 mm, and carvacrol (2000 μg/disk) produced an inhibition zone of 66 mm; Tabak, Armon, and Neeman 1999).

Cao, Urban, and Anderson (2008) studied the role of polyphenolic polymers from commercial cinnamon extract in immune regulation using mouse RAW264.7 macrophages. The authors examined whether cinnamon polyphenol extract (CPE) regulated immune function by affecting expression levels of genes that code for tristetraprolin (TTP/zinc finger protein 36), proinflammatory cytokines, and glucose transporter (GLUT) family proteins, and they compared these effects with that of insulin and lipopolysaccharide. Because TTP downregulates proinflammatory cytokines, it has the potential for use in the prevention and treatment of inflammation-related diseases. In this study, CPE rapidly increased TTP mRNA and protein levels in mouse RAW264.7 macrophages after 30 minutes of treatment, and twofold increase in expression was sustained throughout the 4 hours of treatment. CPE also increased the mRNAs coding for proinflammatory cytokines, such as tumor necrosis factor α, cyclooxygenase-2, and interleukin 6, although the TTP levels were 6- to 3000-fold higher than the proinflammatory cytokine mRNA molecules in the same cells (Cao and Prior 1998). In mammals, glucose is a critically important molecule in the host immune response to injury and infection, which is facilitated by GLUT family proteins, and based on this study, cinnamon increases GLUT expression.

Vascular endothelial growth factor (VEGF) is a critical factor in the induction of angiogenesis. Unfortunately, the side effects associated with most anti-VEGF drugs limit their use, and thus, the

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use of naturally occurring dietary inhibitors derived from diets has great appeal. A water-based extract from cinnamon is a promising effective agent because it directly inhibits kinase activity of purified VEGFR2 as well as mitogen-activated protein kinase- and Stat3-mediated signaling pathway in endothelial cells (ECs; Lu et al. 2010). Impressively, the extract was found to inhibit VEGF-induced EC proliferation, migration and tube formation in vitro, sprout formation from aortic ring ex vivo, and tumor-induced blood vessel formation in vivo. Polyphenols in the extract appear to be responsible for the response; cinnamaldehyde is observed to have little effect on VEGFR2 kinase activity (Lu et al. 2010). While the ability of cinnamon to influence angiogenesis is intriguing, additional studies are warranted.

17.7 CLOVE

Clove is derived from flower buds of the *Eugenia caryophyllata* tree. Several bioactive components are found in clove, including tannins, terpenoids, eugenol, and acetyleugenol (Kluth et al. 2007). Cloves are native to Indonesia and are used in cuisines throughout the world. While no studies have been conducted in humans to date to evaluate use of cloves in cancer prevention, a few studies conducted in mice suggest its effectiveness, especially in modifying cellular detoxification processes. Feeding 40 mg clove per gram of diet to mice resulted in an increase in GST activity compared to those that were not fed the spice. The physiological significance of these findings remains unclear because the increase was approximately 2% above normal in the liver (not significant), 18% in the stomach ($p < .05$), and 33% in the esophagus ($p < .05$; Aruna and Sivaramakrishnan 1990). An increase in GSH concentrations in the stomach also occurred ($p < .05$), suggesting detoxification of clove constituents in the stomach (Aruna and Sivaramakrishnan 1990). In another study, feeding clove (0.5%, 1%, and 2%) to mice for varying durations (10, 20, and 30 days) modified several phase II enzymes associated with carcinogen bioactivation (Kumari 1991). A dose-dependent response was observed for several detoxification enzymes. After 20 days, all exposures resulted in a significant increase in GST and cytochrome b_5 (Kumari 1991). DT-diaphorase was significantly elevated in those that were provided with 1% or 2% clove after 30 days ($p < .0005$) compared to controls. A significant decrease in CYP activity was observed in those provided with any clove after 30 days. No changes were observed in the activity levels of aryl hydrocarbon hydroxylase in response to the clove administration. Malondialdehyde (MDA) formation was measured to monitor radiation-induced lipid peroxidation, and both the concentration of clove provided and the duration of exposure affected the results. Diets containing 2% clove for at least 20 days, or any concentration administered for 30 days, significantly decreased MDA (Kumari 1991). Eugenol, an allyl chain-substituted guaiacol, may be responsible, at least in part, for the induction of the phase II enzymes (Han et al. 2007) and/or serve as an antioxidant (Rajakumar and Rao 1993; Nagababu and Lakshmaiah 1994). Changes in phase I and II enzymes may account for the ability of eugenol to serve as an antimutagen (Miyazawa and Hisama 2003) and to inhibit carcinogen-induced genotoxicity (Han et al. 2007).

Kluth et al. (2007) examined the influence of several spice extracts on phase I and II enzymes in cultured human liver carcinoma and human colon adenocarcinoma cells, and they suggested a shift in the nuclear transcription factor Nrf2 was responsible for the induction. Evidence also exists that clove extracts might interfere with β-catenin activity and thereby decrease colon carcinogenesis, but further studies are needed on this (Aggarwal 2010).

Similar to allspice, clove contains high amounts of eugenol. However, this compound cannot serve to increase gastrointestinal GPx promoter activity, suggesting other compounds in clove may account for its biological activity (Kluth et al. 2007). Overall, the findings to date suggest that tissues adapt to exposures to one or more constituents in cloves. In doing so, clove may improve the ability of selected tissues to handle foreign compounds that might lead to the initiation of carcinogenesis. Based on findings to date, additional clinical studies are warranted to determine the ability of clove to influence drug detoxification pathways.

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17.8 CORIANDER

Coriander (*Coriandrum sativum*) is an herb in the family Apiaceae and is native to southern Europe and northern Africa to southwestern Asia. Although all parts of the plant are edible, its fresh leaves and dried seeds are most frequently used in cooking. Coriander is a common ingredient in many foods throughout the world. One of its principal constituents is linalool. Several animal studies provide evidence that coriander seeds can promote the hepatic antioxidant system. Feeding a 10% coriander seed diet to male Wistar rats for 12 weeks decreased the ability of hexachlorocyclohexane, an organochlorine insecticide, to promote lipid peroxidation (Aruna and Sivaramakrishnan 1990; Anilakumar, Nagaraj, and Santhanam 2001). Coriander can also influence foreign compound metabolism. Feeding Swiss mice with 160-mg coriander seeds per gram diet resulted in GST induction ranging from 20% to 37%, depending on the tissue examined. In another study, Banerjee et al. (1994) observed roughly a doubling in GST activity in Swiss albino mice that were provided with diets containing coriander oil (10 μL coriander oil daily for 2 weeks). No significant changes were observed in CYP or aryl hydrocarbon hydroxylase. Although relatively few studies focus on coriander for its anticancer properties, those that are available suggest coriander may be important (Esiyok, Otles, and Akcicek 2004).

17.9 CUMIN

Cumin (*Cuminum cyminum*) is a flowering plant in the family Apiaceae and is native to the eastern Mediterranean region and India. Thymoquinone (TQ) is the most abundant component of black cumin seed oil. TQ has been reported to exhibit antioxidant, antimicrobial, anti-inflammatory, and chemopreventive properties (Allahghadri et al. 2010; Nader, el-Agamy, and Suddek 2010) and to ameliorate B(*a*)P-induced carcinogenesis in the forestomach. Swiss mice that were fed 160-mg cumin seeds per gram of diet and injected with B(*a*)P to induce chromosome aberrations were able to suppress the aberrations by 83% compared to controls (Aruna and Sivaramakrishnan 1990). Part of this response may be due to cumin's ability to influence phase II enzymes. In another study by Banerjee et al., feeding Swiss mice with 10-μL cumin oil daily for 2 weeks caused a 13% increase in GST levels ($p < .1$). No statistically significant changes were observed in CYP activity, aryl hydrocarbon hydroxylase levels, or sulfhydryl levels in the liver compared to the control group, and thus, the first, and possibly most important, change may be an elevation in GST activity (Banerjee et al. 1994). Aruna et al. (2005) studied male albino Wistar rats to determine the effect of cumin seeds (0.25 g cumin/kg body weight) on oxidative stress induced by alcohol and heated sunflower oil, a source of polyunsaturated fatty acids. The rats' antioxidant status was determined to be near normal when cumin was consumed with alcohol and preheated oil, potentially due to cumin's antioxidant and detoxification properties (Aruna, Rukkumani, and Menon 2005).

Considerable evidence points to the ability of TQ to suppress tumor cell proliferation, including colorectal carcinoma, breast adenocarcinoma, osteosarcoma, ovarian carcinoma, myeloblastic leukemia, and pancreatic carcinoma (Gali-Muhtasib, Roessner, and Schneider-Stock 2006). Normal cells appear to be slightly resistant to TQ (Worthen, Ghosheh, and Crooks 1998). Several mechanisms may explain the ability of TQ to bring about a change in cell division in neoplastic cells, including downregulation in Bcl-xL, cyclin D1, and VEGF (Aggarwal et al. 2008). Considerable evidence points to the ability of TQ to induce free radical formation in tumor cells. Thus, the biological response in tumor cells (pro-oxidants) may be different than that in normal cells (antioxidant; Koka et al. 2010). TQ has also been found to be effective in inhibiting human umbilical vein EC migration, invasion, and tube formation, suggesting its role in angiogenesis (Yi et al. 2008). TQ (6 mg/kg/day) was also found to prevent tumor angiogenesis in a xenograft human prostate cancer (PC-3) model (Yi et al. 2008). The multitude of effects caused by cumin serves as justification for its continued examination as a spice with widespread potential for health promotion.

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17.10 DILL

Dill (*Anethum graveolens*) is a relatively short-lived perennial spice. Dill is an herb that in effect has two components that are dependent on the seasons. In the early spring, dill is used for its leaves and in the autumn for its seeds. The principal constituents of dill weed oil are anethofuran or 3,6-di methyl-2,3,3a,4,5,7a-hydroxobenzofuran, and carvone or *p*-mentha-1,8-dien-2-one (Zheng, Kenney, and Lam 1992). As with other spices, there is evidence that dill promotes drug detoxification mechanisms. Providing 20 mg each of carvone and anethofuran by gavage once every 2 days for a total of three doses increased GST activity in A/J mice (Zheng, Kenney, and Lam 1992). The response depended on the agent and the tissue examined. Anethofuran more than doubled the activity of the detoxifying enzyme GST in the liver ($p < .005$) and forestomach ($p < .005$), and carvone increased GST activity 78% in the forestomach ($p < .05$) and increased GST activity more than twofold in the liver and large intestinal mucosa ($p < .05$) and more than threefold in the small intestinal mucosa ($p < .005$; Zheng, Kenney, and Lam 1992). Because GSH helps maintain cellular oxidation-reduction balance and protects cells against free-radical species, the combination of increased GST and GSH levels results may be particularly helpful in detoxifying foreign compounds, including carcinogens.

17.11 GARLIC

Garlic (*Allium sativum*) is a member of the onion family Alliaceae. Garlic has been used throughout history for both its culinary and medicinal properties. Garlic's distinctive characteristics arise from sulfur, which constitutes almost 1% of its dry weight. The primary sulfur-containing constituents are γ-glutamyl-S-alk(en)yl-L-cysteines and S-alk(en)yl-L-cysteine sulfoxides. Considerable variation in the S-alk(en)ylcysteine sulfoxide content can occur; alliin (S-allylcysteine sulfoxide) is the largest contributor. Alliin concentrations can increase during storage because of the transformation of γ-glutamylcysteines. Although garlic does not typically serve as a major source of essential nutrients, it may contribute to several dietary factors with potential health benefits, including the presence of oligosaccharides, arginine-rich proteins and, depending on soil and growing conditions, selenium and flavonoids.

Preclinical models provide rather compelling evidence that garlic and its associated components can lower the incidence of breast, colon, skin, uterine, esophagus, and lung cancers. However, evidence in human investigations is less compelling. Suppression of nitrosamine formation continues to surface as one of the most likely mechanisms by which garlic retards cancer. The ability of S-allyl cysteine (SAC) and its nonallyl analog S-propyl cysteine to retard N-nitroso compounds formation, but not diallyl disulfide (DADS), dipropyl disulfide, and diallyl sulfide (DAS), reveal the critical role that the cysteine residue plays in inhibition (Milner 2001). Some of the most compelling evidence in humans comes from studies by Mei et al. (1989) demonstrating that ingesting 5 g/day of garlic blocked the enhanced urinary excretion of nitrosoproline resulting from exaggerated nitrate and proline intake. More recent evidence suggests as little as 1 g of garlic may be sufficient to suppress nitroproline formation (Cope et al. 2009).

The ability of garlic to inhibit tumors due to different cancer-inducing agents and in different tissues indicates that a generalized cellular event is likely responsible for the change in tumor incidence and that the response is highly dependent on environmental or other types of biological insults. Because metabolic activation is required for many of these carcinogens, there is likelihood that either phase I or II enzymes are altered. Interestingly, little change in CYP1A1, 1A2, 2B1, or 3A4 activities has been detected following treatment with garlic or related sulfur compounds. However, this lack of responsiveness may relate to the amount and duration of exposure, the quantity of carcinogen administered, or the methods used to assess the cytochrome content or activity. Wu et al. (2002), using immunoblot assays, found that the protein content of CYP1A1, 2B1, and 3A1 was increased by garlic oil and each of several isolated disulfide compounds. Their data demonstrated that the number of sulfur atoms in the allyl compound is inversely related to the depression in these cytochromes.

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Several lipid- and water-soluble organosulfur compounds have been examined for their antiproliferative efficacy. Some of the more commonly used lipid-soluble allyl sulfur compounds in tumorigenesis research are ajoene, DAS, DADS, and diallyl trisulfide (DATS). A breakdown of allicin appears to be necessary for achieving maximum tumor inhibition. Earlier studies reported that lipid-soluble DAS, DADS, and DATS (100 μM) were more effective in suppressing canine tumor cell proliferation than isomolar water-soluble SAC, S-ethyl cysteine and S-propyl cysteine (Knowles and Milner 2001). Undeniably, not all allyl sulfur compounds from garlic are equally effective in retarding tumor proliferation. Allyl sulfur compounds preferentially suppress neoplastic over nonneoplastic cells (Sakamoto, Lawson, and Milner 1997). S-allylmercaptocysteine (SAMC), DAS, and DADS have also been reported to increase the percentage of cells blocked within the G_2/M phase. $p34^{cdc2}$ kinase is a complex that governs the progression of cells from the G_2 phase into the M phase of the cell cycle (Knowles and Milner 2001). Using LNCaP and HCT-116 human cancer cells, Xiao, Zeng, and Singh (2009) demonstrated that checkpoint kinase 1-mediated mitotic arrest resulting from DATS is the key to apoptosis induction. It is becoming increasingly clear that the response to allyl sulfurs relates to their ability to form free radicals rather than to serve as an antioxidant (Antosiewicz et al. 2008). Allyl sulfurs may bring about changes by influencing the genomic expression by affecting histone homeostasis. Allyl mercaptan is a particularly potent inhibitor of histone deacetylase (HDAC; Nian et al. 2009). HDAC inhibition has the potential to derepress epigenetically silenced genes in cancer cells, leading to cell-cycle arrest and apoptosis. Sp3 appears to have a role in driving p21 gene expression after HDAC inhibition by allyl sulfur compounds and coincides with cell-cycle arrest. Alliin has been reported to influence angiogenesis. It causes a dose-dependent inhibition of fibroblast growth factor 2 (FGF-2)-induced human EC tube formation and angiogenesis in the chick chorioallantoic membrane model (Mousa and Mousa 2005). Xiao et al. (2006) suggested the antiangiogenic characteristics of DATS relate to its ability to downregulate VEGF secretion and VEGF receptor-2 protein level and inactivation of Akt kinase. However, while DATS was effective in decreasing prostate cancer multiplicity in the transgenic adenocarcinoma of the mouse prostate model, it did not appear to relate to a change in angiogenesis (Singh et al. 2008).

17.12 GINGER

Ginger (*Zingiber officinale)* is a member of the Zingiberaceae family and is consumed widely not only as a spice but also as a medicinal agent (see also Chapter 7 on ginger). Other members of the family include turmeric and cardamom. Ginger's cultivation appears to have begun in South Asia and has now spread to various parts of the world. It is sometimes called "root ginger" to distinguish it from other products that share the name. The principal constituents of ginger include [6]-gingerol, [6]-paradol, [6]-shogaol (dehydration gingerols), and zingerone. Several studies have investigated ginger's antioxidant properties (Chrubasik, Pittler, and Roufogalis 2005). Gingerol has also been shown to decrease intracellular ROS formation in human keratinocyte cells (Kim et al. 2007), inhibit angiogenesis in human ECs, and limit nitrogen oxide synthase expression and epidermal growth factor–induced cell transformation and AP-1 transcriptional complexes in JB6 cells (Bode et al. 2001; Ippoushi et al. 2003; Davies et al. 2005; Kim et al. 2005).

Feeding NIN/Wistar rats a diet containing up to 0.5–5% ginger for 1 month significantly increased ($p < .05$) several liver antioxidant enzymes, including superoxide dismutase (76–141%), catalase (37–94%), and GPx (11–30%; Kota, Krishna, and Polasa 2008). Lipid and protein oxidation was inhibited in rats consuming ginger, as evidenced by significant decreases ($p < .05$) in liver and kidney levels of MDA (35–59% and 27–59%, respectively) and carbonyl levels (23–36%), compared to controls (Kota, Krishna, and Polasa 2008). Ippoushi et al. (2007) found that AIN-76 basal diets with 2% ginger decreased TBARS by 29% ($p < .05$) and suppressed 8-hydroxy-2′-deoxyguanosine (8-OHdG, a product of oxidative DNA damage) levels in Wistar rats. TBARS was also significantly decreased ($p < .001$) in Wistar rats fed with diets supplemented with 1% ginger following exposure to lindane, a pesticide that is a global pollutant, (Ahmed et al. 2008).

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Various animal models have been used to examine the role of ginger in cancer prevention. For example, Ihlaseh et al. (2006) exposed male Wistar rats to *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine (BNN) and uracil salt to induce tumors resembling human low-grade papillary urothelial neoplasia. Rats fed with a basal diet supplemented with 1% ginger extract for 26 weeks had significantly fewer urothelial lesions compared to the controls or those fed with the diet with 0.5% ginger ($p = .013$; Ihlaseh et al. 2006). However, ginger does not appear effective in all cases, as evidenced by the lack of protection against proliferative lesions in the bladders of Swiss mice fed with a 1% or 2% extract and exposed to BNN/*N*-methyl-*N*-nitrosourea (Bidinotto et al. 2006).

Induction of phase I and II activities may partially account for ginger's anticarcinogenic actions. Banerjee et al. (1994) found that providing 10-μL ginger oil daily for 2 weeks to Swiss mice increased aryl hydrocarbon hydroxylase activity about 25% ($p < .05$) and increased GST by 60% ($p < .01$). No significant increase in GST induction was observed in Swiss mice fed with 160 mg ginger/gram diet (Aruna and Sivaramakrishnan 1990).

Inflammation is a significant risk factor for cancer, including prostate cancer. Mitogen-activated protein kinase phosphatase-5 (MKP5) is implicated as a proinflammatory inhibitor in innate and adaptive immune response in vivo (Zhang et al. 2004). Providing [6]-gingerol upregulated MKP5 expression in normal prostate epithelial cells treated with 50 μM gingerol; likewise, it upregulated MKP5 expression in human prostate cancer cell lines (DU145, PC-3, LNCaP and LAPC-4; Nonn, Duong, and Peehl 2007). Ginger extracts, more so than their individual components, have been shown to inhibit lipopolysaccharide-induced prostaglandin E_2 (PGE_2) production to an extent similar to that of indomethacin, a nonsteroidal anti-inflammatory drug. Subfractions of ginger extract decreased LPS-induced COX-2 mRNA expression levels, although apparently not through the nuclear factor κB (NF-κβ) or activating protein 1 (AP-1) transcription factor pathways, because the ginger extracts did not inhibit TNF-α production (Lantz et al. 2007). [6]-paradol, another active compound in ginger, is reported to induce apoptosis in human promyelocytic leukemia cells, JB6 cells, an oral squamous carcinoma cell line, and Jurkat human T-cell leukemia cells in a dose-dependent manner (Huang, Ma, and Dong 1996; Lee and Surh 1998; Keum et al. 2002; Miyoshi et al. 2003). It is unclear whether [6]-paradol has molecular targets similar to [6]-gingerol.

Ginger also appears to have antitumorigenic properties. Several cell lines have been examined for their sensitivity to ginger. For example, alcoholic extracts of ginger inhibited tumor cell growth for Dalton's lymphocytic ascites tumor cells and human lymphocytes at concentrations of 0.2–1 mg/mL in vitro (Unnikrishnan and Kuttan 1988). In a study of cytotoxic activities of several compounds in ginger against four tumor cell lines (A549, human lung cancer; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; and HCT-15, human colon cancer), [6]-shogaol was the most potent (ED_{50}: 1.05–1.76 μg/mL), and [4]-, [6]-, [8]-, and [10]-gingerol displayed moderate cytotoxicity (ED_{50}: 4.92–30.05; Kim et al. 2008). Adding [6]-gingerol (25 μM) has been reported to inhibit proliferation in rat ascites hepatoma cells AH109A and increase apoptosis at higher concentrations (50 μM; Yagihashi, Miura, and Yagasaki 2008). Likewise, adding [6]-shogoal (60 μM) to COLO295 cells has been reported to increase the expression of GADD153, a gene that promotes apoptosis (Chen et al. 2007). [6]-shogaol (>50 μM) also provokes DNA damage and apoptosis through an oxidative stress-mediated caspase-dependent pathway (Chen et al. 2007). Similarly, incubation of HEp-2 cells with ginger (250 μg/mL, 500 μg/mL, or 1000 μg/mL) resulted in a dose-dependent decrease in nitrite generation, increased production of superoxide, and decreased GSH levels compared to untreated cells, indicating ginger-induced apoptosis through the generation of ROS (Chen et al. 2007).

Ginger is also recognized for its potential usefulness to reduce nausea. To determine whether ginger had antiemetic effects in cisplatin-induced emesis, Manusirivithaya et al. (2004) conducted a randomized, double-blinded, crossover study in 48 gynecologic cancer patients. The addition of ginger (1 g/day) to a standard antiemetic regimen has no advantage in reducing nausea or vomiting in the acute phase of cisplatin-induced emesis. In the delayed phase, ginger and metoclopramide have no statistically significant difference in efficacy (Manusirivithaya et al. 2004). In another study, 1000 mg of ginger was compared to 20-mg intravenous (IV) metoclopramide, and to 4-mg IV ondansetron in

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controlling nausea in patients receiving cyclophosphamide chemotherapy. Ginger was determined to be as effective as metoclopramide, but neither was as effective as ondansetron (Sontakke, Thawani, and Naik 2003).

Overall, while the anticancer findings of ginger are intriguing and several processes may be associated with the observed responses, additional studies are needed to clarify the underlying mechanisms and to determine overall benefits to humans (Pan et al. 2008).

17.13 ROSEMARY

Rosemary (*Rosmarinus officinalis*) is a woody herb with fragrant needle-like leaves. Rosemary is native to the Mediterranean region and possesses a bitter, astringent taste and highly aromatic characteristics that complement a wide variety of foods. Rosemary is a member of the family Lamiaceae, and it contains a number of potentially biologically active compounds, including antioxidants such as carnosic acid and rosmarinic acid. Other bioactive compounds include camphor (up to 20% in dry rosemary leaves), caffeic acid, ursolic acid, betulinic acid, rosmaridiphenol, and rosmanol.

Due to its high antioxidant activity, crude and refined extracts of rosemary are now widely available commercially (Ho et al. 2000). While the data are difficult to interpret, when rosemary is added along with other herbs to a balsamic vinegar preparation used in soups and salads, it appears to provide protection again oxidative stress in humans (Dragan et al. 2007).

Considerable evidence also suggests that rosemary extracts, or its isolated components, can retard chemically induced cancers. For example, topical application of a rosemary extract has been reported to block the initiation and promotion phases of B(*a*)P- and DMBA-mediated skin tumorigenesis (Huang et al. 1994). Likewise, topical application of pure carnosol and ursolic acid also inhibited 12-0-tetradecanoylphorbol 13-acetate (TPA)-induced skin tumor promotion in DMBA-initiated mice (Huang et al. 1994). Adding rosemary or carnosol has also been shown to retard DMBA-induced mammary cancer in rats (Singletary, MacDonald, and Wallig 1996). The depression in tumors may occur because of a change in the types and amounts of DMBA adducts bound to DNA (Amagase et al. 1996). Although not extensively studied, such evidence suggests the ability of rosemary to influence drug-metabolizing enzymes.

Rosemary extracts and the active compounds carnosic acid and rosmarinic acid have been found to inhibit the proliferation of various human cancer cell lines, including NCI-H82 (human, small cell lung carcinoma), DU145 (human prostate carcinoma), Hep-3B (human [black] liver carcinoma), K-562 (human chronic myeloid leukemia), MCF-7 (human breast adenocarcinoma), PC-3 (human prostate adenocarcinoma), and MDA-MB-231 (human breast adenocarcinoma; Yesil-Celiktas et al. 2010). Part of the antitumorigenic properties associated with rosemary may relate to a decrease in TNF-α-induced ROS generation and NF-κB activation, and thus enhanced TNF-α-induced apoptosis (Moon et al. 2010). Carnosol was the most effective in reducing tumor proliferation. Carnosol is also known to induce apoptotic cell death in high-risk pre-B acute lymphoblastic leukemia (ALL; Dorrie, Sapala, and Zunino 2001). At least part of this response may relate to a decrease in Bcl-2. Although carnosol may be effective, it may also interfere with the actions of some other antitumor agents. Zunino and Storms (2009) reported that carnosol decreased the percentage of cell death in the pre-B ALL lines SEM, RS4;11, and REH when combined with cytarabine, methotrexate, or vincristine compared to these chemotherapeutic agents alone. Overall, these data suggest that carnosol, and possibly other constituents in rosemary, may block the terminal apoptotic events induced by some chemotherapeutic drugs and therefore may decrease the effectiveness of some standard therapies for leukemia.

17.14 SAFFRON

Saffron is a spice derived from the flower of the saffron crocus (*Crocus sativus*) plant native to Southwest Asia. It has historically been the world's most expensive spice per unit weight. Saffron

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imparts a bitter taste and hay-like fragrance to food. Saffron likely contains more than 150 volatile and aroma-yielding compounds. A carotenoid, α-crocin, comprises >10% of dry saffron's mass and is responsible for the rich golden-yellow hue created when saffron is added to food dishes. Picrocrocin, a bitter glucoside, is responsible for saffron's flavor.

Significant information points to the ability of saffron to inhibit cancer (Abdullaev 2003). Aqueous saffron preparations have been reported to inhibit chemically induced skin carcinogenesis (Das, Chakrabarty, and Das 2004). Both changes in carcinogen bioactivation and tumor proliferation appear to occur. Saffron infusion given orally either before or after DMBA treatment increased GST, GPx, catalase, and superoxide dismutase in liver (Das, Das, and Saha 2010).

Saffron and crocus also have significant antitumorigenic properties. Similar to other spices, they appear to suppress cell growth in neoplastic cells to a greater extent than in normal cells (Aung et al. 2007). The ability of crocin to decrease cell viability occurs in a concentration- and time-dependent manner (Bakshi et al. 2009). The response is not limited to cells in culture because pancreatic xenografts are also influenced by saffron (4 mg/kg diet for 30 days; Dhar et al. 2009). The effects of tumor suppression also have an impact on the longevity of the host. A significant increase in the life span of Dalton's lymphoma-bearing animals was found in those provided with saffron (Bakshi et al. 2009).

The mechanism by which saffron suppresses tumor proliferation has not been adequately explored, but a shift in caspases and an increase in Bax protein are possible (Mousavi et al. 2009). When a saffron extract (200–2000 μg/mL) was added to MCF-7 cells in culture, there was a marked decrease in cell viability as concentration and duration of exposure increased (IC_{50} of 400 ± 18.5 μg/mL after 48 hours). Analysis of DNA fragmentation by flow cytometry revealed apoptotic cell death in these cells (Mousavi et al. 2009). Saffron-induced apoptosis was inhibited by pan-caspase inhibitors, indicating the importance of this process in determining the response.

17.15 THYME

Thyme is another culinary and medicinal herb. Today, common usage refers to any or all members of the plant genus *Thymus*, also of the Lamiaceae family. Several active agents are reported, including thymol, carvacrol, apigenin, luteolin, tannins, γ-terpinene, and other oils (Aydin, Basaran, and Basaran 2005; Kluth et al. 2007).

Feeding thyme leaves (0.5% or 2.0%) or its phenolic compounds, thymol and carvacrol (50–200 mg/kg), has been reported to enhance xenobiotic-metabolizing enzymes, including phase I enzymes such as 7-ethoxycoumarin O-deethylase and phase II enzymes such as GST and quinone reductase (Sasaki et al. 2005). Admittedly, the isolated components were more effective than feeding the leaf. Kluth et al. (2007) examined the effects of thyme on enzyme induction in cultured human liver carcinoma cells and human colon adenocarcinoma cells. They observed a thyme extract to activate CYP3A4 promoter via PXR and the GI-GPx promoter via the electrophile responsive element, thus providing potential clues about the mechanism by which thymol and carvacrol may influence phase I and II enzyme expression (Kluth et al. 2007). The number of studies on genotoxic effects of thymol and carvacrol are limited, but contradictory. *S. typhimurium* tests have provided some, but not compelling, evidence that thyme is weakly mutagenic (Stammati et al. 1999). In vivo, 0.25% thyme had no detectable effect on mouse embryo development (Domaracky et al. 2007). In comet assays with human lymphocytes, thymol and carvacrol did not induce DNA strand breakage at concentrations lower than 50–100 μM, and were therefore considered safe for consumers (Undeger et al. 2009).

17.16 CONCLUSION

Mounting evidence suggests that cancers are not an inevitable consequence of aging but are preventable diseases. The evidence in this chapter suggests that spices may be factors in one's diet that may

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lower cancer risk and affect tumor behavior. Spices have been consumed for centuries for a variety of purposes, such as flavoring agents, colorants, and preservatives. This chapter only scratches the surface of the overall impact of herbs and spices since there are approximately 180 spices commonly being used for culinary purposes. Without question, evidence exists that multiple processes, including proliferation, apoptosis, angiogenesis, and immunocompetence, can be influenced by one or more spices. While the currently available data are intriguing, considerably more information is needed to determine who will benefit most from exaggerated intake of one or more spices, the effective exposures needed to bring about the desired outcome(s), and what interactions (both positive and negative) exist with other components of the diet or with medications that an individual may regularly consume.

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18 Herbal Treatment for Dermatologic Disorders

Philip D. Shenefelt

CONTENTS

18.1 Introduction 383
18.2 Background and Context 384
18.3 Herbal Treatments for Dermatologic Disorders 385
18.3.1 Acne 385
18.3.2 Alopecia 386
18.3.3 Bacterial and Fungal Infections of Skin 387
18.3.4 Chronic Venous Insufficiency 387
18.3.5 Dermatitis 388
18.3.6 Herpes Simplex 392
18.3.7 Herpes Zoster 392
18.3.8 Hyperhidrosis 392
18.3.9 Pruritus 392
18.3.10 Psoriasis 393
18.3.11 Psychosomatic 394
18.3.12 Scabies 394
18.3.13 Skin Cancer 394
18.3.14 Verruca Vulgaris and Condyloma Accuminata 396
18.3.15 Vitiligo 396
18.3.16 Wounds and Burns 396
18.4 Adverse Effects of Herbal Therapy 397
18.5 Caveats Concerning Herbal Therapy and Dermatologic Surgery 398
18.6 Research Needs 399
18.7 Conclusions 399
References 399

18.1 INTRODUCTION

Herbal therapy for skin disorders has been used for thousands of years. Even our biologically close relatives, the great apes, use herbal self-medication (Huffman 2001). Specific herbs and their uses developed regionally, based on locally available plants and through trade in ethnobotanical remedies. Systems of herbal use developed regionally in Europe, the Middle East (Ghazanfar 1994), Africa, India (Behl and Srivastava 2002), China, Japan, Australia, and the Americas. Two well-known systems still in use are the Ayurvedic herbs in India (Kapoor 1990) and herb combinations developed as part of traditional Chinese medicine (TCM) in China (Xu 2004). In Europe and the United States, use of herbs declined as purified extracts and synthetic chemical drugs became available. In recent years, there has been a resurgence of the use of herbs due to the following reasons: the side effects of chemical drugs became apparent, there was a call to return to nature, natural remedies became a part of the green revolution, and there was a return to organic produce. Herbal

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remedies, including those for skin disorders, are currently gaining popularity among patients and to a lesser degree among physicians. In Asia, especially in China and India, herbal treatments that have been used for centuries are now being studied scientifically. In Germany, the regulatory authority Commission E oversees herbal preparations and their recommended uses (Blumenthal et al. 1998). Currently, the United States does not regulate herbal products except as dietary supplements. There is no standardization of active ingredients, purity, or concentration. There are also no regulations governing which herbs can be marketed for specific indications.

Included in this review of herbal medications are those medications that show scientific evidence for clinical efficacy, as well as the more common herbs found to be useful in the treatment of dermatologic disorders. Information regarding the safety of each herb is also included in this chapter to better enable physicians to decide which herbal therapies they may want to use in practice. Common drug interactions and the side effects of herbal medicines that may be seen in the dermatologic setting are also included in this discussion.

18.2 BACKGROUND AND CONTEXT

In India, records of Ayurvedic medicine date back to about 3000 BC. The system of Ayurvedic medicine combines physiological and holistic principles. It is based on the concept that the human body consists of five energy elements that also make up the universe: (1) earth, (2) water, (3) fire, (4) air, and (5) space. The interactions of these five elements give rise to the three *doshas* (forces), seven *dhatus* (tissues), and three *malas* (waste products). All diseases are attributed to an imbalance among the three *doshas* (Bedi and Shenefelt 2002). Diagnosis is made by an elaborate system of examining the physical findings, pulse, and urine, as well as by an eightfold detailed examination to evaluate both the physical and mental aspects of the condition. The treatment is then tailored to suit an individual based on the findings (Routh and Bhowmik 1999).

Records of TCM date back to about 4000 years. Similar to Ayurvedic medicine, TCM also is aimed at treating the whole person. It is based on the complementary forces *yin* and *yang*. In healthy individuals, yin and yang are in balance, and illness occurs when there is inequality between the forces. The Chinese also recognize five elements: (1) earth, (2) water, (3) fire, (4) air, and (5) metal, each related to specific organs. In addition, they recognize a flow of energy, called *chi* or *qi*, through the body in 14 major meridians. The Chinese evaluate the exchange between the environment and the body, such as food, drink, and air into the body and waste leaving the body. Special attention is given to the physical examination of the tongue, iris, and pulses of the individual to determine the cause of the imbalance and then to determine the appropriate individual treatment. Treatment is usually a mixture of herbs, massage, and acupuncture (Latchman et al. 1994). An entire textbook on dermatology in TCM is available (Xu 2004).

In Western medicine, herbal therapy began as folk medicine. In the United States, it began in the colonial days, when homemade botanicals were used by women at home (Winslow and Kroll 1998). Native American use of botanical treatments also greatly influenced the use of herbal therapy in the United States. Iroquois medical botanicals in the northeastern United States became well known to the colonists (Herrick 1995). In the nineteenth century, these Old World European and Native American traditions were expanded and used by a group of physicians known as the "eclectics." As herbal medicine continued to develop in the United States, it was further influenced by European and Chinese practices (Winston and Dattner 1999).

Herbal therapy has increased in popularity in the past two decades among patients seeking alternative treatments to conventional Western allopathic medicine. The number of visits to alternative medicine practitioners in the United States has grown rapidly and in 1997 it was estimated to be 629 million, surpassing the number of visits to all primary care physicians (Neldner 2000). Approximately US$27 billion was spent on these alternative therapies in 1997, of which US$3.24 billion was spent on herbal therapy (Klepser and Klepser 1999). It has been estimated that about 50% of the population uses some form of alternative medicine. Many patients choose not to tell this

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information to their physicians. The group most likely to use unconventional treatment modalities according to a previous survey consisted of nonblack, college-educated individuals between the ages of 25 and 49 years, having an annual income greater than US$35,000 (Eisenburg et al. 1993). Most patients seek alternatives because conventional therapy has failed to help them sufficiently or because they feel there are fewer side effects with the natural products. The recent increase in the use of alternative medicine has led to more research regarding alternatives and requires education of physicians on the subject to enable them to better inform and care for their patients. In the United States, herbal remedies continue to be sold as dietary supplements, with no standards of potency and efficacy required currently. The Dietary Supplement Health and Education Act of 1994 did set purity standards for some commonly used herbs. In Germany, a regulatory authority known as Commission E extensively reviewed common European botanicals. In all, Commission E evaluated the quality of evidence for the clinical efficacy, safety, and uses of 300 herbal preparations (Blumenthal et al. 1998; Bisset and Wichtl 2001). In Germany, this information has led to standardization of herbal treatments. A number of herbal therapies have stood the test of time for their efficacy in treating dermatologic conditions, with a few having significant scientific evidence of usefulness.

With alternative herbal therapies, an individual patient often treats himself or herself, many times without high-quality professional advice. Patients are advised to ensure the safe use of herbal therapies by deciding on health goals; informing themselves on efficacy, safety, interactions, and usage of the medicine; selecting therapies that are likely to achieve their goals; having a correct diagnosis before using the therapy; consulting reputable practitioners; informing the practitioners about all the remedies they are using; monitoring the effects of the remedies, both positive and negative; waiting patiently for effects to become noticeable; and adjusting doses as needed to accommodate surgery, illness, or changes in conventional therapy (Dunning 2003). Product-labeling information that the patient should look for includes the name and composition of the product, including the parts of the plant and quantity of raw material used, daily dosage and timing of dosages, allergy and other warning statements, quality and safety testing, expiration date, manufacturer, country of manufacture, claims and indications for use, and details on how to store the product (Kron 2002). The *Botanical Safety Handbook* (McGuffin et al. 1997) places herbs in different classes of safety, with Class 1 herbs being safe to consume appropriately, Class 2 safe to consume with restrictions (2a for external use only, 2b not for use in pregnancy, 2c not for use while nursing, and 2d indicating other specific restrictions), Class 3 herbs restricted to use only when supervised by an expert, and Class 4 herbs have insufficient data for classification of safety.

18.3 HERBAL TREATMENTS FOR DERMATOLOGIC DISORDERS

Most common dermatologic disorders have beneficial herbal treatments available. The disorders are listed in alphabetical order below.

18.3.1 Acne

Fruit acids, such as citric, gluconic, gluconolactone, glycolic, malic, and tartaric acids, used topically have demonstrated some effectiveness in treating acne because of their exfoliative properties. In one study, gluconolactone was found to be as effective in clearing inflamed and noninflamed acne lesions as 5% benzoyl peroxide and more effective than placebo (Hunt et al. 1992). Irritation is the main adverse effect of fruit acids, especially in higher concentrations. When contained in the fruit, they are Class 1.

Tannins have natural astringent properties and are used topically to treat acne. Witch hazel (*Hamamelis virginiana*) bark extract is commonly used as a household remedy by making a decoction from 5 to 10 g of herb in 1 cup (0.24 L) of water. Witch hazel is considered very safe to use topically and is Class 1 (McGuffin et al. 1997; Peirce, Fargis, and Scordato 1999). Similar astringents can be made from white oak tree bark or the English walnut tree bark. These preparations should be

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strained before use and can be used two or three times a day. Commercially available preparations are not astringent, as the tannins are lost in the distillation process (Buchness 1998).

Tea tree oil is an essential oil extracted from the leaves of *Melaleuca alternifolia*, a small tree indigenous to Australia. It contains approximately 100 compounds, mainly plant terpenes and their corresponding alcohols (Swords and Hunter 1978). A study of 124 patients compared 5% tea tree oil in a water-based gel with 5% benzoyl peroxide. Although the tea tree oil did not act as rapidly as benzoyl peroxide, it did show statistical improvement in the number of acne lesions at the end of 3 months, and there was a significantly lower incidence of adverse effects such as dryness, irritation, itching, and burning with tea tree oil (44%) than with benzoyl peroxide (79%; Peirce, Fargis, and Scordato 1999). There have been occasional reports of allergic contact dermatitis (de Groot and Weyland 1993; Knight and Hansen 1994; Selvaag, Eriksen, and Thure 1994) and of poisoning if taken internally (Elliot 1993; Moss 1994). However, it is the degradation products of monoterpenes in the tea tree oil that actually appear to be the sensitizing agents (Hausen, Reichling, and Harkenthal 1999). Hence, topical treatment is considered very safe.

Oral administration of vitex (*Vitex agnus-castus*) is effective in treating premenstrual acne. The whole-fruit extract has an amphoteric hormone-regulating effect that is thought to act on follicle-stimulating hormone and luteinizing hormone levels in the pituitary to increase progesterone levels and reduce estrogen levels. It is included in Classes 2b, 2c, and 2d, and may counteract the effectiveness of oral contraceptives. The German Commission E monographs recommend a dose of 40 mg/day. The main adverse effects reported are gastrointestinal tract distress and occurrence of rashes. It should not be taken by pregnant or nursing women (Fleming 2000).

Bitter herbs that stimulate digestive function, including acid secretion, may improve acne (Yarnell and Abascal 2006). Commission E also approved topical bittersweet nightshade (*Solanum dulcamara*; Fleming 2000) and orally administered brewer's yeast (*Saccharomyces cerevisiae*; Fleming 2000, 118) for the treatment of acne because of their antimicrobial effects. Topical duckweed (*Lemna minor*) is used in China to treat acne (Fleming 2000). Herbal mixtures are also used in China both internally and externally to treat acne (Xu 2004).

18.3.2 Alopecia

Essential oils have been studied in a randomized, controlled, double-blind study of 86 patients with alopecia areata (Hey, Jamieson, and Ormerod 1998). A mixture of essential oils including thyme, rosemary, lavender, and cedarwood in carrier oils with grape seed and jojoba (a liquid wax) was massaged into the scalp daily. The control group massaged only the carrier oils into the scalp. Success was evaluated on the basis of sequential photographs, by both a six-point scale and a computerized analysis of areas of alopecia. The treatment group had a statistically significant improvement over the control group (44% vs. 15%). There were no reported adverse effects.

A double-blind study that lasted 6 months and in which 396 patients participated evaluated the topical use of a Chinese herbal formula, Dabao (manufactured by Engelbert & Vialle, Venlo, Netherlands), for the treatment of androgenic alopecia (Kessels et al. 1991). The ingredients of Dabao include 50% ethanol, 42% water, and 8% Chinese herbal extracts, including saffron flowers, mulberry leaves, stemona root, fruits of the pepper plant, sesame leaves, skin of the Szechuan pepper fruit, ginger root, Chinese angelica root, bark of the pseudolarix, and fruit of the hawthorn plant. The ingredients of the placebo included 50% ethanol, 48% water, and 2% odorizing and coloring agents consisting of cherry laurel water, cinnamon water, licorice syrup, sugar syrup, and a solution of burned sugar. In both groups, there was an increase in nonvellus hairs. Although the Dabao group was statistically superior to the placebo group in number of nonvellus hairs, the cosmetic improvement in both groups was minimal. There were no reported adverse effects. Other TCM herbal mixtures have also been used for alopecia areata (Xu 2004).

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18.3.3 Bacterial and Fungal Infections of Skin

Garlic (*Allium sativum*) contains ajoene, which has been demonstrated to exhibit antifungal activity. In a study of 34 patients treated topically with 0.4% ajoene cream once a day for tinea pedis, 79% noted clearing within 7 days and the remainder reported clearing within 14 days. In a 3-month follow-up, all participants remained free of fungus (Ledezma, De Sousa, and Jorquera 1996). Contact dermatitis has occasionally been reported with frequent topical exposure (Fleming 2000). Oral administration should be avoided while breast-feeding as this is regarded as a Class 2c herb (McGuffin et al. 1997). Prolonged bleeding may occur when garlic is taken orally (Fleming 2000).

Tea tree oil (see Section 18.3.1 for a description of tea tree oil) is applied topically for treatment of bacterial and fungal infections. Tea tree oil has shown in vitro activity against a wide variety of microorganisms, including *Propionibacterium acnes, Staphylococcus aureus, Escherichia coli, Candida albicans, Trichophyton mentagrophytes*, and *Trichophyton rubrum* (Beylier 1979; Williams, Home, and Zang 1988). Tea tree oil 10% cream was compared in a randomized, double-blind trial of 104 patients with 1% tolnaftate cream and placebo cream. Although symptomatic relief was comparable in tea tree oil and tolnaftate groups, there was significantly greater mycologic cure in the tolnaftate group (85%) than the tea tree oil group (30%). Cure rates between the tea tree oil and placebo groups were not statistically different (Tong, Altman, and Barnetson 1992). Another randomized, double-blind study of 117 patients compared a solution of 100% tea tree oil with 1% clotrimazole solution in the treatment of onychomycosis. The two groups showed comparable results after 6 months of treatment in terms of mycologic cure (11% for clotrimazole and 18% for tea tree oil), clinical assessment, and subjective rating of appearance and symptoms (61% for clotrimazole and 60% for tea tree oil; Buck, Nidorf, and Addini 1994). Tea tree oil may thus have a role in at least the symptomatic treatment of tinea pedis, onychomycosis, and other superficial wounds. However, it should not be used on burns because of its cytolytic effect on epithelial cells and fibroblasts (Faoagali, George, and Leditschke 1997).

Thyme oil from thyme (*Thymus vulgaris*) has been used topically as an antibacterial and an anticandidal agent (van Wyk et al. 2004), and is Class 1 (McGuffin et al. 1997). The traditional Korean antifungal herb *Galla rhois* was found to have a methanol extract active against *Candida albicans* (Seong 2007). The TCM herbal mixtures for treating bacterial and fungal infections of the skin are extensively discussed by Xu (2004).

18.3.4 Chronic Venous Insufficiency

Chronic venous insufficiency (CVI) and varicosities occur in at least 10–15% of men and 20–25% of women (Callam 1994) and results in and morbidity. Compliance with current treatments such as compression stockings is poor, leading to the search for alternative therapies (Abascal and Yarnell 2007).

The German Commission E approves the oral administration of butcher's broom (*Ruscus aculeatus*) and sweet clover (*Melilotus officinalis*) for relief from symptoms such as pain, heaviness, pruritus, and swelling associated with venous insufficiency. In animal studies, butcher's broom was demonstrated to increase venous tone and to also exhibit diuretic properties, whereas sweet clover was found to increase venous reflux, better termed "venous return" (Fleming 2000). Both butcher's broom, which is Class 1, and sweet clover appear to be safe when used as recommended (McGuffin et al. 1997; Fleming 2000).

Ginkgo (*Ginkgo biloba*) has been used orally in China for centuries and has come to use more recently in Europe and the United States for treating numerous conditions, including heart disease, asthma, vertigo, tinnitus, impotence, cerebral and vascular insufficiency, peripheral vascular disorders, dementia, and other conditions. Research indicates that ginkgo promotes vasodilation, thereby improving many of these conditions. Most research on ginkgo focuses on cerebral insufficiency and

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claudication. Studies suggest ginkgo may be more useful for these vascular disorders than for CVI (Hadley and Petry 1999; Peirce, Fargis, and Scordato 1999). Caution should be used when ginkgo is taken orally, as there have been reports of subarachnoid and intracerebral hemorrhage, as well as increased bleeding time (Fleming 2000), although it is Class 1 (McGuffin et al. 1997).

Several double-blind trials conducted in France studied the effects of grape seed (*Vinus vinifera*) extract on CVI. Grape seed extract contains oligomeric proanthocyanidins, which are bioflavonoids demonstrated to be beneficial by strengthening capillaries. Dosages in the studies varied from 50 mg orally once a day to 100 mg thrice a day. No serious adverse effects were reported (Fleming 2000).

Horse chestnut seed extract (HCSE) is one of the most researched herbal alternatives. Horse chestnut (*Aesculus hippocastanum*) contains the plant compounds known as "terpenes," with the most active component being aescin (Peirce, Fargis, and Scordato 1999). The mechanism of action appears to be related to the inhibition of leukocyte activation, an important pathophysiological mechanism contributing to CVI. Aescin is also thought to decrease vascular leakage by inhibiting elastase and hyaluronase, which are involved in proteoglycan degradation at the capillary endothelium (Pittler and Ernst 1998). Many double-blind, randomized trials of orally administered HCSE have been conducted on patients with CVI. It was demonstrated that HCSE decreases lower-leg volume as well as calf and ankle circumference. Patients also showed decreased symptoms such as fatigue, tenderness, and pruritus. One study showed the relative equivalency of using HCSE compared with grade II compression stockings for treatment of CVI (Diehm 1996). Most of the studies achieved statistically significant results for treatment of CVI with doses of HCSE containing 100–150 mg of aescin per day, most often taken as 50 mg twice a day. Adverse effects reported were minimal and included gastrointestinal tract discomfort, dizziness, headache, and pruritus. Rates of reported adverse effects were from 0.9% to 3.0% and in several studies were not statistically different from rates of adverse effects observed with placebo. Although there are no long-term studies of orally administered HCSE in treating CVI and its sequelae, these results seem promising and offer patients a safe alternative to compression stockings. In Europe, HCSE has also been used in the form of a topical gel, lotion, or ointment to reduce inflammation and discomfort associated with varicose veins, phlebitis, and hemorrhoids (Peirce, Fargis, and Scordato 1999).

It must be noted that the seeds of horse chestnut tree are poisonous and must be specially prepared by a reputable manufacturer to remove all toxins. Once the toxins have been removed, it is considered relatively safe when taken orally. There has been one case report of drug-induced lupus attributed to Venocuran (manufactured by Knoll AG, Ludwigshafen, Germany), a drug for venous insufficiency containing HCSE (Peirce, Fargis, and Scordato 1999). Contact dermatitis has occasionally been reported when HSCE was used topically (Bisset and Wichtl 2001).

Witch hazel (*H. virginiana*) contains considerable amounts of tannin (see the details of preparation in Section 18.3.1), making it a useful astringent. It has been used topically to soothe inflammation of the skin and mucous membranes in disorders such as varicose veins and hemorrhoids. Animal research suggests that witch hazel extract has local styptic and vasoconstrictive effects. The alcohol fluid extract has also been found to cause venous constriction in rabbits. It is often used orally for CVI in Europe. Although it appears safe when taken orally and is included in Class 1, the efficacy of such treatment has not been studied well in humans (McGuffin et al. 1997; Blumenthal et al. 1998). Various TCM herbal mixes for treating stasis dermatitis are listed by Xu (2004).

18.3.5 Dermatitis

Arnica is derived from the dried flowers of *Arnica montana* or other arnica species. Although oral administration can cause severe health hazards even in small amounts, preparations for external use are very safe and effective. Arnica has been used for centuries as an anti-inflammatory drug to rub into sore muscles and joints, bruises, insect bites, boils, inflamed gums, acne eruptions, and hemorrhoids. It is also an ingredient found in many seborrheic dermatitis and psoriasis preparations. It is approved by Commission E for topical treatment of skin inflammation (Blumenthal et

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al. 1998). When used as a compress, 1 tablespoon (tbsp; 15 mL) of tincture is mixed with 0.5 L of water; if used as an infusion, 2 g of dried arnica is mixed with 100 mL of water. Cream or ointment preparations should contain a maximum of 15% arnica oil or 20–25% tincture (Bisset and Wichtl 2001; Peirce, Fargis, and Scordato 1999). The active ingredients of arnica are the sesquiterpene lactones such as helanalin, 11α,13-dihydrohelenalin, chamissonolid, and their ester derivatives. These components reduce inflammation by inhibiting the transcription factor nuclear factor κB (NF-κB). The factor NF-κB controls the transcription of many genes, including cytokines such as interleukin (IL)-1, IL-2, IL-6, IL-8, and tumor necrosis factor α, as well as adhesion molecules such as intercellular adhesion molecule 1, vascular cellular adhesion molecule 1, and endothelial leukocyte adhesion molecule 1. It also inhibits many genes responsible for antigen presentation and activation of cyclooxygenase 2 (Lyss et al. 1997). There are reports of contact dermatitis caused by arnica. There are also several reports of irritation when arnica is used at stronger concentrations or for longer periods than are recommended. It is not recommended for use on open wounds or broken skin, and is included in Class 2d (McGuffin et al. 1997). It is important to buy arnica from a reputable source, because it is a protected species in some countries and other plants are substituted fraudulently.

German chamomile (*Matricaria recutita*), a member of the daisy family, has been used for centuries, both internally and externally, for treating many conditions, especially gastrointestinal tract symptoms, oral or skin inflammation, as well as dermatitis. A tea is made by using 2–3 teaspoons (tsp; 10–15 mL) of dried flowers per cup of water and is taken internally or used as a compress. Topical preparations with cream or ointment bases are also used and researched in Germany (Bisset and Wichtl 2001). Studies have demonstrated that topical chamomile is comparable with 0.25% hydrocortisone and shows improvement in sodium lauryl sulfate–induced contact dermatitis (Brown and Dattner 1998). A small double-blind trial found that chamomile significantly decreased the surface area of wounds and, in animal studies, healing time was found to be reduced with chamomile. Chamomile also shows in vitro antimicrobial activities (Peirce, Fargis, and Scordato 1999). The main adverse effect reported is allergic contact dermatitis. Chamomile is considered safe to use topically and orally, and is included in Class 1 (McGuffin et al. 1997). The anti-inflammatory, wound-healing, and antimicrobial effects of German chamomile are attributed to an essential blue oil that contains sesquiterpene alcohol, α-bisabolol, chamazulene, and flavinoids. These substances showed anti-inflammatory and antispasmodic properties in animal studies, due in part to the inhibition of cyclooxygenase and lipoxygenase in vitro. The flavinoids also act by inhibiting histamine release from antigen-stimulated human basophilic polymorphonuclear leukocytes (Brown and Dattner 1998). The substance α-isabolol also demonstrated promotion of granulation tissue in wound healing (Peirce, Fargis, and Scordato 1999). Bittersweet nightshade (*S. dulcamara*) and brewer's yeast (*S. cerevisiae*) are thought to have similar anti-inflammatory and antibacterial effects.

Herbal medicine derived from TCM for the treatment of atopic dermatitis has been reported effective by British studies. In TCM, the body is treated as a whole and the aim of therapy is to restore harmony to the functions of the body (Atherton et al. 1992). A mixture of various herbs is individually formulated for a patient (Sheehan et al. 1992), making it difficult to undertake randomized, controlled trials. Two randomized, placebo-controlled crossover trials were performed in England to study the effects of standardized oral herbal TCM in the treatment of atopic dermatitis cases for which traditional Western therapy had failed (Sheehan et al. 1992; Sheehan and Atherton 1992; Armstrong and Ernest 1999). The investigators were aided by a Chinese physician who created a standardized mixture of 10 herbs useful for treating atopic dermatitis characterized by erythema, lichenification, and plaques of dermatitis in the absence of active exudation or clinical infection. The 10 herbs used were *Potentilla Chinensis*, Class 1; *Tribulus terrestris*; *Rehmannia glutinosa*, Class 2d; *Lophatherum gracile*; *Clematis armandii*, Class 1; *Ledebouriella saseloides*, Class 1; *Dictamnus dasycarpus*; *Paeonia lactiflora*, Class 1; *Schizonepeta tenuifolia*; and *Glycyrrhizia glabra*, Class 1 (Sheehan and Atherton 1992; McGuffin et al. 1997). These herbs were placed in sachets and boiled to make a decoction that was orally administered daily as a tea. The placebo consisted of a decoction made from several herbs with similar smells and tastes that have no known efficacy in

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the treatment of atopic dermatitis. The first study with 37 children demonstrated a median decrease in erythema score of 51.0% in the treatment group compared with only a 6.1% improvement in the placebo group. The percentage surface involvement also decreased by 63.1% and 6.2% for the herb-treated and placebo groups, respectively. In this initial study, no serious adverse effects were found. These 37 children were offered continued treatment with the TCM herbal mixture and were then followed up for 1 year (Sheehan and Atherton 1994). Eighteen children completed the year of treatment and showed 90% reduction in eczema activity scores. The children who withdrew from the study did so because of lack of further response to treatment, unpalatability of the tea, or difficulty in preparation of the treatment. By the end of 1 year, seven patients were able to discontinue therapy without relapse. Asymptomatic elevation of aspartate aminotransferase level was noted in two patients, the levels returning to normal after discontinuing treatment. No serious adverse effects were observed. The design was similar in the other study that involved 31 adult patients with atopic dermatitis (Sheehan et al. 1992). The decrease in erythema and surface damage was statistically superior in the herb-treated group compared with the placebo group. There was also subjective improvement in itching and sleep. These patients also were followed up for 1 year, with reports of continued improvement and no serious adverse effects, although the patients who discontinued treatment noted a relapse in their condition (Sheehan and Atherton 1994). Although the sample sizes were limited, initial results were promising for patients for whom standard therapy had failed. The main limiting issue seemed to be the taste and the preparation of the decoction. It should be emphasized that although no serious adverse effects were noted in this study, careful monitoring of complete blood cell count and liver function is recommended, as liver failure and even death have been reported with these TCM herbs when baseline laboratory values were not followed (Graham-Brown 1992; Mostefa-Kara et al. 1992; Koo and Arain 1998). It is known that the specific herbs used in these studies have anti-inflammatory, antibacterial, antifungal, antihistaminic, immunosuppressant, and corticosteroid-like effects. A few of the ingredients are also smooth muscle relaxants, and inhibit the platelet-activating factor. Several studies have attempted to elucidate the mechanism of action of this group of 10 herbs (Zemophyte, manufactured by Phytotech Limited, Godmanchester, England) in treating atopic dermatitis. Patients with atopic dermatitis are known to have elevated levels of the low-affinity IgE receptor CD23 expressed on circulating monocytes. In studies of IL-4-induced CD23 expression on monocytes, there appeared to be a reduction in CD23 expression when the cells were exposed to the aqueous herb extracts (Latchman et al. 1994, 1996). Another study examined immunologic markers for T cells, macrophages, Langerhans cells, and low-affinity and high-affinity IgE receptors in biopsy specimens of lesional skin treated with Zemophyte compared with biopsy specimens of nonlesional skin (Xu et al. 1997). The investigators found clinical improvement similar to that seen in the aforementioned Sheehan studies, and also found that the improvement was associated with a statistically significant reduction in CD23 antigen–presenting cells. However, an attempt to replicate the Zemophyte double-blind randomized placebo-controlled study in Hong Kong failed to achieve a statistically significant effect of Zemophyte over placebo (Fung et al. 1999). A different TCM herbal mixture called PentaHerbs formula, with *Paeonia suffruticosa* root bark, Class 1; *Phellodentron chinensis* bark, Class 2b; *Lonicera japonica* flower, Class 1; *Mentha haplocalux* aerial part, Class 1; and *Atractylodes lancea* rhizome Class 1 in a ratio of 2:2:2:1:2, known clinically to be useful in the management of atopic dermatitis, was tested on rat peritoneal mast cells and found to suppress histamine release and prostaglandin D2 synthesis (Chan et al. 2008). The bark of the birch tree (*Betula platyphylla* var. *japonica*), which is used to treat atopic dermatitis, was studied in NC/Nga mice. It decreased scratching and skin inflammation, as well as decreasing IgE and IL-4 messenger ribonucleic acid (mRNA) levels, suggesting that it suppresses the T-helper 2 cellular response (Kim et al. 2008). Other TCM herbal mixes for dermatitis are listed by Xu (2004).

Jewelweed (*Impatiens biflora*) is alleged to be useful topically for treating poison ivy contact dermatitis, but research results are conflicting. In one study, treatment with jewelweed was found to be comparable with standard treatment for poison ivy contact dermatitis, and in 108 of 115 patients

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studied, the symptoms cleared within 2–3 days (Lipton 1958). However, in another study, jewelweed extract failed to decrease symptoms of poison ivy dermatitis (Guin and Reynolds 1980). In yet another study, no prophylactic effect of jewelweed in treating poison ivy dermatitis was reported (Long, Ballentine, and Marks 1997). Jewelweed has been said to be most effective if applied to the area where the poison ivy touched as soon as possible after contact, but this aspect was not addressed by the aforementioned studies. There have been no reports of topical jewelweed causing adverse effects (Peirce, Fargis, and Scordato 1999, 365).

Several herbs contain a substance called "mucilage," which is useful topically to soothe and act as an emollient on skin. Heartseases (*Viola tricolor*), Class 1; marshmallow (*Althea officinalis*); English plantain (*Plantago lanceolata*), Class 1; fenugreek (*Trigonella foenum-gaecum*), Class 2b; mullein (*Verbascum thapsus*), Class 1; slippery elm (*Ulmus fulva*), Class 1; and flax (*Linum usitatissimum*) contain mucilages, which act as emollients on and soothe the skin. Mucilage quickly swells into a gooey mass when exposed to water, thereby ameliorating dry or mildly inflamed skin. Mucilage also dries as a mild adhesive and can be used as an herbal bandage for minor wounds (McGuffin et al. 1997; Peirce, Fargis, and Scordato 1999; Fleming 2000).

Oats (*Avena sativa*) have been used topically in baths for hundreds of years for their soothing and antipruritic properties, and they are approved for this use by the German regulatory authority Commission E and are listed as Class 1 (McGuffin et al. 1997; Fleming 2000; Bisset and Wichtl 2001). Colloidal oatmeal turns to a gooey sticky mass when mixed with liquid which can be used to coat the skin and sealing in moisture. This soothing and moisturizing property is attributed to the gluten content of the plant. This can be useful in treating atopic dermatitis as well as idiopathic pruritus of the elderly.

Pansy flower (*V. tricolor* hybrids) infusion is recommended as a nontoxic treatment for seborrheic dermatitis, especially in infants. The infusion is made by mixing 1–2 tsp of flowers per cup of water and is used as a wet dressing. Salicylic acid in concentrations of about 0.3% appears to be the active ingredient. It also contains saponins and mucilage, which have softening and soothing effects. No adverse effects have been reported with topical use, and pansy is included in Class 1 (McGuffin et al. 1997; Peirce, Fargis, and Scordato 1999).

In treating dermatitis, tannins used topically act by coagulating the surface proteins of cells and exudates, thereby reducing permeability and secretion. The precipitated proteins also form a protective layer on the skin (Brown and Dattner 1998). Tannins may also have antimicrobial properties. Tannins found in agrimony (*Agrimonia eupatoria*), Class 1; jambolan bark (*Syzygium cumini*), Class 1; oak bark (*Quercus robur*), Class 2d; English walnut leaf (*Juglans regia*), Class 2d; Labrador tea (*Ledum groenlandicum*); goldenrod (*Solidago* spp.), Class 2d; lady's mantle (*Alchemilla* spp.), Class 1; lavender (*Lavandula angustifolia*), Class 1; mullein (*Verbascum thapsus*), Class 1; rhatany (*Krameria* spp.), Class 1; Chinese rhubarb (*Rheum officinale*), Class 2b, 2c, 2d; yellow dock (*Rumex crispus*), Class 2d; witch hazel bark (*H. virginiana*), Class 1; and St. John's wort (*Hypericum montana*), Class 2d, act as astringents. Oat straw (*A. sativa*) included in Class 1 is also approved for its soothing and antipruritic qualities (McGuffin et al. 1997; Blumenthal et al. 1998; Peirce, Fargis, and Scordato 1999; Fleming 2000; Bisset and Wichtl 2001). One study showed that a witch hazel extract in a phosphatidyl choline base was less effective in reducing erythema from ultraviolet (UV) radiation and cellophane tape stripping in 24 healthy patients than 1% hydrocortisone (Korting et al. 1993). In another clinical trial, one group with atopic dermatitis ($n = 36$) and another group with contact dermatitis ($n = 80$) compared witch hazel extract with control. In the atopic group, the witch hazel was slightly superior in reducing inflammation and itching. There are also anecdotal reports of witch hazel's usefulness in treating atopic dermatitis (Brown and Dattner 1998).

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18.3.6 Herpes Simplex

Lemon balm (*Melissa officinalis*) is a lemon-scented member of the mint family. An essential oil can be steam-distilled from the cut leaves. Topical uses include treatment of herpes simplex and minor wounds. In a randomized, double-blind trial of 116 patients with herpes simplex lesions, 96% reported complete clearing of lesions at day 8 after using 1% balm extract cream five times a day (Wobling and Leonhardt 1994). In another trial where balm extract was placed on lesions within 72 hours of the onset of symptoms, the size of the lesions and healing time were found to be statistically better in the group treated with balm (Brown and Dattner 1998). Tannin and polyphenols appear to be responsible for the antiviral effect of the balm (Peirce, Fargis, and Scordato 1999). Balm is included in Class 1, and is very safe to use both topically and orally (McGuffin et al. 1997; Peirce, Fargis, and Scordato 1999).

Other herbal preparations that have reported in-vitro activity against herpes simplex include *Echinacea* spp., sweet marjoram, peppermint, and propolis, although clinical studies for the latter three have not yet been performed (Peirce, Fargis, and Scordato 1999). A small, randomized, placebo-controlled crossover clinical trial found no statistically significant differences between *Echinacea* extract of 800 mg twice per day for 6 months and placebo controls in treating recurrent genital herpes (Basch et al. 2005). The TCM herbal mixtures for treating herpes simplex are listed by Xu (2004).

18.3.7 Herpes Zoster

Capsaicin, the main ingredient in cayenne pepper (*Capiscum frutescens*, Class 1 internally but Class 2d externally; McGuffin et al. 1997) is available as a cream for the treatment of postherpetic neuralgia. It is applied four or five times a day and initially causes a burning sensation. With continued use, it depletes substance P in the regional peripheral nerves, reducing pain. In China, herpes zoster is commonly treated topically with hibiscus (*Hibiscus sabdariffa*; Fleming 2000). Hibiscus has been proved to be a very safe Class 1 herb, both topically and orally (McGuffin et al. 1997). The TCM herbal mixtures for herpes zoster are listed by Xu (2004).

Herpes zoster and postherpetic neuralgia have been treated with a topical licorice (*Glycyrrhiza glabra, G. uralensis*) Class 1 gel preparation (Lininger 2000). Glycyrrhizen, one of the active components of licorice, has been demonstrated to inhibit the replication of varicella zoster in vitro (Baba and Shigeta 1987). There are so far no clinical studies to support this. Topical use is reported to be very safe, but care should be taken when it is taken orally as it is included in both Classes 2b and 2d (McGuffin et al. 1997).

18.3.8 Hyperhidrosis

By precipitating surface proteins, topical tannins can reduce the openings of sweat ducts and thus reduce sweating locally. Tannins also have antimicrobial properties that help to reduce odorous bacterial by-products (van Wyk and Wink 2004). See Section 18.3.5 for information about specific sources of tannins. Black tea also contains tannins.

18.3.9 Pruritus

Camphor is derived from the camphor tree (*Cinnamomum camphora*) Classes 2b and 2d distillate of the wood (McGuffin et al. 1997, 30). It is toxic in large doses. As an antipruritic, it can be added to lotions or creams at one-half percent. Menthol is derived from Japanese mint (*M. arvensis*), which is included in Class 1 (McGuffin et al. 1997). It has a cooling, antipruritic, and antibacterial effect. Lotions and creams typically contain 1–5% essential oil. As noted in Section 18.3.5, oats also have a soothing, antipruritic effect.

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Tars derived from birch (*Betula* spp.), beech (*Fagus* spp.), or juniper (*Juniperus* spp.) trees (van Wyk and Wink 2004) are antipruritic and antiproliferative. They are used in a 5–10% concentration in creams, gels, and soaps. They are photosensitizing compounds, and judicious exposure to sunlight can be beneficial.

18.3.10 Psoriasis

Aloe vera (*Aloe vera*), which is Class 1 internally and Class 2d externally (McGuffin et al. 1997), has been used for centuries in wound healing and was recently found to be a potential treatment for psoriasis. In a double-blind placebo-controlled study, 60 patients with slight to moderate plaque psoriasis were treated topically with either 0.5% hydrophilic aloe cream or placebo. The aloe-treated group showed statistically significant improvement (83.3%) compared with the placebo group (6.6%). There were no adverse effects reported in the treatment group (Syed et al. 1996).

Capsaicin is the main ingredient in cayenne pepper (*C. frutescens*), which is Class 1 internally but Class 2d externally (McGuffin et al. 1997); it has also been studied for the treatment of psoriasis. In vitro, capsaicin was found to inhibit phorbol ester–induced activation of transcription factors NF-κB and AP-1 (Surh et al. 2000). Two trials showed that 0.025% cream used topically is effective in treating psoriasis. The first study showed a significant decrease in scaling and erythema during a 6-week period in 44 patients with moderate and severe psoriasis (Bernstein et al. 1986). The second was a double-blind study of 197 patients in whom psoriasis was treated with the capsaicin cream four times daily for 6 weeks, with a significant decrease in scaling, thickness, erythema, and pruritus (Ellis et al. 1993). The main adverse effect reported was a brief burning sensation at the application site. Capsaicin is contraindicated on injured skin or near the eyes, and the German authority Commission E suggests it should not be used for more than 2 consecutive days, with a 14-day lapse between applications.

A survey of patients with psoriasis at a large university dermatology practice revealed that 51% of patients used one or more alternative therapeutic modalities (Fleischer et al. 1996). This is consistent with previous Norwegian surveys of patients with psoriasis (Jensen 1990). Herbal therapy is one of the most frequently chosen alternative therapies. Psoriasis has been treated for centuries with herbal preparations, both topical and oral. There are many herbal preparations composed of furocoumarins, which act as psoralens when combined with ultraviolet A (UV-A, 320–400 nm). Furocoumarins derived from *Ammi majus* and related plants that produce 8-methoxy-psoralen when applied topically or taken orally intercalate with DNA. Further, when coupled with exposure to UV-A from the sun or a an ultraviolet light-box, the photoactivation causes cross-linkages with the thymine in the DNA, inducing cell death (van Wyk and Wink 2004). This, in turn, inhibits hyperproliferation in psoriatic lesions.

One commonly used TCM, Radix *Angelicae dahurica*, included in Class 1 (McGuffin et al. 1997), contains the furocoumarins imperatorin, isoimperatorin, and alloimperatorin. In a study involving 300 patients with psoriasis, this TCM, taken orally, was combined with UV-A therapy and was compared with the standard treatment of psoralen—UV-A with methoxsalen. The efficacy of the two treatments was equivalent; however, there were fewer adverse effects such as nausea and dizziness in the group treated with TCM and UV-A (Koo and Arain 1998). In addition, there are topical preparations made from herbs that show systemic efficacy against psoriasis, but are too toxic when given systemically (Ng 1998). Topical TCM of the plant *Camptotheca acuminata* in an open trial including 92 patients with psoriasis found that this TCM was statistically more effective than 1% hydrocortisone. A disadvantage was that allergic contact dermatitis was seen in 9–15% of the patients in the TCM group. Comparison of TCM mixtures in clinical trials is difficult, because the mixture of herbs prescribed varies individually depending on the subtype of psoriasis ("blood-heat" type, "blood deficiency dryness" type, and "blood stasis" type), which is determined in TCM by many findings, including lesions of psoriasis, the pulse, and the condition of the tongue (Koo and Arain 1998).

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Some types of TCM may act in part on the microcirculation of the psoriatic lesion (Zhang and Gu 2007). Additional TCM herbal mixtures for psoriasis are listed by Xu (2004).

About 5% curcumin is present in turmeric (*Curcuma longa*), which is included in Classes 2b and 2d (McGuffin et al. 1997; see also Chapter 13 on turmeric). Turmeric has been used for centuries in India to provide glow and luster to the skin. It has antimicrobial, antioxidant, astringent, and other useful effects that help to heal wounds and reduce scarring (Chaturvedi 2009). In vitro, the purified turmeric extract curcumin has been found to inhibit phorbol ester–induced activation of transcription factors NF-κB and AP-1 (Surh et al. 2000). The resulting suppression of phosphorylase kinase activity correlates with the resolution of psoriasis when curcumin is applied topically to the lesions (Heng et al. 2000). Microencapsulation of curcumin reduces the yellow staining produced by application of topical curcumin on the skin, while prolonging the bioavailability of curcumin (Aziz, Peh, and Tan 2007).

Tars have been used for centuries to treat psoriasis. Tars derived from birch (*Betula* spp.), beech (*Fagus* spp.), or juniper (*Juniperus* spp.) trees (van Wyk and Wink 2004) are antipruritic and antiproliferative. They are used in a 5–10% concentration in creams, gels, and soaps. They are photosensitizing compounds, so judicious exposure to sunlight can be beneficial, or they can be used in conjunction with ultraviolet B (UV-B; 250–320 nm) or narrowband UV-B (311 nm).

18.3.11 Psychosomatic

Depression and anxiety can cause skin problems. Kava kava (*Piper methysticum*) has moderate anxiolytic effects, but its use is not recommended due to its potential hepatotoxicity. It is included in Classes 2b, 2c, and 2d (McGuffin et al. 1997). Lavender oil aromatherapy (*Lavendula* spp.) has been demonstrated to produce significant reduction in anxiety. This may in part be a conditioned response, and it is important that the first exposure to lavender oil is a pleasant and relaxing one. It is Class 1 (McGuffin et al. 1997). Lemon balm (*M. officinalis*) is approved by the German authority Commission E for treating nervousness and insomnia. It is also Class 1 (McGuffin et al. 1997). Magnolia bark (*Magnolia obovata*) has moderate anxiolytic effects. It contains honokiol and magnolol, which have antioxidant and anti-inflammatory (Kuribara, Stavinoha, and Maruyama 1998) effects. It is Class 2b (McGuffin et al. 1997). Passion flower (*Passiflora incarnata*) is approved by Commission E for treating nervousness and insomnia. It is Class 1 (McGuffin et al. 1997). St. John's wort (*H. perforatum*) is approved by Commission E for treating depression. It is helpful for treating mild to moderate depression but not for severe depression (Linde et al. 1996). It has significant interactions with the metabolism of a number of other drugs by inducing cytochrome P450 isoform 3A4, and is Class 2d (McGuffin et al. 1997). Valerian (*Valariana* spp.) is approved by Commission E for treating insomnia caused by nervousness. It is Class 1 (McGuffin et al. 1997).

18.3.12 Scabies

Anise (*Pimpinella anisum*) seeds are a source of an essential oil that displays antibacterial and insecticidal activity in vitro and is used topically to treat scabies and head lice. It should not be used in pregnancy and is Class 2b (McGuffin et al. 1997). Neem (*Azadirachta indica*) is indigenous to India, and every part of the plant is used medicinally. In a study of more than 800 villagers in India, a paste of neem and turmeric applied topically was reported to treat chronic ulcers and scabies (Peirce, Fargis, and Scordato 1999). It seems to be safe for use in adults, but can be poisonous to children (Peirce, Fargis, and Scordato 1999). Numerous other herbs have been used for centuries in India and China to treat scabies (Fleming 2000).

18.3.13 Skin Cancer

Red ginseng (*Panax ginseng*) is a classic TCM. In a recent study, red ginseng extracts used topically were found to inhibit chemically induced skin tumors in mice. This is thought to be due to

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the immuno-modulating properties of red ginseng (Cheng, Lin and Lei et al. 1998). It is Class 2d (McGuffin et al. 1997).

Propolis is a resinous material gathered by honeybees from the buds and bark of certain plants and trees. Propolis has been used for centuries for its antimicrobial, anti-inflammatory, analgesic, and antitumor effects, which are thought to result from the flavinoid and related phenolic acids components. A tumoricidal component, clerodane diterpenoid, has also been isolated. This compound was studied regarding its topical effects on skin tumorigenesis in mice. Clerodane diterpenoid appeared to reduce the incidence of chemically induced dysplastic papillomas by inhibiting the synthesis of DNA in a de novo pathway and by suppressing the growth of tumors by decreasing DNA synthesis in a salvage pathway (Mitamura et al. 1996).

Rosemary (*Rosmarinus officinalis*) extract is reputed to have antioxidant activity. A methanol extract of the leaves was evaluated for its effects on skin tumors in mice. It was found that topically applied rosemary inhibited induction and promotion of skin tumors in mice treated with known chemical carcinogens. Although the exact mechanism of action is still under study, it appears that several components of the extract are important in this process. This finding suggests that it was not the antioxidant properties alone that were beneficial in the prevention of skin tumors (Huang et al. 1994). Rosemary should not be used in pregnancy as it is a Class 2b herb (McGuffin et al. 1997).

Silymarin is a flavinoid isolated from milk thistle (*Silybum marianum*), and is approved by the German Commission E for treating liver disease because of its antioxidant properties. An experiment was performed to assess whether this antioxidant effect would protect against tumor promotion. Topically applied silymarin was found to possess highly protective effects against chemically induced skin tumor promotion in mice. This may involve inhibition of promoter-induced edema, hyperplasia, and proliferation, as well as the oxidant state (Lahiri-Chatterjee et al. 1999). These results are promising, yet more research involving human models is needed. Silymarin is safe to use topically and orally when used appropriately, and is Class 1 (McGuffin et al. 1997).

Tea is manufactured from the leaf and bud of *Camellia sinensis* (see also Chapter 12 on tea). The majority of tea consumed worldwide is in the form of black tea, which is Class 2d (McGuffin et al. 1997). Green tea has been found in several mouse models to have anti-inflammatory and antitumorigenic properties. The polyphenolic constituent (–)-epigallocatechin-3-gallate is thought to be the active ingredient. Numerous studies of green tea and skin cancer were reviewed (Katiyar, Ahmad, and Mukhtar 2000). It was found that topical application or oral consumption of green tea protects against inflammation, chemical carcinogenesis, and photocarcinogenesis. Green tea demonstrated the blocking of many mediators in the inflammatory process important in the early steps of skin tumor promotion. It also appears that there is inhibition of biochemical markers of chemical carcinogenesis, inhibition of UV-induced oxidative stress, and prevention of UV-induced immunosuppression (Katiyar, Ahmad, and Mukhtar 2000) as a result of action of green tea. Green tea also protects against psoralen UV-A–induced photochemical damage to the skin (Zhao Jin and Yaping et al. 1999). Many cosmetics and skin care products have been recently supplemented with green tea, but more research in humans is needed to understand the true benefits. Black tea may also play a role in the prevention of skin tumors. It appears that theaflavins are the components active in chemoprevention (Nomura et al. 2000). Several studies provide evidence that topical application of the constituents of black tea can decrease UV-B-induced erythema, inhibit tumor initiation, and act as an antitumor promoter (Javed, Mehrotra, and Shukla 1998; Zhao Zhang and Jin et al. 1999). Oral administration of black tea was also found to inhibit tumor proliferation and promote tumor apoptosis in nonmalignant and malignant skin tumors (Lu et al. 1997). A survey of older patients compared tea consumption and history of squamous cell carcinoma. There was a lower risk of squamous cell carcinoma in patients who regularly consumed hot black tea than in nonconsumers (Hakim, Harris, and Weisgerber 2000). Different studies comparing the effectiveness of black and green teas in protecting against UV-induced skin tumors give conflicting findings as to which is more beneficial (Wang et al. 1994; Huang et al. 1997; Record and Dreosti 1998; Lou et al. 1999). Caffeinated teas seem to be more protective than decaffeinated teas, and caffeine by itself has some

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inhibitory effects on UV-B-induced carcinogenesis (Wang et al. 1994; Huang et al. 1997; Lou et al. 1999).

18.3.14 Verruca Vulgaris and Condyloma Accuminata

Podophyllin, used to treat condyloma acuminata, is extracted from the root of the American mayapple (*Podophyllum peltatum*; Fleming 2000). It should not be used during pregnancy and is Class 2b externally and toxic internally (McGuffin et al. 1997). Commission E approves bittersweet nightshade (*S. dulcamara*), Classes 2b and 2c, and oat straw (*A. sativa*), Class 1, for the treatment of common warts (McGuffin et al. 1997; Fleming 2000). Calotropis (*Calotropis procera*) is used in India, and greater celandine (*Chelidonium majus*), Classes 2b, 2c, and 2d (McGuffin et al. 1997, 28), is used in China for the treatment of warts (Fleming 2000). Bittersweet nightshade and celandine should also be avoided in pregnancy and while breast-feeding (Fleming 2000).

18.3.15 Vitiligo

Ginkgo (*G. biloba*) was found to be effective in a small study for treating limited, slowly spreading vitiligo (Parsad, Pandhi, and Juneja 2003). Caution should be used when ginkgo is taken orally, as there have been reports of subarachnoid and intracerebral hemorrhage, as well as increased bleeding time (Fleming 2000); but the herb is included in Class 1 (McGuffin et al. 1997).

Psoralens, such as the furanocoumarins derived from *A. majus* and related plants that produce 8-methoxy-psoralen, when applied topically or taken orally, intercalate with DNA. As noted in Section 18.3.10, with photoactivation they can induce cell death (van Wyk and Wink 2004). By thus reducing inflammatory cells while stimulating melanogenesis, the treatment often induces repigmentation of vitiliginous skin.

18.3.16 Wounds and Burns

Aloe vera (*A. vera*) leaves produce a gel and a juice or latex. The gel is obtained from the central core of the leaf and has been used topically for centuries for the treatment of wounds and burns. The juice or latex is a bitter yellow fluid extracted from the inner leaf skin and is generally sold dried as a powder that has very potent laxative effects (Peirce, Fargis, and Scordato 1999). Several case reports and animal studies demonstrate that aloe vera decreases burning, itching, and scarring associated with radiation dermatitis (Klein and Penneys 1988). Aloe vera was also found to accelerate healing of chronic leg ulcers, surgically induced wounds, and frostbite. The mechanism of action has been studied in vivo in animal studies. Aloe vera decreases thromboxane A_2, thromboxane B_2, and prostaglandin 2α, which cause vasoconstriction and platelet aggregation. By increasing dermal perfusion, tissue loss from ischemia is reduced (Klein and Penneys 1988). In vitro studies have also demonstrated a carboxypeptidase that inactivates bradykinin, decreasing pain at the treatment site (Fujita and Shosike 1976). Salicylic acid present in aloe vera acts as an analgesic and anti-inflammatory agent by inhibiting prostaglandin production (Robinson, Heggers, and Hagstrom 1982). Magnesium lactate is also present in aloe vera and is thought to be antipruritic by inhibiting histidine decarboxylase, which controls the conversion of histidine to histamine in mast cells (Klein and Penneys 1988). Reduction in inflammation is also thought to result from the immunomodulatory properties of the gel polysaccharides present, especially the acetylated mannans (Reynolds and Dweck 1999). Aloe vera also demonstrates bactericidal and antifungal activity in vitro. The main adverse effect of topical aloe vera gel is that it causes allergic contact dermatitis. There are also reports of delayed healing after laparotomy or a Cesarean section. Taken orally, aloe vera is considered very safe when used properly. It is Class 1 internally and Class 2d externally (McGuffin et al. 1997).

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Asiaticoside in low concentrations has been found to enhance the healing of burn wounds, with evidence suggesting that enhanced angiogenesis may occur as a result of stimulation of vascular endothelial growth factor production (Kimura et al. 2008).

Honey has been used topically for centuries to assist healing of wounds, including burns, decubitus ulcers, and infected wounds (Greenwood 1993). It has been found in vitro to have antibacterial and antifungal activity against organisms that commonly infect surgical wounds (Efam and Udoh 1992). A study was performed on nine infants with large, open, culture-positive postoperative wound infections for whom standard treatment consisting of appropriate intravenous antibiotics and cleansing with chlorhexidine for more than 14 days had failed. The wounds were then treated with 5–10 mL of fresh, unprocessed honey twice a day. There was marked clinical improvement by day 5, and by day 21, the wounds were all closed, clean, and sterile (Vardi et al. 1998). In a randomized controlled trial, honey-impregnated gauze was compared with a polyurethane film (OpSite, manufactured by Smith & Nephew, North Humberside, England) for partial-thickness burns. The honey-treated wounds healed statistically earlier, with a mean of 10.8 days versus 15.3 days for film-treated wounds and with equal numbers of complications such as infection, excessive granulation, and contracture compared with the polyurethane-film-treated wounds (Subrahmanyam 1993). The wound-healing properties of honey are believed to result from the debriding properties of the enzyme catalase, absorption of edema due to honey's hygroscopic properties, its ability to promote granulation and reepithelialization from the wound edges, and its antimicrobial properties (Efam 1988). There have been no reports of significant adverse effects, although there are reports of contact dermatitis to honey (Efam 1988).

Marigold (*Calendula officinalis*) has been used topically since ancient times and is approved by the German regulatory authority Commission E as an antiseptic and for wound healing (Bisset and Wichtl 2001). A topical preparation of marigold continues to be recommended for the treatment of wounds, ulcers, burns, boils, rashes, chapped hands, herpes zoster, and varicose veins. Marigold gargles are used for mouth and throat inflammation (Peirce 1999). Marigold is also widely used as a topical treatment for diaper dermatitis and other mild skin inflammations (Brown and Dattner 1998). The treatment consists of an application several times a day of an ointment or a cream made by mixing 2–5 g of the flower heads with 100 g of ointment. A gargle or lotion is made by mixing 1–2 tsp (5–10 mL) of tincture with 0.25–0.5 L of water (Peirce 1999). The main adverse event is allergic contact dermatitis. No serious adverse effects have been reported, and it is considered safe to use both topically and orally. It is Class 1 (McGuffin et al. 1997). The anti-inflammatory effects of marigold are ascribed to the presence of triterpenoids. In animal studies, *Calendula* was suggested to stimulate granulation and increase glycoproteins and collagen at wound sites (Brown and Dattner 1998). Marigold also shows in vitro antimicrobial and immune-modulating properties (Peirce 1999).

There are many herbs containing tannins that act as astringents, helping to dry oozing and bleeding wounds. Some of the more commonly reported tannin-containing herbs that may be helpful for the topical treatment of wounds include English walnut leaf, goldenrod, Labrador tea, lavender, mullein, oak bark, rhatany, Chinese rhubarb, St. John's wort, and yellow dock (see Section 18.3.5 for a list of scientific plant names and ratings of toxicity; Peirce 1999).

18.4 ADVERSE EFFECTS OF HERBAL THERAPY

Herbal therapies vary greatly in their safety class ratings. For example, some are consumed as foods and have high safety ratings, whereas others are highly biologically active and toxic and must be used very carefully. The safety classes of the herbs mentioned in this chapter are addressed in each section, and further discussion of interactions of herbal therapies that may be encountered in dermatology is detailed in the remaining sections of the chapter. Many cutaneous reactions to herbal preparations have been reported, with the most common cutaneous adverse event being allergic contact dermatitis. More serious cutaneous reactions have been reported. Two patients developed

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erythroderma after using topical herbal treatments for psoriasis and atopic dermatitis, and one patient developed Stevens–Johnson syndrome after taking "golden health blood-purifying tablets," which contained multiple herbs, including red clover, burdock, queen's delight, poke root, prickly ash, sassafras bark, and *Passiflora* (Monk 1986). Bullous and nodular lichen planus were reported to be induced by ingestion of native African herbal medicines (Soyinka 1973). A young woman was also described with leukemia-related Sweet syndrome elicited by a pathergic response to topical arnica cream (Delmonte et al. 1998).

Serious systemic adverse effects have been reported with the use of TCM herbal mixtures for the treatment of dermatologic disorders. The most common are hepatotoxic effects. Although most patients recover without serious consequences as long as the medication is stopped, there have been reports of patients with acute liver failure leading even to death. There are also reports of renal failure and agranulocytosis (Graham-Brown 1992; Mostefa-Kara et al. 1992; Koo and Arain 1998). One patient was described with adult respiratory distress syndrome after administration of a TCM, kamisyoyo-san, for seborrheic dermatitis (Shota et al. 1961). A patient was reported with reversible dilated cardiomyopathy after receiving treatment for her atopic dermatitis with a Chinese herbal tea (Ferguson, Chalmers, and Rowlands 1997). There are also reports of Chinese and Indian herbal medicines containing as contaminants heavy metals, such as lead, arsenic, and mercury. Prescription medications have also been found in over-the-counter herbal formulations from other countries. Some herbs are mislabeled or misidentified.

There are many possible drug interactions with herbs and prescription medications. It is crucial for patients to share information about what herbs, supplements, and other over-the-counter remedies they are taking or applying to their skin with their physicians. The most important drug interactions in the dermatologic setting are the immune-upmodulating effects of *Echinacea, Astragalus*, licorice, alfalfa sprouts, and vitamin E, and zinc may decrease the efficacy of corticosteroids and immunosuppressants (Miller 1998). Some herbs are reported to cause hepatic damage, and they should not be used in combination with medications such as methotrexate. These include many of the ingredients in TCM preparations, as well as *Echinacea*, chaparral, germander, ragwort, and life root (Ferguson, Chalmers, and Rowlands 1997; Borins 1998). Herbs containing γ-linolenic acid, such as evening primrose oil, which has been used for treating dermatitis, psoriasis, and xerosis, lower the seizure threshold; thus, dosages of anticonvulsants may need to be increased (Ferguson, Chalmers, and Rowlands 1997). Rue (*Ruta graveolens*) and other herbs containing psoralens can cause phototoxic reactions externally on the skin (Eickhorst, Deleo, and Csaposs 2007). In addition to making them aware of the adverse effects already discussed, patients should be counseled on the relative lack of regulation for herbal medicines. There are minimal quality-control requirements currently in place in the United States to ensure the purity, concentration, or safety of herbal supplements. Although herb manufacturers are restricted from making efficacy statements, there are no regulations on claims for what symptoms these herbs can alleviate. In the United States, there are also minimal regulations on which herbs can be restricted in formulations (Shaw 1998).

18.5 CAVEATS CONCERNING HERBAL THERAPY AND DERMATOLOGIC SURGERY

Herbs may affect blood coagulation. A number of medicinal herbs contain coumarin, salicylate, or other platelet-inhibiting substances that can increase the risk of interoperative and postoperative bleeding. Some coumarin-containing herbs include danshen (*Salvia miltiorrhiza*), dong quai (*Angelica sinensis*), horse chestnut bark (*Aesculius hippocastanum*), sweet clover (*M. officinalis*), sweet vernal (*Anthoxanthum odoratum*), sweet-scented bedstraw (*Galium triflorum*), tonka beans (*Dipteryx odorata*), vanilla leaf (*Trilisa odoratissima*), and woodruff (*Asperula odorata*). Salicylate-containing herbs include black cohosh (*Cimifuga racemosa*), meadowsweet (*Spirea ulmaria*), poplar bark (*Populus* spp.), sweet birch bark (*Betula* spp.), willow bark (*Salix* spp.), and wintergreen (*Gaultheria procumbens*). Other platelet function inhibitors include bromelain (*Ananas comosus*), cayenne (*C. frutescens*),

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Chinese skullcap (*Scutullaria baicalensis*), feverfew (*Tanacetum parthenium*), garlic (*A. sativum*), ginger (*Zingiber officinale*), ginkgo (*G. biloba*), ginseng (*Panex ginseng*), onion (*A. cepa*), papain (*Carica papaya*), reishi fruit (*Ganoderma lucidum*), and turmeric (*C. longa*; Pribitkin 2005).

Herbs may also affect blood pressure. Potentially hypertensive plants include black cohosh, ephedra or ma huang (*Ephedra* spp.), licorice (*G. glabra*), and yohimbe (*Pausinystalia yohimbe*). Potentially hypotensive plants include garlic (Pribitkin 2005).

18.6 RESEARCH NEEDS

Further research into the efficacy, safety, optimal uses, and standardization of herbal remedies is clearly needed. Inhibiting factors in the United States include the nonpatentability of herbal materials in a system in which the typical costs of double-blind testing for Food and Drug Administration (FDA) approval of drugs range in the millions of dollars, requiring patentability for private enterprises to attain a profit. Since herbal remedies currently remain in the category of dietary supplements, a different mechanism of funding for research is needed. The funding for complementary and alternative medicines research provided through the National Institutes of Health is meager compared with private and public funding of research for conventional drugs.

18.7 CONCLUSIONS

Many herbal therapies have been used for centuries, which show good anecdotal results. A few randomized, controlled trials have also demonstrated significant results in the use of herbal therapies for the treatment of dermatologic disorders. Some countries, such as Germany, now require standardization of herbal preparations and specific recommendations as to the use and efficacy of herbs in the treatment of disease. It is important to know what common herbal alternatives exist and which potential adverse effects or interactions can occur to permit more effective counseling of patients.

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19 Diabetes and Herbal (Botanical) Medicine

William T. Cefalu, Jaqueline M. Stephens, and David M. Ribnicky

CONTENTS

19.1 Introduction 405
19.2 Historical Use of Botanicals 406
19.3 Proposed Mechanisms of Botanical Actions 407
19.4 Insulin Resistance in Type 2 Diabetes 408
19.5 Botanicals and Mechanisms Relevant to Type 2 Diabetes 409
19.5.1 Bitter Melon (*Momordica charantia*) 409
19.5.2 Fenugreek (*Trigonella foenum-graecum*) 410
19.5.3 Gymnema (*Gymnema sylvestre*) 410
19.5.4 Hoodia (*Hoodia gordonii*) 411
19.5.5 Prickly Pear Cactus (*Opuntia* spp.) 411
19.5.6 Ginseng (*Panax* spp.) 411
19.5.7 Cinnamon (*Cinnamomum cassia, Verum,* and Others) 412
19.5.8 Russian Tarragon (*Artemisia dracunculus* L.) 412
19.5.9 Garlic (*Allium sativum*) 412
19.5.10 Ginkgo (*Ginkgo biloba*) 413
19.5.11 Ivy Gourd 413
19.5.12 Aloe (*Aloe vera*) 413
19.6 Conclusions 414
Acknowledgments 414
References 414

19.1 INTRODUCTION

The epidemic rise in the number of new cases of diabetes is one of the most alarming statistics regarding health issues on a worldwide basis. The major concern regarding this observation relates to the development of the chronic complications associated with the condition. Specifically, the complications of diabetes have been classified as either microvascular—retinopathy, nephropathy, and neuropathy—or macrovascular—cardiovascular disease (CVD), cerebrovascular accidents (CVA), and peripheral vascular disease (PVD). It is well recognized that the complications cause considerable morbidity and mortality worldwide and, as such, negatively affect the quality of life in individuals with diabetes, with an increase in disability and death. The costs of caring for diabetes and its related complications are staggering. For example, in the United States alone, the total estimated financial burden of diabetes was US$174 billion in 2007, and it is expected to be US$330 billion by 2020 due to the expected increase in new cases.

In an individual with diabetes, on a clinical level the major objective is to design a regimen that will improve the metabolic factors associated with the development and progression of

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complications. As such, it is well recognized that a primary strategy is to achieve the target levels recommended for blood pressure, lipids, and glycemia. This strategy may consist of lifestyle modification alone, but more commonly it consists of lifestyle management, that is, dietary modification and enhanced physical activity, combined with pharmacological intervention from agents in multiple classes (Riddle 2005). However, as providers caring for patients with diabetes, we recognize that the patients are very interested in alternative (complementary) strategies that consist of dietary supplementation with over-the-counter agents. Supplementing conventional approaches to medical care with alternative means is extensively practiced by a large number of patients. Interestingly, more times than not, these practices appear to be undertaken without consultation with a medical provider. It is also recognized that this is not a trivial practice and not without substantial financial cost. Specifically, data from the Food and Drug Administration (FDA) of the United States suggest that more than 29,000 different nutritional supplements are available to consumers. It also appears that more than US$12 billion per year may be spent on supplements (Neuhouser 2003; Gibson and Taylor 2005).

Patients may choose to supplement their pharmacological regimen with dietary supplementation in many forms, for example, vitamin and/or mineral mixtures, but the most popular supplements taken by the patients are those considered to be from natural products, that is, herbal or botanical sources. Unfortunately, considerable controversy exists regarding the efficacy of dietary supplements in general and of botanical supplements, particularly regarding pathophysiological factors related to the treatment of patients with type 2 diabetes. The controversy exists because reported efficacy data for many of the natural products are only in the form of uncontrolled studies and anecdotal reports. Poor quality control measures may also cause inconsistent effects for certain natural products. Currently, there is a paucity of consistent and reproducible efficacy data in humans to suggest any recommendations for most botanical or bioactive supplements as adjunct treatments for risk factors related to metabolic syndrome or type 2 diabetes. Firm recommendations for general use would also require an understanding of the mechanism of action, which is not known for most botanicals.

19.2 HISTORICAL USE OF BOTANICALS

From the patient perspective, it is considered very acceptable to include herbal or botanical extracts as part of the medical intervention based on the recognition that the herbal intervention is considered to be natural and that the practice may have been part of the culture for many generations. In this regard, the use of plants and plant extracts to treat a specific disease and/or disease symptoms appears to have been part of medical care as observed for thousands of years. Although the use of plant extracts is no longer a major aspect of medical care as practiced in Western populations, it is still extremely popular in large numbers of the world's population, particularly in Asia and Europe (Griggs 1981). However, for medicine as practiced in Western countries, one observation that appears to be forgotten is that many of the pharmaceutical agents currently prescribed appear to have been derived from natural compounds found in traditional medicinal plants (Evans 2003). As a specific example, biguanide metformin is considered one of the first-line agents used for the treatment of type 2 diabetes, and its use can be traced to the traditional use of *Galega officinalis* to treat diabetes and the subsequent search to identify active compounds with reduced toxicity (Cusi and Defronzo 1998). However, it has been reported that more than 1200 traditional plants may have been used for real or perceived benefit of medicinal purposes for the treatment of diabetes (Marles and Farnsworth 1995; Jung et al. 2006). In this regard, the reader is referred to a number of published reviews on the topic that contain information related to the following: (1) the botanical source of the extract; (2) the history of use by the population; (3) the specific geographic region in the world for which its use has been documented; (4) the proposed benefit of the extract; (5) known side effects; and (6) proposed mechanisms of action (Griggs 1981; Marles and Farnsworth 1995; Roman-Ramos, Flores-Saenz, and Alarcon-Aguilar 1995; Oubre et al. 1997; Dey, Attele, and Yuan 2002; Grover, Yadov and Yats 2002; Shapiro and Gong 2002; Evans 2003; Jung et al. 2006).

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19.3 PROPOSED MECHANISMS OF BOTANICAL ACTIONS

Despite the historical use of botanicals to treat diabetes and its related symptoms, one of the major concerns for this area of study is the paucity of definitive and consistent data on efficacy, and more importantly, a lack of knowledge about precise mechanism(s) of action. These are significant limitations, and in large part these limitations explain why there is considerable skepticism regarding the effectiveness of herbal remedies in Western medicine. However, there is growing evidence in this area, and if a botanical is demonstrated to have a favorable effect on a given mechanism, that will provide the rationale for further and more definitive studies on a particular botanical.

The physiological parameters that regulate glucose metabolism and the pathophysiological changes that occur and that give rise to diabetes have been studied for years. These involve the interplay and function of multiple peripheral tissues, such as liver, muscle, and adipose tissue. In order to exert an effect, botanicals may theoretically modulate glucose at several different levels in multiple tissues (Table 19.1; Cefalu and Ribnicky 2009). Thus, based on reported abnormalities for type 2 diabetes, botanicals could be proposed to affect the whole-body metabolism by modulating adipocyte function and thus, regulating endocrine secretions that play a role to enhance the skeletal muscle insulin action. In addition, based on the known abnormalities, botanicals may regulate hepatic processes, that is, hepatic gluconeogenesis, and may affect the whole-body glucose levels. In this regard, a specific agent termed a "biguanide" (metformin) and derived from botanical sources appears to improve hyperglycemia by regulating hepatic processes. Type 2 diabetes is clearly a disorder that involves insulin secretory defects, and enhancing pancreatic β-cell function is another proposed pathway by which botanicals may theoretically work. Enhancing insulin secretory function may not be solely an acute effect. But as intensively pursued in preclinical and clinical trials, if a particular agent is shown to enhance proliferation and/or modulate apoptosis of islet tissue, this may markedly impact the natural progression of diabetes. There is no evidence to date that any of

TABLE 19.1
Postulated Mechanisms by Which Botanicals May Alter Whole-Body Carbohydrate Metabolism

Tissue/Organ	Postulated Mechanisms
Adipocytes/adipocyte function	Adipogenesis
	Modulating adipocyte secretion, for example, adiponectin, leptin, resistin
	Lipolysis
Liver	Hepatic Metabolism
	Modulating gluconeogenesis and Glycogenolysis
	Enhancing hepatic sensitivity
Central nervous system	Satiety
Skeletal muscle	Insulin receptor binding/receptor number
	Insulin signaling—increased tyrosine or reduced serine phosphorylation
	Reducing phosphatase levels
	Intracellular protein content and/or degradation
	Glucose transporter function
	AMPK kinase activity
Gastrointestinal tract	Modulating incretin secretion/physiology
	Gastric emptying
	Gastrointestinal glucose absorption: sodium-glucose transporter-2 inhibition
Pancreas	Increasing glucose-stimulated insulin release
	Enhancing B-cell sensitivity to glucose
	B-cell mass: proliferation? Apoptosis

Source: Cefalu and Ribnicky 2009. *Obes Weight Manag* 5:277–81. With permission.

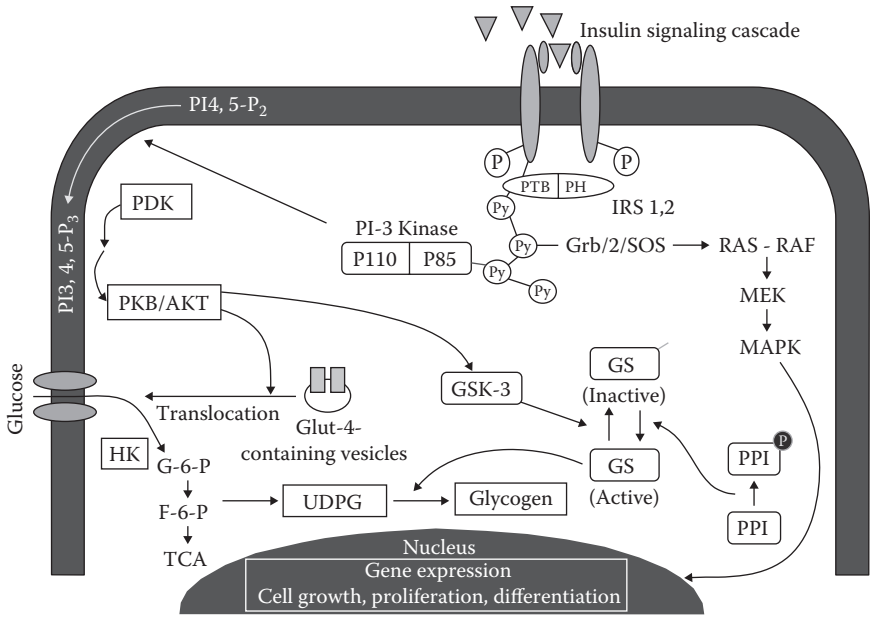
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these postulated effects are consistently noted with any botanical supplement presently available. Finally, another postulated pathway by which botanicals may work is by direct regulation of insulin action in peripheral tissues such as skeletal muscle and adipose tissue. In this regard, there is evidence to support the botanical modulation of these processes.

19.4 INSULIN RESISTANCE IN TYPE 2 DIABETES

One of the major abnormalities in obesity and type 2 diabetes is insulin resistance. Insulin resistance has been shown to be present in prediabetes, and at this stage of the natural history of diabetes, insulin secretion is observed to be increased, that is, hyperinsulinemia, in order to compensate for the insulin resistance. Given the compensation, glucose levels remain at or near the normal level. However, when insulin secretory function begins to decline, full compensation of insulin resistance is not observed, and hyperglycemia is noted at that time. Clearly, insulin resistance is a key pathophysiological feature of type 2 diabetes and is strongly associated with cardiovascular risk factors and accelerated atherosclerosis. Given the central role of insulin resistance to diabetes, one of the most desirable goals of treatment for subjects with type 2 diabetes is directed at increasing the insulin sensitivity in vivo. Caloric restriction and enhanced physical activity are well known to enhance insulin sensitivity. Unfortunately, maintenance of lifestyle intervention for patients is difficult in the long term. Therefore, a very attractive approach to improve the insulin sensitivity has been proposed with the use of botanical supplementation.

Insulin action in peripheral tissues, such as adipose tissue and muscle, involves receptor binding and enhanced intracellular signaling. The initial step is the binding of insulin to the α-subunit of its receptor. This binding leads to autophosphorylation of specific tyrosine residues of the β-subunit and enhanced tyrosine kinase activity of the receptor toward other protein substrates (Cefalu 2001; Figure 19.1). Enhanced insulin receptor tyrosine kinase activation results in tyrosine phosphorylation of insulin receptor substrates, activation of PI-3 kinase, and the resulting cellular processes associated



IRS = Insulin receptor substrate; GSK-3 = Glycogen synthase kinase-3; GS = Glycogen synthase; HK = Hexokinase; G-6-P = Glucose-6 phosphate; F-6-P = Fructose-6-phosphate; PKB = Protein kinase B; P85/P110 = PI-3 kinase; UDPG = Uridine disphosphate glucose.

FIGURE 19.1 The insulin signaling cascade. (From Cefalu, W. T. 2001. *Exp Biol Med (Maywood)* 226:13–26.)

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with insulin action (glucose transport, GLUT-4 translocation, glycogen synthesis, protein synthesis, antilipolysis, and gene expression; Cefalu 2001). On theoretical grounds, a botanical may be capable of altering insulin action by modulating any of the steps in the insulin receptor signaling cascade.

19.5 BOTANICALS AND MECHANISMS RELEVANT TO TYPE 2 DIABETES

A limited list of selected botanicals that are reported to alter carbohydrate metabolism is given in Table 19.2. However, it is important to note that consistent documentation of a glucose- or insulin-lowering effects has not yet been shown for any specific botanical. Each botanical having historical use, current use in herbal supplements, or potential for clinical efficacy based on proposed mechanisms is briefly described in Sections 19.5.1 through 19.5.12.

19.5.1 Bitter Melon (*Momordica charantia*)

Bitter melon is a traditional plant of Asian origin that has been a popular botanical proposed for treatment of diabetes and diabetes-related complications (Leung et al. 2009). The mechanism of action is believed to be secondary to multiple bioactives, one of which, polypeptide-p, is reported to have a structure similar to insulin as found in animals, and as such, is proposed to have glucose-lowering effects (Basch, Gabardi, and Ulbricht 2003; Evans 2003; Grover and Yadav 2004; Krawinkel and Keding 2006). Specifically, bitter melon fruit contains cucurbitane-type triterpenoids, steroidal saponins called "charantins," insulinlike peptides, and alkaloids, which are postulated to have effects on carbohydrate metabolism (Leung et al. 2009). As reported, clinical results with the use of bitter melon are inconsistent, as only about half the studies demonstrate efficacy. Clearly, there is controversy regarding the reported observations, and there are concerns with study design and the adequacy of statistical analyses.

Another variable that may contribute to inconsistent results is the preparation of the test material. Test material is comprised of fresh juice, dried whole fruit, fresh fruit, dried seedless fruit, seeds, aqueous extract, methanolic extract, or tablets (Ahmad et al. 1999; Rathi, Grover, and Vats 2002;

TABLE 19.2
Selected Botanical Therapeutics and Proposed Action on Carbohydrate Metabolism

Botanical	Scientific Name	Proposed Actions
Hoodia	*Hoodia gordonii*	WL, decreases appetite
Prickly pear cactus	*Opuntia* spp.	↓LDL; ↓TG; ↓PPG; ↑IS
Cinnamon	*Cinnamomum cassia, Cinnamomum verum*, and others	↑IS; ↓FPG; ↓PPG; ↓BP; ↓LDL; ↓TG
Russian tarragon	*Artemisia dracunculus* L.	↑IS; ↓PPG
Bitter melon	*Momordica charantia*	↑IS; ↓FPG; ↓PPG; ↓LDL; ↓TG
Fenugreek	*Trigonella foenum-graecum*	↑IS; ↓FPG; ↓LDL; ↓TG
Gymnema	*Gymnema sylvestre*	↑IS; ↓FPG; ↓PPG ↓LDL; ↓TG; ↑Ins sec
Garlic	*Allium sativum*	↓BP; ↓LDL
Ginkgo	*Ginkgo biloba*	↓BP
Ginseng	*Panax* spp.	↓BP
Aloe	*Aloe vera*	↑IS; ↓FPG
Ivy gourd Coccinia indica		↑IS; ↓FPG

Note: ↓BP = lowers blood pressure; ↓LDL = lowers LDL-cholesterol; ↓TG = lowers triglycerides; ↓FPG = lowers fasting blood glucose; ↓PPG = lowers postprandial blood glucose ↑IS = increases insulin sensitivity; WL = weight loss; and ↑Ins sec = increases insulin secretion.

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Grover and Yadav 2004; Leung et al. 2009). Such variation would greatly affect the bioactive content of the preparation and the bioavailability of the active compounds. Ahmad et al. (1999) reported on a relatively large ($n = 100$) intervention study with freshly prepared bitter melon fruit given to type 2 diabetic patients after a 3-day washout period of oral medications. The investigators reported an overall decrease in fasting glucose and postprandial glucose (Ahmad et al. 1999). Other data have suggested the beneficial effects of bitter melon for related complications of diabetes, such as renal disease, neuropathy, gastrointestinal disturbances, and ophthalmologic complications, that is, cataracts, in addition to a possible beneficial effect on dyslipidemia (Ahmed et al. 2001; Grover et al. 2001; Grover, Yadav, and Vats 2002; Grover, Rathi, and Vats 2002; Rathi et al. 2002; Chaturvedi 2005; Fernandes et al. 2007).

19.5.2 Fenugreek (*Trigonella foenum-graecum*)

Fenugreek has a long and storied history of medicinal use and has been used worldwide for the treatment of diabetes (Basch et al. 2003; Evans 2003). Specifically, fenugreek is described as a leguminous herb that is cultivated in India and North Africa. The seeds are used as a food ingredient and spice, and they are reported to contain high amounts of protein and fiber. Fenugreek is reported to have hypoglycemic and hypocholesterolemic actions in both animal and human studies (Srinivasan 2006). The clinical effects of fenugreek, and particularly, the hypoglycemic effects, may be secondary to the fiber content, which potentially may affect gastric emptying and may result in a decrease in postprandial blood glucose levels. Many other bioactive compounds, such as the alkaloid trigonelline and steroidal saponins, have been reported. 4-hydroxyisoleucine is considered to be an active compound in fenugreek and reportedly has an insulinlike effect (Broca et al. 1999, 2000).

As noted with several herbal preparations, inconsistent clinical results also have been observed with fenugreek that may have resulted from inadequate study design, lack of precise end points, underpowered studies, or variability in the test substance. However, fenugreek seed powder has been reported to favorably affect the glycemic index of food and glucose tolerance in both control and diabetic subjects (Gopalpura, Jayanthi, and Dubey 2009). Additional studies have suggested that treatment of diabetic subjects for 8 weeks resulted in improvements in fasting glucose and dyslipidemia (triglycerides; Kassaian et al. 2009). Interestingly, more consistent results are obtained when fenugreek is provided at larger doses of 10–20 g/day, and this may be related to an effect on digestive processes (Srinivasan 2006).

19.5.3 Gymnema (*Gymnema sylvestre*)

Gymnema sylvestre, known as gurmar, is native to Africa, Middle East, and India, and it has historical use in the treatment of diabetes and is commonly used (Grover, Yadov, and Vats 2002). The gymnema leaf or its extract is reported to be the most commonly used preparation of the plant. Potential antidiabetic compounds include oleanane triterpenoid saponins (i.e., gymnemic acids), dammarane saponins called gymnemosides, and a polypeptide called gurmarin (Porchezhian and Dobriyal 2003). There are extensive studies in animal models. Particularly, the effect of *Gymnema sylvestre* extract on carbohydrate metabolism has been suggested to be secondary to improving glucose uptake in peripheral tissues and increasing insulin secretion and β cell number in the pancreas (Dey, Attele, and Yuan 2002). However, there has been a paucity of definitive clinical studies that would allow one to provide clear guidelines on efficacy and safety (Leach 2007). In addition to the proposed systemic hypoglycemic activity in vivo, gymnema preparations are postulated to suppress the taste sensation of sweet, decrease the uptake of glucose from the small intestine, improve glucose metabolism, decrease HbA1c, and improve insulin secretion and dyslipidemia (Baskaran et al. 1990; Porchezhian and Dobriyal 2003; Ramkumar et al. 2008; Daisy, Eliza, and Mohamed Farook 2009). Clearly, completion of well-designed clinical studies is needed before definitive recommendations can be made for *Gymnema*.

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19.5.4 Hoodia (*Hoodia gordonii*)

The prevalence and incidence of obesity worldwide have reached epidemic proportions. Specifically, obesity is a key pathophysiological feature that contributes to the development of the metabolic syndrome and type 2 diabetes. In general, lifestyle modifications such as dietary restriction and enhanced physical activity are very effective in promoting weight loss and decreasing rates of progression of metabolic syndrome to type 2 diabetes (Knowler et al. 2002). However, lifestyle modifications alone are rarely sustained over a long-term period. Thus, a botanical that effectively alters energy balance, that is, increasing energy expenditure or lowering energy intake, would be of great interest to public health. In this regard, hoodia is currently marketed as an appetite suppressant component of many diet products. Data suggest that the botanical appears to have an anorexic effect in preclinical studies, and a steroidal pregnane glycoside (P57AS3) has been purified from the plant and is suggested to be the responsible anorexic compound (MacLean and Luo 2004). However, there are more than 30 pregnane glycosides identified from hoodia, all of which potentially could contribute to the overall clinical effect (Shukla et al. 2009). Nonetheless, it is important to note that there is no published or definitive clinical evidence demonstrating that hoodia does effectively reduce appetite. There is another concern that relates to the adulteration of hoodia products currently available on the market (Avula et al. 2008).

19.5.5 Prickly Pear Cactus (*Opuntia* spp.)

Prickly pear is a common cactus, and its fleshy stems and pearlike fruits are consumed as both medicine and food. It is a widely known and a commonly used herbal treatment for glucose control in Central and South America (Roman-Ramos, Flores-Saenz, and Alarcon-Aguilar 1995; Evans 2003). Prickly pear cactus is reported to have a very high-soluble fiber and pectin content that may prevent the absorption of sugars (Marles and Farnsworth 1995). This may be the most likely reason for regulatory effects on blood glucose on a whole-body level, but other mechanisms also have been suggested (Marles and Farnsworth 1995; Roman-Ramos, Flores-Saenz, and Alarcon-Aguilar 1995; Evans 2003). Isorhamnetic-3-glucoside is reported to be one of the many active flavonoids isolated from *Opuntia* (Ginestra et al. 2009). In preclinical studies, *Opuntia* spp., pectin, seed oil, and powder significantly lowered total cholesterol, low-density lipoprotein (LDL)-cholesterol, and triglyceride levels (Fernandez et al. 1992; Li et al. 2005; Ennouri et al. 2006; Oh and Lim 2006). Favorable effects on dyslipidemia were confirmed in a pilot study of 24 nondiabetic male subjects. Specifically, *Opuntia robusta* pectin lowered total cholesterol by 12%, LDL cholesterol by 15%, triglycerides by 12%, blood glucose by 11%, and insulin levels by 11% (Wolfram et al. 2002). Two controlled short-term studies of 14 and 22 human subjects, respectively, reported decreased fasting glucose and insulin levels in patients with type 2 diabetes (Frati et al. 1990; El Kossori et al. 1998).

19.5.6 Ginseng (*Panax* spp.)

Ginseng has been a very popular botanical that has been suggested to control diabetes (Griggs 1981; Marles and Farnsworth 1995; Vogler, Pittler, and Ernst 1999; Evans 2003). A review of controlled trials (Vogler, Pittler, and Ernst 1999) using ginseng extracts (mostly *Panax ginseng* [Asian ginseng] and *Panax quinquefolius* [American ginseng]) concluded that there was insufficient evidence to support the efficacy for lipid or glycemic indications. Buettner et al. (2006) summarized a comprehensive database analysis of the reported studies of ginseng (*Panax* spp.) for efficacy related to cardiovascular risk factors, including blood pressure, lipid profiles, and blood glucose. The overall analysis suggested that ginseng was noted to slightly decrease blood pressure compared with placebo (range: 0–4%), but they observed mixed results for an effect on lipids. Furthermore, they found several studies showing that ginseng lowers blood glucose, but overall they concluded that the results were inconsistent (Buettner et al. 2006).

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19.5.7 Cinnamon (*Cinnamomum cassia, Verum,* and Others)

Cinnamon has not only been used historically for the treatment of diabetes but is a supplement that is gaining in popularity, and many cinnamon products are currently available as dietary supplements. The bioactives considered to be responsible for antidiabetic effects are not precisely known, but polyphenol type-A polymers are believed to represent some of the active components of cinnamon that may have insulin-mimetic effects (Jarvill-Taylor, Anderson, and Graves 2001; Anderson et al. 2004). As such, there are data suggesting an effect in preclinical studies that have evaluated cinnamon in murine models of diabetes. There is a growing database of clinical studies on cinnamon, but similar to clinical results as observed with other herbal products, the results are not entirely consistent. Again, selection criteria of the cohort, clinical end points selected, appropriate dose, and source of cinnamon bioactives are all factors that potentially contribute to the variable clinical results (Dugoua et al. 2007). However, studies have suggested a positive effect in some settings. Specifically, Crawford (2009) evaluated 109 diabetic patients who were previously treated with diet and exercise. The intervention consisted of 1 g/day dose of cinnamon for 90 days and seemed to be effective to significantly lower antecedent glycemia, as assessed with HbA1c, in the treatment group relative to the control group. Other studies also suggested beneficial effects on glucose and lipids (Khan et al. 2003; Anderson 2008; Mang et al. 2006), whereas other investigations failed to reveal an effect on glycemia or lipids (Altschuler et al. 2007; Blevins et al. 2007). Other effects of cinnamon on cardiovascular risk factors such as antihypertensive effects have been suggested in preclinical and small clinical trials evaluating subjects with metabolic syndrome (Preuss et al. 2006; Ziegenfuss et al. 2006).

19.5.8 Russian Tarragon (*Artemisia dracunculus* L.)

Over the recent past, an ethanolic extract of *Artemisia dracunculus* L. (commonly known as Russian tarragon) has been demonstrated to have antidiabetic properties. Its antidiabetic effects were shown in several preclinical studies evaluating both chemically induced (i.e., streptozotocin-induced) and genetically diabetic (i.e., KK-A(y) mice) murine models (Ribnicky et al. 2006). Bioactives that have been identified as part of the extract include 4,5-di-O-caffeolquinic acid, davidigenin, 6-demethoxycapillarison, and 2′, 4′-dihydroxy-4-methoxydihydrochalcone as aldose reductase inhibitors (Logendra et al. 2006) and 2′, 4′-dihydroxy-4-methoxydihydrochalcone, 2′, 4-dihydroxy-4′-methoxydihydrochalcone, and sakuranetin as protein tyrosine phosphatase-1B inhibitors (Wang et al. 2008), whereas 6-demethoxycapillarisin and 2′, 4′-dihydroxy-4-methoxydihydrochalcone inhibited PEPCK gene expression in cultured liver cells (Govorko et al. 2007). Both in vitro and preclinical studies strongly suggest that the primary effect of the extract is to favorably affect insulin signaling in the muscle (Wang et al. 2008, 2010). The improved cellular signaling has been related to enhanced whole-body insulin sensitivity. A pilot human trial in a small number of insulin-resistant subjects suggested that the alcoholic extract of *Artemisia dracunculus* L. enhanced insulin sensitivity in subjects randomized to the *Artemisia* extract compared to the baseline value, but it was not significantly changed compared to the placebo group. No changes in insulin sensitivity at the end of the study were noted compared to baseline in the placebo group. In addition, no changes in body weight or body fat composition between the treatment groups were observed. Of note, this pilot study was the first to show that specific bioactive compounds from the botanical extract of *Artemisia dracunculus* L. (i.e., davidigenin, chalcone, and sakuranetin) that had been noted to have significant effects in vitro from preclinical studies could be identified in the plasma of subjects after extract ingestion (Ribnicky et al. in press).

19.5.9 Garlic (*Allium sativum*)

Garlic is one of the more intriguing herbal remedies used historically. The range of beneficial effects of garlic is very broad and has been traditionally used as an antithrombotic, antihypertensive,

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cholesterol-lowering, antioxidant, antimutagenic, and antimicrobial agent. As would be expected for an herbal remedy proposed to have such broad effects, there has been a tremendous amount of research interest into its actions. In particular, a number of preclinical and clinical studies report the hypotensive effect of garlic, which appears to be more consistent in animal studies, as opposed to clinical studies (Ali et al. 2000; Sharifi, Darabi, and Akbarloo 2003; Cruz et al. 2007). The precise mechanism of action by which garlic lowers blood pressure is not known. However, it is proposed that garlic modulates endothelial production of nitric oxide (NO), inhibits angiotensin-converting enzyme (ACE) activity, decreases the production of vasoconstrictive agents thromboxane-B2 and prostaglandin-E2 and has potent free-radical scavenging activity (Al-Qattan et al. 2001; Ku et al. 2002; Sharifi, Darabi, and Akbarloo 2003; Medina-Campos et al. 2007). As with other herbal preparations, the variability in the clinical results may stem from differences in garlic preparations used for study or the specific content of bioactives represented in the preparation. Some bioactives have been reported to include unstable sulfur-containing compounds, polyphenols, flavonoids, anthocyanins, tannins, and others (Rahman and Lowe 2006; Chen et al. 2009).

A recent meta-analysis was conducted on clinical studies over the past half-century. The meta-analysis was based on 11 clinical studies between 1955 and 2007, and it included true placebo groups, used garlic-only preparations, and reported mean systolic and/or diastolic blood pressure (SBP/DBP) and standard deviations in their statistical analyses. This analysis concluded that individuals treated with garlic had better outcomes, and superior effects in lowering blood pressure in hypertensive individuals were observed compared with the placebo-treated group. The mean (SD) decrease in blood pressure reported in the hypertensive subgroup was 8.4 (2.8) mm Hg ($n = 4$; $p < .001$) for systolic and 7.3 (1.5) mm Hg for diastolic ($n = 3$; $p < .001$) blood pressure (Ried et al. 2008).

19.5.10 Ginkgo (*Ginkgo biloba*)

Ginkgo, a popular herbal remedy for centuries in China, has also become popular in Europe and America. One of the proposed indications has been to improve circulation. The focus of several studies has been to evaluate ginkgo leaf extract and measure the modulation of calcium levels in the endothelium and vasodilation (Chen et al. 2009). Ginkgo was reported to have a hypotensive effect in preclinical studies (Kubota et al. 2006a, 2006b; Koltermann et al. 2007). However, other studies have demonstrated that long term intake may not be useful (Tada et al. 2008). Clinical data have also suggested that ginkgo may lower blood pressure in healthy subjects over a treatment course of 3 months and within a single treatment for temporary stress-induced hypertension (Kudolo 2000; Jezova et al. 2002). However, controversy exists as other clinical studies have failed to confirm an effect (Chen et al. 2009).

19.5.11 Ivy Gourd

Historically, ivy gourd was used in Ayurvedic medicine, a traditional East Indian healing system, to treat glycosuria. Reports suggest that active compounds in the plant may mimic the action of insulin and suppress the activity of certain enzymes involved in glucose production. Clinical research studies with ivy gourd extract have suggested its effect on fasting and postprandial blood glucose levels of the treated patient groups (Kuriyan et al. 2007).

19.5.12 Aloe (*Aloe vera*)

Aloe vera has also been used in the medicinal treatment of diabetes in India and the Arabian peninsula (Vogler and Ernst 1999; Evans 2003). The gel, which is obtained from the inner portion of the leaves, may contain glucomannan, a water-soluble fiber that reportedly has hypoglycemic and insulin-sensitizing actions (Vuksan et al. 1999, 2000). Preclinical studies have reported inconsistent results (Yeh et al. 2003). However, small-scale clinical research trials suggested an improvement in

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fasting glucose levels with the extract (Bunyapraphatsara et al. 1996; Yongchaiyudha et al. 1996; Yeh et al. 2003). In a comprehensive review of the effects of herbals on glycemia, Yeh et al. (2003) concluded that the preliminary data suggest a potential effect of *Aloe vera* in glycemic control; however, further validation is needed.

19.6 CONCLUSIONS

Botanical extracts have been widely used as medicinal agents throughout human history. Many are now available in commercial supplements and are promoted for general health benefits or for prevention and treatment of specific diseases. As such, the public's interest in the potential benefit of botanical supplements on carbohydrate metabolism is quite high. The advantage of a botanical extract is that if botanicals are shown to be effective to improve metabolism and/or risk factors on a clinical level, these remedies, in general, are commonly available and therefore could potentially aid the general public with regard to obesity and diabetes. Unfortunately, although most of the popular botanicals have a long history in folk medicine, there is a paucity of definitive clinical data, particularly as it relates to consistently improving carbohydrate metabolism. There is insufficient evidence, based on currently available data, to actively recommend the use of any particular botanical product to treat either high blood glucose or other related risk factors. However, there are active investigations in many areas for which botanical preparations are consistent, and defined clinical studies are still ongoing. We need to await the results of these carefully conducted studies.

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20 Bioactive Components in Herbal Medicine
Experimental Approaches

Foo-tim Chau, Kwok-pui Fung, Chi-man Koon, Kit-man Lau, Shui-yin Wei, and Ping-chung Leung

CONTENTS

20.1 Introduction 419
20.2 The Efficacy-Driven Approach to Research in Herbal Medicines and the Role of Chemometrics Fingerprinting: An Overview 420
20.3 An Herbal Formulation for the Promotion of Cardiovascular Well-Being: Danshen and Gegen 421
20.3.1 Introduction 421
20.3.2 Efficacy Testing in Experimental Study (Animal and In Vitro Testing) 422
20.3.3 Efficacy Testing in Clinical Study 424
20.3.4 Summary of the Cardioprotective Effects of the Danshen–Gegen Formulation 426
20.4 Quality Control of Herbs: The Chemometrics Approach 428
20.4.1 Background 428
20.4.2 The Pattern-Oriented Approach in Chemometrics 429
20.4.3 Separation Techniques Used in the Multicompound Chemometric Approach 429
20.4.4 Application of Chemometric Approach in the Quality Control of Herbs 432
20.4.5 Example with Danshen, Gegen, and Danshen–Gegen Formulation 433
20.4.6 Summary 434
20.5 Concluding Remarks 434
Acknowledgments 435
References 435

20.1 INTRODUCTION

Modern medicine is a deductive science, whereas traditional Chinese medicine (TCM) is inductive. Deductive medicine has a specific focus, but as a consequence the general need of the individual might be neglected. TCM does not relate to very specific targets or problems, but aims at improving the general well-being of the individual by maintaining an effective balance between various physiological functions. This holistic approach, by which the individual is kept in a biologically balanced state, allows the mobilization of biological reserves to take care of physiological problems (Campion 1993). In contrast, the deductive approach relies on accurate targets, mandates specialization, and is largely disease focused rather than patient focused. Neglect of holistic care by modern medicine is one of the important reasons behind the increasing support for alternative care and over-the-counter health preparations (Eisenberg et al. 1993). If the two divergent systems of medical science can be harmonized, more clinical problems can be solved. Holistic care to promote physiological balance to allow spontaneous adjustment and the building up of better bodily defenses could supplement

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aggressive single-target modern medicine to remove a specific problem. Such harmonization of the two systems could lead to better and more holistic treatment of individuals within a modern medicine setting that is science based and evidence based. This requires research into efficacy that goes beyond specific targets. However, it also requires research to ensure the quality of herbal medicines, which are often misidentified, contaminated, or adulterated.

20.2 THE EFFICACY-DRIVEN APPROACH TO RESEARCH IN HERBAL MEDICINES AND THE ROLE OF CHEMOMETRICS FINGERPRINTING: AN OVERVIEW

Research on herbal medicine in the past century has focused on many aspects, including pharmacognosy, quality control, and laboratory and clinical tests for efficacy. Many resources have focused on the identification of active components in herbs for drug development. One remarkably successful example in China was the discovery of the derivatives of artemisinin (*qinghao*). This was used traditionally as an herbal treatment for fever and chills, and has been found to be effective against malarial and other parasites and to have cytotoxic effects against some cancers (Valecha and Tripath 1997). Two other examples, from France, are the cytotoxic drugs vincristine, from the periwinkle flower, and taxol, extracted from the bark of the yew tree (National Centre, 1999). However, tremendous resources and facilities are required for such major successes, and there have been many failures. Botanicals are complex and varied mixtures. Extraction and identification of putative active components in the whole herb or herbal formulation require innovative approaches as well as much laboratory investment. Evidence of clinical efficacy is needed, and the overall investment is very costly, although success is not guaranteed. A more cost-effective approach to the search for bioactive components in herbs is needed.

For an approach to be generally accepted in the development and evaluation of herbal medicines, it must address several aspects of an herbal medicine, including efficacy, safety, quality and consistency of composition, and mode of action. A particular research need in this field is to understand the quality of the herbs, which can vary widely. In addition, herbs can be wrongly identified and contamination or adulteration of herbs is common. Herbs need to be thoroughly authenticated. A basic chemical record of quality control can be established through chromatographic studies, and the species details related to the origin of production are established with DNA fingerprinting. Quality in relation to the absence of pesticides, heavy metals, fungi, and microbes is also important. Every batch of an herb should be subjected to screening and counterchecking against records of standard extracts provided by the relevant academic institution in China (Zhan and Lin 2002). However, existing standards for labeling samples of medicinal herbs are far from satisfactory. New technologies can create chemometric profiles of a known herb for quality assessment, as well as helping with the identification and quantitation of biologically active chemical components. This practice takes quality control of herbal medicine to a more effective level and will support its use in modern medicine (Mok and Chau 2006; Zeng et al. 2008).

Ensuring the quality and consistency of herbal medicine is very important, but questions of efficacy and modes of action also must be addressed. An investigative approach that combines biochemical, biological, and chemometric testing in a "layered" system offers a useful platform for the overall evaluation of herbal medicines. Parallel testing of characteristics or biological effects with chemometric screening is performed on crude herbal extracts, before moving to parallel testing of further fractionations and their combinations (Figure 20.1). The aim of the chemometric analysis is to identify biologically active chemical groups, not the compound, and offer a chemical fingerprinting technique that leads to the identification of more target compounds with known and partially known chemical properties. The chemometric fingerprint analysis technique makes possible the comparison of chemical compositions of different samples using all the detected components through their entire chromatograms obtained from liquid chromatography, gas chromatography,

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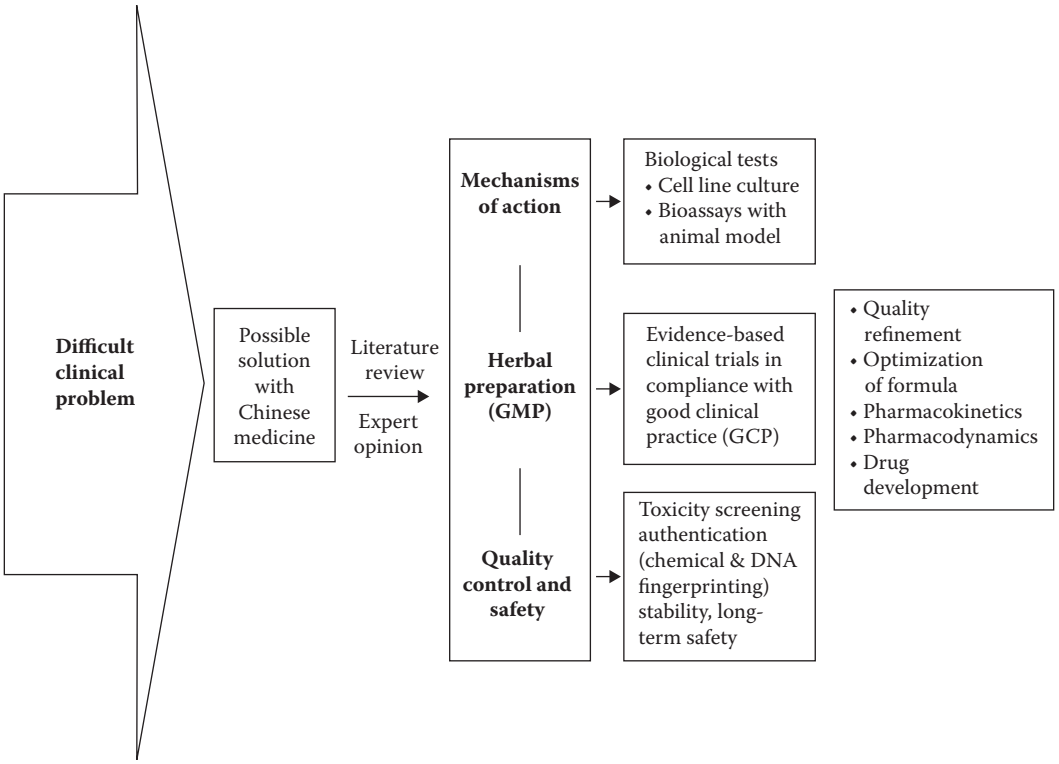


FIGURE 20.1 The efficacy-driven approach to evaluation of herbal medicine.

mass spectrometry (MS), and others. These techniques also help to discover new biomarkers and active ingredients as well as estimate bioactivity levels more efficiently and effectively (Idborg-Bojorkman et al. 2003; Wang, Wang, and Cheng 2006; Dumarey et al. 2008; Chau et al. 2009).

In summary, the development and evaluation of herbal medicines requires a comprehensive research approach that encompasses chemometric testing of herbs for quality, consistency, and identification of bioactive components, as well as experimental procedures and clinical trials to confirm the efficacy and mode of action. An example of this comprehensive testing as applied to a formulation for the promotion of cardiovascular well-being is given in Section 20.3.

20.3 AN HERBAL FORMULATION FOR THE PROMOTION OF CARDIOVASCULAR WELL-BEING: DANSHEN AND GEGEN

20.3.1 Introduction

Atherosclerosis-induced heart attacks and strokes are leading causes of morbidity and mortality (Muhlestin 2000; Dalal, Evans, and Campbell 2004). Current primary and secondary prevention strategies emphasize control of various atherosclerotic risk factors, including smoking, hypertension, hypercholesterolemia, diabetes mellitus, obesity, inflammation, and homocysteine (Graham et al. 1997; Ridker et al. 1998; Schnyder et al. 2002). Radix Salviae miltiorrhizae (danshen) and Radix puerariae (gegen) are two herbal medicines used in controlling angina and other cardiac symptoms in the Chinese materia medica (Fan, O'Keefe, and Powell 1984; Ji, Tan, and Zhu 2000). Modern pharmacological studies suggest therapeutic values of these herbal preparations, including lowering of blood pressure and lipids (Zheng et al. 2002), antioxidation (Fan, O'Keefe, and Powell 1984; Zhang and Fang 1997; Jiang, Lau, and Lamm 2005), and the promotion of microcirculation

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(Lei and Chiou 1986; Fung et al. 1993). Therefore, danshen and gegen are worthy of study as cardioprotective agents, and clinical, experimental, and chemometric testing was performed on a dansen–gegen (DG) formulation for efficacy and chemometric fingerprinting.

20.3.2 Efficacy Testing in Experimental Study (Animal and In Vitro Testing)

Antioxidant effects, protection from ischemia–reperfusion injury, effects on blood pressure, and vasodilatory effects have been investigated on the DG formulation using a variety of in vitro and ex vivo models.

The antioxidant effects are discussed first. The compound 2-2′-azo-bis (2-amidinopropane) dihydrochloride (AAPH) is a water-soluble radical-chain initiator that can induce lipid oxidation (Yamamoto et al. 1984; Niki et al. 1986; Miki et al. 1987). The AAPH-induced red blood cell hemolysis assay and AAPH induced cardiomyocyte cell death test are convenient in vitro experiments for the study of antioxidant activity. In the presence of AAPH, the membrane lipids of red blood cells and cardiomyocytes are readily oxidized, resulting in hemolysis and cell death, respectively. The DG formulation inhibited AAPH-induced red blood cell hemolysis and cardiomyocyte cell death in a dose-dependent manner (Leung 2003; Lam et al. 2005; Lam 2006).

The ischemia–reperfusion injury effects are discussed next. In ischemia, oxygen supply is decreased to an extent that is insufficient for maintaining normal metabolism (Hearse 1994). Reperfusion restores the oxygen content but causes injury to the organ at the same time because of the large amount of superoxide produced from the action of xanthine oxidase on the accumulated hypoxanthine (McCord, Roy, and Schaffer 1985; Manning et al. 1988). This ischemia–reperfusion injury occurs in patients undergoing percutaneous transluminal coronary angioplasty (PTCA). To mimic this in ex vivo studies, isolated rat hearts are cannulated on a Langendorff apparatus and subjected to controlled ischemia and reperfusion. The protective effect of DG formulation on isolated rat hearts against ischemia–reperfusion injury was reflected from contractile force recovery, coronary flow rate recovery, and the lower release of enzymes (e.g., creatine kinase and lactate dehydrogenase) from cardiac tissue (Leung 2003).

The antihypertensive effects of DG formulation are as follows: The endothelium plays an important role in regulating vascular tone and blood pressure. It continually releases endothelium-derived relaxing factors (EDRFs) and endothelium-derived contracting factors (EDCFs) to modulate underlying vascular smooth muscle cell (vSMC) function. The EDRFs are comprised of nitric oxide (NO), prostacyclin (PGI_2), and endothelium-derived hyperpolarizing factor (EDHF), whereas EDCFs include superoxide anion ($O_2^{\bullet-}$), endothelin, thromboxane A_2 (TXA_2), and prostaglandin H_2 (PGH_2). A balance between EDRF and EDCF is maintained in normal physiological situations (Palmer, Ferrige, and Moncada 1987; Akpaffiong and Taylor 1998). To investigate the vasodilative effect of DG formulation, one ex vivo and one in vivo model were employed. In the ex vivo study, rat aortas were isolated and cut into pieces of 2 mm in length. The rings were then mounted on organ baths, containing 37°C Krebs solution aerated with 95% O_2 and 5% CO_2, and connected to a force transducer to record isometric force. After 1 hr. of equilibration and pretesting with phenylephrine (an α-adrenergic agonist for vSMC contraction testing) and then with acetylcholine (an endothelial muscarinic receptor agonist for endothelium-induced relaxation testing), the aortic rings were rinsed with Krebs solution and allowed to equilibrate for 1. Using this model, DG formulation dose-dependently induced vasodilation of isolated rat aorta (Yam 2005). For in vivo testing, spontaneous hypertensive rats (SHRs) were used, a system in which results consistently correlate efficacy to humans (Lund-Johansen 1990; Doggrell and Brown 1998). Generally, SHRs are prehypertensive at the first 6–8 weeks and hypertension develops over the next 12–14 weeks (i.e., at an age of 6–22 weeks; McGuire and Twietmeyer 1985). Blood pressure of SHRs can be measured by the rat tail-cuff noninvasive blood pressure system.

In testing the DG formulation, conscious rats were put into a transparent container with temperature control and allowed to calm down for at least 15 min. before measurement. An occlusion cuff and a sensor cuff were put onto the rats' tails. Blood pressure was recorded by the inflation and

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deflation of the occlusion cuff. Any agitation of the rat could be compensated by another 15-minute calming period before the next measurement. Herbal samples were intragastrically administered to the SHRs once daily for 3 months and blood pressure was measured at 4-week intervals. The DG formulation was found to attenuate hypertension progression in SHRs either at the prehypertensive stage or at the stage of sustained hypertension (Lam et al. 2005; Lam 2006).

The DG formulation shows significant anti-atherosclerosis effects. Atherosclerosis is a continuous pathological process that takes years to develop and progress. It is now possible to assess such vascular abnormalities objectively and noninvasively with ultrasound assessment. For these, measurement of carotid intima-media thickening (IMT) is highly reproducible, correlates well with the severity and extent of coronary artery disease, and is predictive of stroke and coronary events (O'Leary et al. 1999; Simon et al. 2002). Similarly, vascular reactivity (flow-mediated dilation) of the brachial artery as an indicator of overall endothelial function is an emerging sensitive marker of early subclinical atherosclerosis and a reliable index of cardiovascular health (Behrendt and Ganz 2002).

Pathological processes of atherosclerosis include accumulation of modified lipids, mainly oxidized low-density lipoprotein (oxLDL), foam cell formation, endothelial cell dysfunction and activation, increase in expression of adhesion molecules, activation and recruitment of inflammatory cells, and induction of proliferation and migration of vSMCs (Gerrity 1981; Pesonen 1989; Steinberg et al. 1989; Spiteller 2005). The LDL oxidation is a lipid-peroxidation process, and antioxidants that can prevent its initiation and cause chain breaking should have a major impact not only on the oxidation resistance of LDL but also on the development of atherosclerosis. The most frequently used assay to assess in vitro oxidation resistance of LDL is the measurement of conjugated dienes formation in isolated LDL, with copper ions as the pro-oxidant (Chomard et al. 1998; Anderson et al. 2001). The DG formulation prevented LDL from being oxidized in a dose-dependent manner, and salvianolic acid B (SAB; Koon 2006), one of the bioactive components from danshen, was at least two times more potent than vitamin C in preventing LDL oxidation. To test its effects on monocyte adhesion, expression of adhesion molecules on the surface of cultured human umbilical vein endothelial cells (HUVECs) is stimulated by tumor necrosis factor (TNF)-α or interleukin (IL)-1β. The next step is the pulsing of human monocytes, THP-1, by tritiated thymidine. After coincubation of the two cell types for 1 hour, the cultured cells are washed thrice with phosphate buffered saline (PBS) to wash away nonadhered monocytes and the cell lysate is collected for scintillation counting. In this assay system, DG formulation was found to decrease the adhesion of THP-1 to HUVECs in a dose-dependent manner (Koon 2006).

The inhibitory effect of DG formulation in foam cell formation was examined using lipid loading of human monocyte–derived macrophages (HMDMs). White cells concentrates were obtained from the peripheral blood of healthy donors, and monocytes were isolated via counterflow centrifugation elutriation. After maturation of the monocytes, lipid loading was achieved by incubation of the HMDMs with acetylated LDL (AcLDL) in culture medium. During lipid loading, the cells were treated with DG formulation. Afterward, cell extracts were prepared and free cholesterol and cholesteryl esters were separated and quantified via reverse-phase high-performance liquid chromatography (HPLC). It was found that DG treatment has significant effects on foam cell formation in vitro by decreasing the accumulation of cholesterol and its esters in a dose-dependent manner (Sieveking et al. 2005).

Vascular proliferation contributes to diffuse intimal thickening in large and medium-sized arteries where the development of atherosclerosis has occurred. Intimal thickening begins after birth and it is composed of vSMCs completely surrounded by an extracellular matrix (ECM) and covered by a monolayer of endothelial cells (Pesonen 1989). Sometimes, macrophages can be found beneath the endothelium layer. And it was found that the ECM in intimal thickening is synthesized by vSMCs (Campbell et al. 1991). Therefore, vSMC proliferation is an important process for plaque formation in primary atherosclerosis (Ross 1995). The DG formulation was found to inhibit platelet-derived growth factor (PDGF)–induced vSMC proliferation by mediating G1/S cell-cycle arrest. The main component that governs the transition from G1 phase to S phase, cyclin D, was found to be downregulated by DG formulation in both protein and messenger ribonucleic acid (mRNA) levels (Koon 2006). DG formulation also showed antimigratory effects against PDGF-induced vSMCs migration (Koon 2006).

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Although the atherogenic effect of dietary cholesterol was first demonstrated in rabbits, it has also been observed in several other animals, including pigs and guinea pigs. The New Zealand white rabbit was employed as the animal model for testing whether supplementation of DG formulation could stop the cholesterol-induced progression of atherosclerosis. Fresh aortas were excised, cut longitudinally, and immersed in a solution of Sudan III (saturated solution in 70% alcohol; Hara et al. 1999) for 45 minutes to identify atheromatous lesions on the surface of the aorta. It was reported that DG supplementation significantly decreased the area of atheroma in a dose-dependent manner (Koon 2006).

20.3.3 Efficacy Testing in Clinical Study

One hundred men ($n = 87$) and women ($n = 13$) aged 40–70 years (mean [standard deviation or SD] age of 58[8] years) were recruited. They had angiographically documented coronary artery disease (>50% reduction in luminal diameter) in at least one vessel and stable angina status. Patients with recent myocardial infarction (within 3 months) and unstable angina (within 6 months), stroke (within 1 year), significant renal insufficiency (plasma creatinine >140 μmol/L), or history of significant drug hypersensitivity were excluded. Eligible coronary patients were screened in the cardiac catheterization laboratory and cardiac clinic in the Prince of Wales Hospital, Shatin, Hong Kong, People's Republic of China. After signing written informed consent, the patients were randomized to take either 6 capsules of DG formulation (3 g) per day or 6 capsules of appearance-identical placebo capsules daily, in double-blind and parallel fashion for 24 weeks. Other medications were not changed. Clinical visits for progress and tolerability monitoring were arranged at 4-week intervals. Routine hematological, biochemical, and other blood tests (folate, homocysteine, vitamin B12, and proinflammatory biomarkers) and ultrasound vascular imaging were performed at baseline and on completion of the trial.

The formulation used was as follows: Danshen and gegen raw herbs (in a ratio of 7:3 by weight) supplied from Sichuan farms were prepared, authenticated (Sun, Shaw, and Fung 2007), and extracted in one batch following good manufacturing practices at the Hong Kong Institute of Biotechnology, Science Park, Hong Kong, People's Republic of China. The prepared herbs were subjected to aqueous extraction (with an herb:water ratio of 1:10) at 100°C twice for 60 minutes and once for 30 minutes, spray dried at −660 mmHg and 50–60°C, and the dried powder encapsulated (500 mg/capsule) after an accelerated stability test. A dosage of 3 g/day of the DG formulation was chosen for the clinical study, based on the recommendation from Chinese materia medica (Fan, O'Keefe, and Powell 1984; Ji, Tan, and Zhu 2000) and our previous in vitro experiment (Fung et al. 1993).

The endothelial function of the brachial artery was studied at baseline using high-resolution ultrasound, as described in Section 20.3.2 (Woo et al. 1993, 2004). In brief, the diameter of the brachial artery was measured on B-mode ultrasound images using a linear array transducer (L10-5 median frequency: 7.5 MHz) and a standard Advanced Technology Laboratories 3000 ultrasound system (Bothell, Washington, USA) and forearm tourniquet cuff placement to induce hyperemia after deflation (Celermajer et al. 1992; Woo et al. 1993; Corretti et al. 2002; Woo et al. 2004; Deanfield et al. 2005). Scans were acquired at rest, during post-tourniquet-reactive hyperemia (to induce flow-mediated endothelium-dependent dilation [FMD]; Celermajer et al. 1992), and 4 minutes after administration of 400 μg sublingual glyceryltrinitrate (GTN; for endothelium-independent dilation). The FMD is predominantly due to endothelial NO release (Jannides et al. 1995), and the endothelial responses of the brachial artery measured by this method correlate significantly with coronary endothelial function in the same subjects (Anderson et al. 1995) and with the severity of coronary atherosclerosis (Schroeder et al. 1999). The accuracy, reproducibility, and low interobserver error for this measurement of arterial physiology have been demonstrated previously in the literature (Celermajer et al. 1992; Woo et al. 1993; Sorensen

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et al. 1995; Woo et al. 2004). All carotid scans were performed by a single and blinded operator after a predetermined and standardized scanning protocol for the right and left carotid arteries, as described previously (Salonen and Salonen 1991; Bots et al. 1997). All scans were recorded on super-VHS (video hyperspace) videotape for subsequent offline measurement of intima-media thickness (IMT) by a blinded investigator, using a verified automatic edge-detecting and measurement software package as described previously (Woo et al. 1999). The intraobserver variability for the mean IMT was 0.03 ± 0.01 mm (coefficient of variation [CV]: 1.0%).

After the double-blind trial phase, all patients were offered an option of continuing open-label DG (1.5 g/day) for an additional 6 months for lower dose-titration and consolidation of the therapeutic effects. Ten placebo patients who had not opted for open-label DG after the double-blind phase but had consented to be followed up were restudied at 1 year for comparison (negative control). The study protocol was approved by the institutional ethics committee on human research of the Chinese University of Hong Kong, People's Republic of China, in compliance with the Declaration of Helsinki. Data were processed to give group mean values and standard deviations where appropriate. In case of normal distribution, possible intergroup differences were identified with a Student's t-test. Otherwise, the possible intergroup differences were assessed by a Kruskal–Wallis's test. In case of detecting any significant intergroup differences, the subsequent identification of the groups was carried out with a Wilcoxon Rank–Sum test. The primary study end points were FMD, GTN, and IMT, whereas other, nonprimary outcome variables were compared with Bonferroni adjustment for multiple comparisons. Multivariate linear regression analysis was carried out to assess the major determinants of FMD changes, including age, changes in total and LDL cholesterols, and treatment groups. A p value less than 0.05 is considered statistically significant. The statistical analyses were made with SPSS for Microsoft Windows 10.0. On our pilot finding of impaired FMD in the range of 5.0 ± 1.2% and of a mean interobserver relative difference of 3% in FMD over time, and assuming a 10% dropout rate in 24 weeks, enrollment of 100 coronary artery disease (CAD) patients can detect a 12% relative improvement from baseline in brachial FMD after DG treatment and in FMD difference between DG- and placebo-treated groups with 80% power and $\alpha = 0.05$.

In total, 92 patients finished the 24-week, double-blind phase study. Eight patients dropped out (two withdrew consent, two had adverse events while on placebo, one had poor compliance, and three defaulted on nonmedical grounds). Baseline characteristics, clinical features, cardiac medication, and vascular function in the two groups were found to be similar (Table 20.1).

After 24 weeks, when compared with the baseline, there were no significant changes in blood pressure, blood folate, homocysteine, C-reactive protein, and other proinflammatory markers levels in either group. A small decrease in total and LDL cholesterols ($p < .05$) in the DG group and a mild decrease in LDL cholesterol in the placebo group were observed ($p < .05$; Table 20.2).

The FMD and GTN of the brachial artery improved significantly with DG ($p < .01$), although FMD also was significantly improved after placebo treatment also ($p < .05$). However, FMD was significantly higher after DG treatment than after placebo treatment ($p < .001$) (Figure 20.2a and b). A slight decrease in carotid IMT ($p < .05$) was seen after 24 weeks of DG but not after placebo treatment (Table 20.3, Figure 20.3a and b). On multivariate analysis, improvement in FMD was related to DG treatment ($\beta = 0.32$, $R = 0.3$, $p = .03$), but not to age or changes in total or LDL cholesterols.

The study drugs were well tolerated in both groups, with no significant symptomatic complaints and no derangement in hematological or routine biochemical profiles. Eight severe adverse events were reported, mostly in the placebo group, due to the occurrence of chest pain, left heart failure, sciatica, gastrointestinal bleeding, and a road traffic accident. The 45 patients who opted to take 6-month open-label DG treatment were not significantly different in their baseline characteristics from the remaining patients. Sustained improvement of their brachial FMD (5.2 ± 1.3 to 5.8 ± 1.0%; $p < .0001$) and carotid IMT (0.92 ± 0.23 to 0.89 ± 0.25 mm; $p < .0001$) at 1 year from baseline was observed. In the 10 placebo patients who had not opted for open-label DG, a nonsignificant trend

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TABLE 20.1
Characteristics of Danshen–Gegen and Placebo Groups

	DG Group ($n = 50$)	Placebo Group ($n = 50$)
Age (years)	58.1 ± 7.8	58.2 ± 8.5
Male sex (%)	86(43/50)	88 (44/50)
Active smoker (%)	28	22
	Cardiac Medication	
Statin (%)	74	78
ACEI (%)	40	38
β-blockers (%)	84	84
Aspirin (%)	98	100
SBP (mmHg)	128.9 ± 14.6	127.1 ± 15.2
DBP (mmHg)	78.3 ± 7.5	76.6 ± 6.9
BMI (kg/m^2)	24.7 ± 4.6	25.1 ± 2.5
Glucose (mmol/L)	5.7 ± 1.0	5.3 ± 0.9
Creatinine (μmol/L)	97.5 ± 13.7	96.9 ± 14.8
TC (mmol/L)	4.8 ± 0.8	4.7 ± 0.9
HDL cholesterol (mmol/L)	1.3 ± 0.3	1.2 ± 0.2
TG (mmol/L)	1.8 ± 1.0	1.6 ± 0.8
Folate (nmol/L)	39.5 ± 23.2	35.0 ± 17.8
Homocysteine (μmol/L)	9.5 ± 2.3	10.1 ± 2.7
FMD (%)	5.33 ± 1.21	5.30 ± 1.12
IMT (mm)	0.98 ± 0.29	0.99 ± 0.33
GTN (%)	15.9 ± 2.5	15.7 ± 2.7

Note: Expressions are in the form of mean ±SD; ACEI: angiotensin I converting enzyme inhibitor; BMI: body mass index; DBP: diastolic blood pressure; FMD: flow-mediated dilation; GTN: glyceryltrinitrate-induced dilation; HDL: high-density lipoprotein; IMT: intima-media thickness; SBP: systolic blood pressure; TC: total cholesterol.

of mean carotid IMT deterioration (1.17 ± 0.57 to 1.20 mm ± 0.57; $p > .05$) was seen; they were restudied after 6 months.

20.3.4 Summary of the Cardioprotective Effects of the Danshen–Gegen Formulation

Based on the results of a variety of in vitro, ex vivo, and in vivo biological assays, the beneficial effects of DG formulation have been demonstrated to resemble those of antioxidants, vasodilators, antihypertensives, and antiatherosclerotics. In the in vitro systems, DG formulation could directly scavenge free radicals and prevent oxidative stress exerted on red blood cells, cardiomyocytes, and LDL. Moreover, DG formulation exhibited ex vivo vasodilatory effects on precontracted rat aortas and suppressed further progression of severe hypertension in primary hypertensive rats. Also, DG formulation inhibited the pathological processes of atherosclerosis, including vSMC proliferation and migration as well as monocyte–endothelial cell adhesions. Furthermore, the in vivo antiatherosclerotic effects of DG formulation were demonstrated in the rabbit model. The clinical trial showed the DG formulation was well tolerated and treatment of patients with CAD was associated with sustained improvements in FMD and IMT, although no marked changes were seen in the biochemical markers of CAD risk such as lipids or homocysteine. In total, these findings provide credible and reliable experimental evidence in support of DG formulation for cardiovascular well-being. However, for clinical use, herbs and herbal formulations of consistently high quality are needed, and this requires effective quality control.

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TABLE 20.2
Results of Placebo-Controlled Trial

	DG Group ($n = 45$)		Placebo Group ($n = 47$)	
	Baseline	**After 24 Weeks**	**Baseline**	**After 24 Weeks**
SBP (mmHg)	128.8 ± 15.1	128.7 ± 16.0	127.3 ± 15.3	121.2 ± 13.9
DBP (mmHg)	78.3 ± 7.6	77.5 ± 6.8	76.5 ± 7.0	74.2 ± 8.4
Total cholesterol (mmol/L)	4.8 ± 0.9	4.6 ± 0.8*	4.7 ± 0.9	4.5 ± 0.9
LDL cholesterol (mmol/L)	2.7 ± 0.9	2.6 ± 0.7*	2.8 ± 0.8	2.5 ± 0.7*
HDL cholesterol (mmol/L)	1.3 ± 0.3	1.3 ± 0.3	1.2 ± 0.2	1.2 ± 0.2
Triglyceride (mmol/L)	1.7 ± 1.0	1.7 ± 1.0	1.6 ± 0.8	1.7 ± 1.2
Folate (nmol/L)	39.7 ± 23.4	31.0 ± 20.9	35.0 ± 17.8	32.8 ± 17.5
B12 (pmol/L)	319.6 ± 109.1	374.7 ± 229.0	316.8 ± 124.5	350.4 ± 148.0
Homocysteine (μ mol/L)	9.6 ± 2.4	10.7 ± 2.4	10.1 ± 2.7	10.9 ± 3.2
sICAM-1 (ng/mL)	482.9 ± 125.9	510.6 ± 167.8	486.2 ± 156.4	479.6 ± 154.0
sVCAM-1 (ng/mL)	820.5 ± 205.4	833.9 ± 248.0	833.6 ± 239.8	847.0 ± 244.2
E-selectin (ng/mL)	24.1 ± 11.7	24.5 ± 13.4	27.0 ± 13.7	25.0 ± 13.0
hsCRP (mg/mL)[a]	1.1(2.5)	1.3(1.7)	1.2(1.8)	1.1(2.1)
Fibrinogen (g/L)	4.3 ± 1.1	4.2 ± 0.6	4.3 ± 0.9	4.2 ± 1.1

Note: All expressions, unless otherwise specified, are in the form mean ±SD; B12: vitamin B12; DBP: diastolic blood pressure; hs-CRP: high-sensitivity C-reactive protein; sICAM-1: soluble intercellular adhesion molecules-1; sVCAM-1: soluble vascular cell adhesion molecules-1; SBP: systolic blood pressure.

[a] Data are median (interquartile range).

*$p < .05$ when compared with baseline, after Bonferroni adjustment.

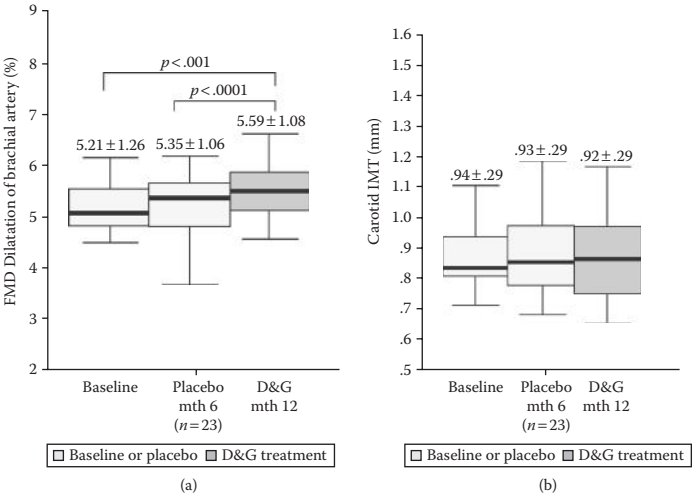


FIGURE 20.2 Changes of (a) brachial flow-mediated dilation and (b) intima-media thickness after 6 months of placebo and subsequent 6 months of open-label herbal medicine (DG) treatment. In each box plot, the bottom and top of the box represent the twenty-fifth percentile and the seventy-fifth percentile, respectively. The line across each box represents the median value, and the vertical lines encompass the entire range of values for each group of the participants.

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TABLE 20.3
Changes in Vascular Profiles as Primary Efficacy End Points in a Danshen–Gegen (DG) Trial

	DG Group ($n = 45$)		Placebo Group ($n = 47$)	
	Baseline	**After 24 Weeks**	**Baseline**	**After 24 Weeks**
Hyperemia (%)	899 ± 106	901 ± 97	887 ± 160	883 ± 148
FMD (%)	5.35 ± 1.21	5.91 ± 0.95***††	5.33 ± 1.13	5.48 ± 0.97*
IMT (mm)	0.98 ± 0.30	0.96 ± 0.32*†	0.98 ± 0.34	0.98 ± 0.34
GTN (%)	15.9 ± 2.6	16.6 ± 2.4**	15.8 ± 2.6	16.1 ± 2.6
FMD/GTN	0.34 ± 0.08	0.36 ± 0.06*	0.35 ± 0.09	0.35 ± 0.09

Note: All expressions, unless otherwise specified, are in the form mean ±SD; FMD: flow-mediated dilation of brachial artery; GTN: glyceryltrinitrate-induced dilation of brachial artery; IMT: carotid intima-media thickness; Reactive hyperaemia was calculated as the maximum flow recorded in the first 15 seconds after cuff deflation divided by the flow during the resting (baseline) scan.

*$p < .05$; **$p < .01$; and ***$p < .001$ when compared with baseline.

†p value < .05 and ††$p < .001$ when compared with placebo group.

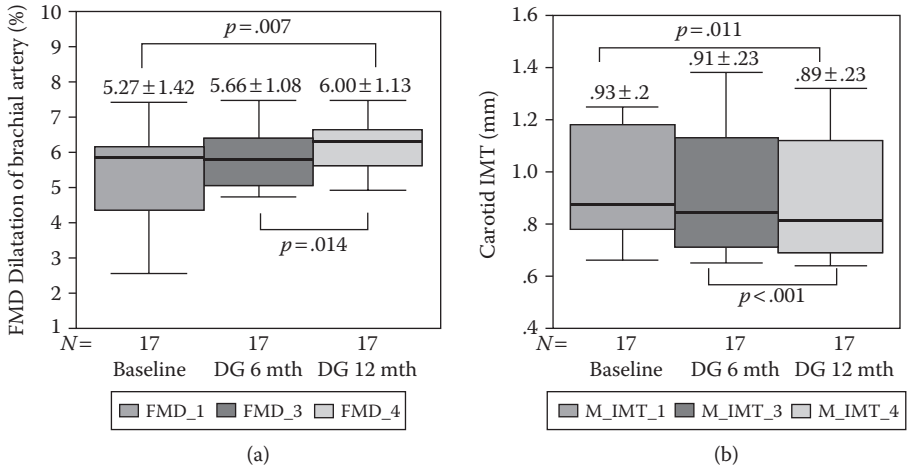


FIGURE 20.3 Changes of (a) brachial flow-mediated dilation and (b) intima-media thickness after 6 and 12 months of herbal medicine (DG) treatment. Changes in carotid IMT at 24 weeks after placebo and herbal medicine (DG) treatment are shown. In each box plot, the bottom and top of the box represent the twenty-fifth percentile and the seventy-fifth percentile, respectively. The line across each box represents the median value, and the vertical lines encompass the entire range of values for each group of the participants.

20.4 QUALITY CONTROL OF HERBS: THE CHEMOMETRICS APPROACH

20.4.1 Background

In general, one to a few representative chemical constituents with known chemical structures or "markers" are used for authentication of herbal medicines (Zschocke, ClaBen-Houben, and Bauer 2001b; Upton 2003a; State Pharmacopoeia Commission of People's Republic of China 2005; United States Pharmacopoeial Conversion 2009). Unknown herbs are identified and the identity of a known herb is confirmed by the markers found within it. The same approach is applied very often to identify

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the component herbs within a combination preparation, such as the DG formulation. Furthermore, to ensure the quality of a formulation, it is essential to maintain the correct amounts of each herb. In the DG formulation, the major classes of the chemical constituents of danshen are phenolic acid and diterpene (Zhu et al. 2007), while that of gegen is isoflavonoid (Chen et al. 2007; Jiang, Lau, and Lamm 2005). According to the Chinese Pharmacopoeia (State Pharmacopoeia Commission of People's Republic of China 2005), the recommended markers of danshen are tanshinone IIA, a diterpene, and SAB, a phenolic acid. These components are reported to promote blood circulation (Gu et al. 2004; Gao et al. 2009). The isoflavonoid, puerarin, is the marker for gegen, and is an antioxidant (Chung et al. 2008).

20.4.2 The Pattern-Oriented Approach in Chemometrics

The use of a few markers for the authentication of herbal medicine has been widely accepted by authorized agencies and by the pharmaceutical industry (CDER 2004; Zeng et al. 2008). However, some markers are not unique and may be found in other herbs also. For instance, Z-ligustilide, the widely used marker of *Radix Angelicae sinensis*, danggui (Zschocke, Classen-Houben, and Bauer 2001a), can also be found in *Radix Ligustici chuanxiong* (Zschocke, ClaBen-Houben, and Bauer 2001c). Moreover, only one marker, that is, salvianic acid A, is recommended for the Chinese medicine combination preparation *fufang danshen diwan,* with three component herbs (State Pharmacopoeia Commission of P. R. China 2005). Ginseng (Qian, Guo and Li 2009) and *Ginkgo biloba* (Upton 2003b) are a few examples of herbs with more than 10 chemical standards available. Furthermore, for many herbs, there are no reference materials and markers are not established. Therefore, from the quality control point of view, the previously accepted marker approach is barely satisfactory, and a multicompound approach is now adopted for authentication of herbal medicines (Mok and Chau 2006; Zeng et al. 2008). This approach makes use of chemical constituents with known chemical structures and also those on whom only partial chemical information is available, for example, retention times, mass spectra, and ultraviolet (UV) spectra. This approach provides a more accurate and reliable means for quality control. The marker and multicompound approaches are grouped together and referred to as a "compound-oriented approach" (Zeng et al. 2008; Figure 20.4).

20.4.3 Separation Techniques Used in the Multicompound Chemometric Approach

The use of advanced hyphenated separation techniques is one of the key factors that makes the multicompound approach feasible (Chau et al. 2004; Zeng et al. 2008). An example is the coupling of an HPLC or its faster version and a rapid-resolution liquid chromatography (RRLC) with a multichannel diode array detector (DAD), which can acquire an absorption spectrum over a wide wavelength range for every retention time, greatly increasing the amount of data obtained. Figure 20.5a shows the three-dimensional (3D) chromatogram of the DG formulation obtained within 60 minutes by HPLC–DAD

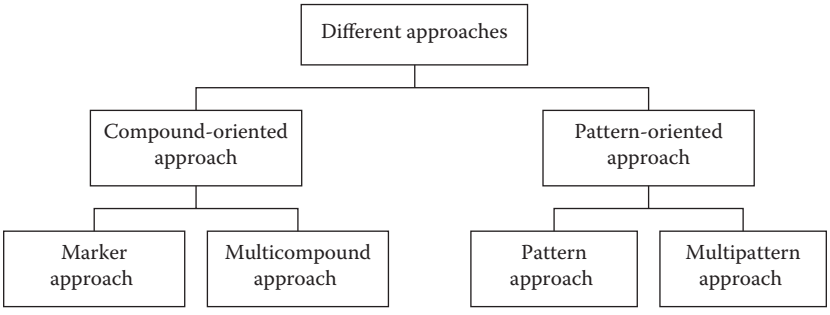
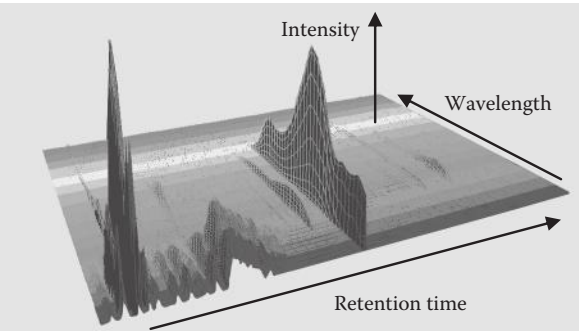
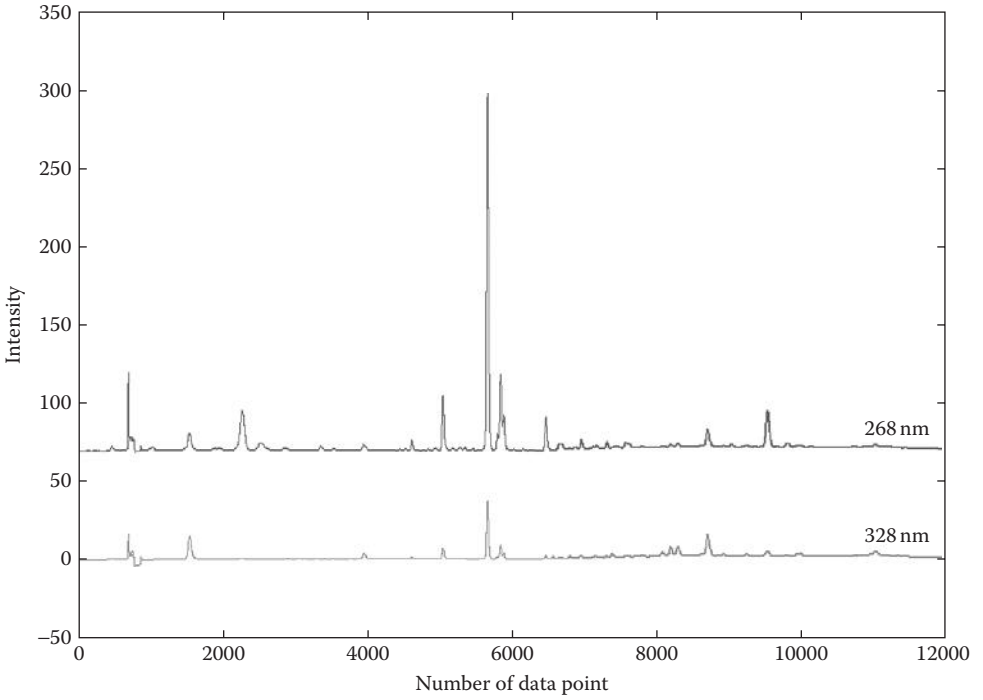


FIGURE 20.4 Different approaches for the quality control of herbal medicine.

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(a)



(b)

FIGURE 20.5 (See color insert.) Chromatograms of DG formulation: (a) the three-dimensional chromatogram of DG formulation from 245 to 400 nm, and (b) the two-dimensional chromatograms of DG formulation measured at wavelengths of 268 and 328 nm.

(the 1100 instrument of Agilent Technology, Inc., California) in the spectral range of 245–400 nm. The total number of spectrochromatographic data points obtained is 1.27 million. Figure 20.5b gives the commonly used two-dimensional (2D) chromatograms of the same herbal medicine measured at 268 and 328 nm, with each peak representing a chemical. Each of these 2D chromatograms consists of 12,000 data points, which is hundredfold less than that of the 3D chromatogram. As the chemical constituents are sensitive to the wavelength selected, the two 2D chromatograms do not have the same profile structure, and care has to be taken in determining the chemical composition of herbal medicine by selecting the right wavelength. The 3D chromatogram provides information across the spectrum, providing more information for identification. In quantitative analysis, the wavelength of the highest absorptivity is usually selected for the component concerned as it produces the most accurate results. However, DAD has its limitation: it cannot detect those ingredients that have no or very

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low absorption. In this situation, MS and other techniques are used, for example, gas chromatography (GC)-MS, liquid chromatography (LC)-MS, and liquid chromatography-tandem mass spectrometry (LC-MS/MS). There are different classes of chemical compounds present in herbal medicine. In chemical fingerprinting, HPLC is commonly utilized to analyze more polar compounds, whereas GC is good for volatile compounds. This leads to the use of more than one kind of fingerprint in a multipattern approach instead of just one in the single-pattern approach for assessment (Mok and Chau 2006; Zeng et al. 2008).

The huge amount of data obtained from hyphenated instruments embeds valuable information. How to dig or mine it out is a great challenge to scientists. In recent years, chemometrics has been found to be very useful in this aspect. The development of new chemometrics data-processing methods boosts the application of multicompound and other approaches in the study of herbal medicine. Very often, a chromatogram of an herbal medicine has overlapping peaks. It is not easy to separate them well by adjusting the experimental conditions alone. Here, chemometric resolution methods (CRMs) and other mathematical tools, such as heuristic evolving latent projection (HELP), multicomponent spectral correlative chromatography (MSCC), window factor analysis (WFA), subwindow factor analysis (SFA), augmented evolving window orthogonal projection (AEWOP), principal component analysis (PCA), and partial least squares (PLS), can help (Otto 1999; Malinowski 2002; Chau et al. 2004; Mok and Chau 2006; Zeng et al. 2008). For instance, the chemical compositions of the head, body, and tail of danggui were studied in detail by GC-MS and chemometrics (Wei et al. 2008). The tools SFA and AEWOP were applied to resolve the GC-MS data sets of these parts to get the pure mass spectra of many chemical ingredients. Up to 42 common components were found, and 35 were identified by matching their resolved spectra with those available in the National Institute of Standards and Technology (NIST) MS database and the Wiley Registry of Mass Spectral data (McLafferty 1989). This approach provides a better understanding on the variation in different parts of danggui. Through LC-DAD-atmospheric pressure chemical ionization mass spectrometry (LC-DAD-APCI-MS) and data analysis using such specialized tools, Wang et al. (2005) investigated the absorption and metabolite components in plasma samples from a rabbit that was given an oral solution of danggui. More than 32 chemical components were discovered in both danggui and biofluid samples, but over 10 components were detected only in plasma. The different chemical patterns provide information about what danggui ingredients were absorbed and what metabolites were generated.

In the year 2000, the State Food and Drug Administration (SFDA) of the People's Republic of China announced that all the commercial injectable Chinese medicines were required to have their chemical fingerprints submitted for approval (SFDA 2000). Here, "fingerprint" refers to patterns like the chromatogram, spectrum, and others. The use of this fingerprint incorporates the concept of photoequivalence (Tyler 1999). From an authentication point of view, a chromatogram and spectrum contain information of all the chemical components of a sample that can be detected by the utilized instrument or method. Of course, the appearance of a pattern depends very much on the tools used, and the spectral pattern of an herb or herbal mixture may look very complicated with overlapping profile structures. This can make data analysis and interpretation difficult without the use of chemometric tools to resolve the pattern and reveal interesting chemical features.

By nature, the application of the pattern or fingerprint approach for quality control is very different from that of the conventional compound-oriented approach, which uses known markers and target compounds. Fingerprinting makes use of all the components at the same time, even though their chemical properties may not be fully known. Therefore, all the bioactive and inactive ingredients are recorded within the fingerprint. This is very important because the biological activity of a Chinese medicine is usually induced by more than one constituent, and there may be important interactions among constituents (Xie 1998; Xie 2000; Mok and Chau 2006). In the fingerprint approach for qualitative analysis, the retention times of components from different samples as found in the entire or selected regions of their chromatograms are utilized for comparison. Using the "true" retention time to identify an unknown compound is a common practice of analytical chemists.

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Chromatograms under study have to be run under identical experimental conditions, and data preprocessing is used to minimize the noise level and to do the peak alignment so as to get the true chromatogram of a sample, after which quantitative analysis can be carried out. The content of a constituent in a certain sample is usually determined by its chromatographic peak height or area. As for the pattern approach, a common assessment scheme is to evaluate how similar the components of different samples are to one another. The similarity index (SI) parameter provides a quantitative measure of this kind. The index is usually represented by the correlation coefficients between the fingerprints under study (Yi et al. 2009). In this way, every data point corresponding to a retention time and related height is considered in the comparison. If the SI value is close to 1.0, then the chemical compositions of the two samples concerned are almost identical to each other. If one of them is an unknown herb and the other one is a known or reference herb, then the unknown herb is identified or authenticated. Furthermore, the use of SI incorporates a fuzzy logic concept in assessing the quality of the sample because it allows tolerance of the natural variations and differences between different samples of the same Chinese medicine.

Using the whole fingerprint approach emphasizes the integral chemical feature of a system with the help of a high-throughput measurement technique. The demand of SFDA has triggered other organizations, including the World Health Organization (WHO; Department of Technical Cooperation for Essential Drugs and Medicines Policy 2005), Food and Drug Administration (FDA) of the United States (CDER 2004), and European Agency for the Evaluation of Medicinal Products (EMEA; CDER 2004), to consider chemical fingerprinting as a means of screening botanical and related products (Mok and Chau 2006; Zeng et al. 2008).

To improve quality monitoring, an effort has been made to put both chemical and bioactivity information together (SFDA 2000; Upton 2003a; Cheng, Wang, and Wang 2006; Dong et al. 2006; Lu et al. 2006; Dumarey et al. 2008; Zeng et al. 2008). This is another kind of multipattern approach. Together with the single-pattern approach mentioned earlier in this section, they are grouped as a pattern-oriented approach (Figure 20.4), in contrast to the compound-oriented approach mentioned earlier in this section (Zeng et al. 2008).

20.4.4 Application of Chemometric Approach in the Quality Control of Herbs

Two different batches of DG (as described and used in the clinical study in Section 20.3.3) were prepared in 2002 and 2005, but they were based on the same prescription. The authentication procedures as recommended by the Chinese Pharmacopoeia 2000 (State Pharmacopoeia Commission of People's Republic of China 2000) were applied to these Chinese medicine samples. They are briefly described here. They cover the markers used, chemical analysis procedures and techniques, as well as related experimental conditions. Thin-layer chromatography (TLC) and HPLC were the basic analysis techniques used. Some of these procedures were modified by us or taken from the literature so as to acquire more information and more accurate results with a minimal number of experiments. In addition, the HPLC-DAD and LC-DAD-MS hyphenated instruments were used to acquire 3D chromatograms of the samples in order to get more information for chemometric data analysis. Other target compounds of sodium danshensu, daidzin, daidzein, and SAB were also included. For those compounds with reference standards available, different concentrations of the standards were prepared and calibration curves were set up by plotting chromatographic peak area against concentration. Through these, the concentrations of these compounds in unknown samples were determined.

The compound-oriented and pattern approaches were applied to the HPLC-DAD and LC-DAD-MS data of the samples. We also looked into the UV or MS spectral data at those retention times of interest to further confirm the identification. In cases of profiles with overlapping or "embedded" peaks, CRMs and other chemometric methods were applied to resolve them, as described previously in a study on the Chinese medicine formulation ping-wei powder (Gong et al. 2001). It is worthwhile to look into another useful application of CRM here. In our experience, CRM can help to obtain more chemical information from a single experiment, thereby shortening

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the analysis time. As more chemical information, like retention times and UV or MS spectra of the components of an herbal medicine, can be obtained through the combination of experimental study and chemometric data analysis, quality control is carried out more efficiently, more effectively, and more reliably. Even components for whom only partial information is available can be included for the purpose. This is very helpful in dealing with a Chinese medicine formulation with more than one component herb. We adopted this way throughout our project and some of the results obtained will be presented in Section 20.4.5.

The HPLC-DAD and LC-MS fingerprints of the DG samples were also utilized for quality control in the pattern-oriented approach in this work. In applying the whole fingerprint to do the job, the chromatograms themselves have to be preprocessed to get the true ones as mentioned in Section 20.4.3. Here, data preprocessing includes noise removal, peak alignment, background shift, and other activities. Afterward, the similarity of these true chromatographic patterns was obtained quantitatively through correlation analysis to give the related SIs for assessment. Moreover, the mean chromatogram based on those from different samples of the same Chinese medicine was also obtained. This served as the representative or the "standard" fingerprint of the medicine, provided the different individual chromatograms were not too diverse. All chemometrics data analyses were carried out by the computer-aided similarity evaluation (CASE) system method, which was coded and run under the MATLAB® environment (Wang et al. 2008). A CRM was then applied to the preprocessed fingerprints to resolve overlapping peak clusters to get more accurate pure spectra and retention times of the components involved.

20.4.5 Example with Danshen, Gegen, and Danshen–Gegen Formulation

The TLC separation technique was applied to investigate the Chinese medicine samples of danshen, gegen, the reference herb of dashen, as well as the two batches of DG. The two markers tanshinone IIA and puerarin were used in the TLC study for quality control. Visible light was utilized to detect these two markers in the developed TLC plates. These markers were found in all the samples concerned.

The HPLC provides better separation power of chemical components than TLC, and the markers considered in the TLC work were further investigated by HPLC-DAD and LC-MS. Again the respective markers of danshen and gegen (tanshinone IIA and puerarin) were identified in the single herb samples. These component herbs were also found in the DG products based on these two markers and the other target compounds in Section 20.4.1. In doing so, we compared both their retention times obtained and the observed UV spectra at the same times in different chromatograms.

The stability of the DG product using both the marker and the pattern approach was then evaluated. Stability is very important to assure the consistency of the quality of encapsulated herbs such as the DG capsules used in the clinical trial mentioned in Section 20.3.3. Here, we scrutinized the variation in the chemical compositions of the DG samples before and after 3 months of "accelerated aging" treatment, which is commonly used to establish the shelf life of a product. All samples were analyzed by HPLC-DAD with the sample preparation and experimental procedures all exactly the same. Results showed that the marker contents at time 0 and after 3 months were 0.42 and 0.45 mg/g, respectively, for puerarin and 0.07 and 0.03 mg/g, respectively, for tanshinone IIA. This indicates the product was stable.

We also expanded stability testing using all the detected chemical compounds in these samples for comparison via the pattern approach. By visual inspection, the time-0 and 3-month chromatograms were very similar (result not shown), but in order to determine how similar they are quantitatively, their similarity index (SI) was calculated and was found to be 0.99. Here, the profile structure used for comparison included the peak height or intensity measured at every retention time. It should be noted that the HPLC-UV chromatographic height is related directly to the content of a component in the sample concerned, although different peaks indicate different components. Therefore, this approach to stability testing simultaneously investigates loss of individual compo-

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nents (by peak height comparison) as well as their transformation into other components (by SI comparison).

It is worth mentioning another application of SI here, and that is performance monitoring of the separation instrument. Multiple successive injections of the same extract give SIs that should be in high agreement (based on our experience, at least >90% to be acceptable).

In testing the 2002 and 2005 batches of DG and their chemical compositions, an LC-DAD-MS study was carried out. Again, we used both the multicompound and pattern approach to scrutinize the variation of their chemical components based on the 3D chromatograms obtained. The target compounds (markers) used were sodium danshensu, SAB, puerarin, daidzin, and daidzein. The HPLC-UV chromatograms acquired at 280 and 254 nm were chosen, respectively, for the first two compounds and the remaining three in quantitative analysis. Calibration curves were set up based on the chromatographic peak areas of individual compounds. All had values of linear regression coefficient >0.95. The contents (in milligram per gram) of markers in the 2002 and 2005 batches of DG were determined to be, respectively, 5.54 and 6.25 for sodium danshensu, 10.89 and 12.18 for puerarin, 1.47 and 1.72 for daidzin, 1.72 and 2.36 for SAB, and 0.87 and 1.03 for daidzein, indicating acceptable stability.

The chromatograms of the two DG products (not shown here) exhibited complicated profiles in the early elution phase. To look into their chemical composition in more detail, chemometric CRM and alternate moving window factor analysis (AMWFA) methods were applied to their LC-MS chromatograms to resolve the overlapping peaks. In this way, a total of 28 common components were identified based on how close their retention times were. In addition, comparison was made on the resolved mass spectra. The matching factors of common components were all >91% with nine components of the two batches matching at >99%. Therefore, using the multicompound approach, a more detailed comparison of the chemical compositions of the 2002 and 2005 DG batches was revealed.

The whole HPLC-UV fingerprints of the two batches of DG were also used for similarity assessment. First, data preprocessing was carried out on the HPLC-UV chromatograms acquired. The calculated SI values at 254 and 280 nm were 88% and 87%, respectively, indicating a high similarity in the chemical compositions of the two batches.

20.4.6 Summary

Authentication of the samples of DG combination preparation and its component herbs danshen and gegen were studied in detail using the traditional marker approach by using TLC, HPLC-DAD, and LC-DAD-MS. In addition, the multicompound approach, as well as the newly developed pattern or fingerprint approach, coupled with chemometric data processing, was performed. By applying CRM and other mathematical methods to two batches of DG, 28 common components were found by comparing their resolved retention times and pure MS spectra obtained from the respective LC-DAD-MS chromatograms. This improves the quality control of complex systems like Chinese medicine combination preparation via the multicomponent approach. Furthermore, all the components of the two batches as detected by LC-DAD-MS were used in the pattern approach for assessing their chemical compositions and the related contents of components. The HPLC-UV SI value of the two batches (from 2002 and 2005) was high, and the stability of the DG products was assessed using the same approach.

20.5 CONCLUDING REMARKS

There is a need for more objective and scientific ways to authenticate individual herbs, identify chemical constituents, detect adulteration or contamination of herbs, and monitor the quality of herbs and herbal medicines. There is also a need to check the consistency of different batches of herbs used in clinical studies and to identify bioactive components in herbs reported to have physiological effects. New technologies are now providing chemical fingerprinting of herbs for such purposes. This chemometric approach enables standardized formulations or "fingerprint models" to be

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produced, and these are described in this chapter along with their application and clinical studies of an herbal medicine containing danshen and gegen.

The chemometric approach is developing rapidly, and lends itself to the study of various herbs and foods. For example, different types of green tea have been studied, and models have been built using the chromatographic fingerprints and antioxidant capacities obtained (Dumarey et al. 2008). Wang, Wang, and Cheng (2006) proposed a quantitative composition–activity relationship to botanical drug design. Chau et al. (2009) developed the quantitative pattern–activity relationship (QPAR) technique and applied it to discover the antioxidative active regions in the fingerprint of the Chinese medicine gegen. Therefore, in addition to standardizing herbal medicines and assessing quality, the chemometrics approach can help identify a drug lead from plants and herbs.

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21 Ethics of Using Herbal Medicine as Primary or Adjunct Treatment and Issues of Drug–Herb Interaction

Lauren Girard and Sunita Vohra

CONTENTS

21.1 Introduction 439
21.2 Ethical Principles Relating to Treatment Decisions 439
21.3 Epidemiology of Herbal Medicine Use 441
21.4 Pharmacology of Herbal Medicines 441
21.4.1 Efficacy 442
21.4.2 Safety 443
21.5 Herbal Medicines as Primary Treatment 444
21.6 Herbal Medicines as Adjunct Treatment 444
21.7 Issues of Drug–Herb Interaction 444
21.8 Recommendations 445
21.8.1 Recommendations for Clinical Care 445
21.8.2 Recommendations for Research into Herbal Medicine 447
21.8.3 Recommendations for Education in Herbal Medicine 447
21.9 Conclusions 448
Acknowledgments 448
References 448

21.1 INTRODUCTION

This chapter intends to provide a brief overview of the basic ethical principles that are applicable to natural health products (NHPs), including herbal medicines. Beneficence, nonmalfeasance (nonmaleficence), and patient autonomy are important pillars of biomedical ethics that also apply to the realm of natural medicines (Kemper and Cohen 2004). The data about herbal medicine utilization, pharmacology, safety, and efficacy are discussed, as well as conditions in which herbal medicines may be used as primary versus adjunct treatment. Issues of NHP–drug interactions are reviewed, as they may be synergistic or antagonistic and must be considered whenever concomitant use of products occurs. Gaps in knowledge are identified, and recommendations are suggested to further explore the issues related to NHP–drug interactions.

21.2 ETHICAL PRINCIPLES RELATING TO TREATMENT DECISIONS

Bioethical principles such as beneficence, nonmalfeasance, patient autonomy, justice, and public accountability are considered to be an essential part of conventional medicine, as they guide

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clinicians' interactions with patients such that patients' interests are of primary importance. As complementary and alternative medicine (CAM), including the use of herbal medicines, becomes increasingly popular in the Western world, it is becoming more and more apparent that these same bioethical principles are applicable to these alternate forms of health care (Kemper and Cohen 2004). This chapter discusses the core ethical principles of beneficence, nonmalfeasance, and autonomy as they relate to the use of herbal medicine.

Beneficence is the bioethical principle that describes the clinician's obligation to promote the well-being of a patient; it is active because clinicians must take appropriate measures to ensure that some positive outcome will occur (Beauchamp and Childress 2009). There are two types of beneficence: utility and positive. Utility beneficence refers to an individual's effort to consider all the advantages and hazards to a patient in order to achieve the best solution, whereas positive beneficence necessitates that one's actions help others; prohibiting others from experiencing harm is an example of positive beneficence (Beauchamp and Childress 2009).

Clinicians have an obligation to practice beneficence, regardless of the domain of medicine being discussed. Fulfillment of this obligation requires that the clinician promotes any therapy that is safe and effective, regardless of whether it is conventional, complementary, or alternative (Institute of Medicine (U.S.) Committee on the Use of Complementary and Alternative Medicine 2005). In order to advise the patient, a physician must assess the safety regarding a treatment and determine whether there is evidence-based information about its efficacy (Kemper, Vohra, and Walls 2008).

Nonmalfeasance describes the responsibility to not hurt others (Beauchamp and Childress 2009). This ethical principle is often associated with that of beneficence, but there are important distinctions that must be made, because one's duty to prevent harm is not the same as the duty to promote well-being (Beauchamp and Childress 2009). Although NHPs are considered natural and benign, one should realize that "natural" is not synonymous with safe (Ernst 1998). Harm is often possible when patients use NHPs, so clinicians must intervene when necessary to practice nonmalfeasance. Health care practitioners must be cognizant of the potential risks and the potential benefits before recommending herbal medicines.

Patient autonomy is a cornerstone of conventional medicine that is also applicable to the use of NHPs. In most parts of the world, consumer access to herbal medicines is not prescription controlled, allowing for widespread use. While self-care is a component of patient autonomy, another key element is that the patient has enough information to make an informed treatment decision (Ernst and Cohen 2001). Researchers have consistently found that patients get their information about NHPs from relatives, friends, magazines, and the Internet (Gardiner and Riley 2007; Khader et al. 2008; Low 2009), all of which are perceived as less reputable than other sources (Health Canada and Ipsos Reid 2005). Clinicians have a responsibility to find information for themselves so that they can share it with patients (Committee on Children with Disabilities 2001), making them important sources of information. Typically, clinicians are not the primary source of information (Health Canada and Ipsos Reid 2005).

Patient access to NHPs depends on how they are regulated and therefore varies by jurisdiction. For instance, NHPs in Canada are regulated by the Natural Health Product Directorate (NHPD; Health Canada 2005), whereas in Europe they are regulated by the Committee on Herbal Medicinal Products (European Medicines Agency 2008); the Dietary Supplement Health and Education Act (DSHEA) enacted by the Food and Drug Administration (FDA) is the American counterpart (U.S. Food and Drug Administration 2009a). Although one of the major goals of DSHEA is to increase patient autonomy, this has led to concern among many health care professionals as it allows products to be purchased regardless of their therapeutic effect or their potential for harm. As such, some clinicians feel it does a poor job of allowing for informed consent (Institute of Medicine (U.S.) Committee on the Use of Complementary and Alternative Medicine 2005), which is a cornerstone of ethical use.

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Legal issues regarding the use of herbal medicines are often consistent with those of conventional treatment. For example, when obtaining informed consent, all information relevant to the treatment must be discussed; failure to do so could result in liability as determined by a judicial system (Ernst and Cohen 2001).

21.3 EPIDEMIOLOGY OF HERBAL MEDICINE USE

Herbal products have been used as medicine for millennia by some cultures (Halberstein 2005), and their use has continued to the current day and age. In recent years, the use of herbal medicine has increased dramatically in the Western world and has maintained its popularity among many African and Asian cultures (Eisenberg et al. 1998; Health Canada and Ipsos Reid 2005; World Health Organization 2008).

All age groups report NHP use, whether pediatric, young and middle-aged adults, or the elderly (Kelly et al. 2005; Stasio et al. 2008; Barnes, Bloom, and Nahin 2009). Users are women, (Kelly et al. 2005), higher-paid people (Tanaka et al. 2008), well educated (Kelly et al. 2005), and white (Kelly et al. 2005). However, given the popularity of herbal medicines in all age groups, such descriptors may no longer apply, and therefore, clinicians should be encouraged to ask all patients about their NHP use.

There are various reasons for the increased popularity of NHPs among consumers in the Western world. Users reported varied reasons for use, including to preserve good health (Wheaton et al. 2005), to treat sickness (Wheaton et al. 2005), to ameliorate the side effects of another medication (Yeh et al. 2000), and because conventional medication(s) did not work or were highly priced (Kennedy 2005). Essentially, herbal medicines are an important component of modern health care; if effective, they may promote autonomy in that there are numerous diverse products allowing patients to choose one that meets their specific needs and preferences.

21.4 PHARMACOLOGY OF HERBAL MEDICINES

It is beyond the scope of this chapter to discuss the pharmacology of all herbal medicines and the wide range of conditions for which they are used. However, it is worth noting that given the sheer number of herbal medicines, as well as considerable heterogeneity within and between brands, it is not feasible to evaluate all products for their pharmacology. Although some herbal medicines have had extensive investigation (e.g., St. John's wort and echinacea), most of them have not. Even those that have been relatively well studied often have little information in special populations, such as pediatric, pregnant or lactating women, or geriatric populations (Brulotte and Vohra 2008), and therefore, caution should be used when recommending herbal medicines to these populations. The complexity of studying NHPs is compounded by the fact that many products may have multiple active phytoconstituents, some of which are unknown (Institute of Medicine (U.S.) Committee on the Use of Complementary and Alternative Medicine 2005).

It is also important to realize that herbal medicines have been the basis of many pharmaceutical medications. A common source of new chemical entities is plant isolates that have been modified (Raskin et al. 2002), and estimates suggest that a quarter of conventional pharmaceuticals are plant based (Rates 2001). Vinblastine, for example, is derived from periwinkle, a plant commonly found in Europe and Northern Africa (Ghosh, Thapliyal, and Gurumurthi 2009). This drug is primarily used as a chemotherapeutic agent in many types of cancer (Ghosh, Thapliyal, and Gurumurthi 2009).

Herbal medicines are important sources of new drugs because plants produce secondary metabolites, which may be used as lead compounds (Carpinella and Rai 2009). Furthermore, the number of chemical compounds for which the biological activity is well understood by scientists is a fraction of the sheer number of unidentified chemical components that have potential health effects (Carpinella and Rai 2009). These novel compounds offer a remarkable opportunity for drug discovery. Within

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the scope of ethical NHP use, it seems appropriate that any profit that is achieved from using flora to make pharmaceuticals should be shared among the plant owners (Mendelsohn and Balick 1995). In particular, when researchers plan on studying traditional medicines, it is important that the advantages revealed through study are returned to the particular group from which the knowledge is taken (King, Carlson, and Moran 1996).

21.4.1 Efficacy

NHPs may be as varied as conventional pharmaceuticals in their physiological effects, with specific NHPs purported as effective for supporting the immune system (e.g., ginseng; Predy et al. 2005), the cardiovascular system (e.g., hawthorn; Pittler, Guo, and Ernst 2008), and the gastrointestinal system (e.g., peppermint; May, Kohler, and Schneider 2000). As such, it is beyond the scope of this chapter to review the specific efficacy of all NHPs; rather, guiding principles regarding efficacy and safety evaluation will be discussed, as these are key elements in informed consent.

Herbal medicine is often evaluated in a similar fashion to that of conventional pharmaceuticals through randomized controlled trials (RCTs) and systematic reviews (SRs). However, if such research does not consider the differences between NHPs and pharmaceuticals, then it may be less meaningful than intended. One major difference between NHPs and pharmaceuticals is heterogeneity. Heterogeneity of NHP is due to a combination of variability in factors such as temperature, soil, and growing conditions, and it often results in an inconsistent amount of active ingredients from one batch to the next (Bent and Ko 2004). These differences affect NHPs to a profound degree in comparison with conventional pharmaceuticals, posing a major obstacle for researchers as it may be difficult to determine which product and what dose to study. Because standardization may elude NHPs, researchers have recommended that products must be described thoroughly and be subjected to quality assurance in order to make the results of studies involving these products more consistent (Ernst 2005).

Although some reports suggest that there is limited research on the efficacy of NHPs (Bent and Ko 2004), RCTs for herbal medicines do exist, including for populations that typically receive less study. When compared to reports of conventional RCTs, reporting quality on trials of herbal medicine slightly exceeded that of conventional medicine (Gagnier, DeMelo et al. 2006). Despite this, on average NHP trials report less than half the required items as described by a modified version of the CONSORT checklist (Gagnier, DeMelo et al. 2006). In order to further improve the reporting of herbal medicines, an extension of the CONSORT statements was developed specifically for this topic (Gagnier, Boon et al. 2006). Much emphasis was placed on properly describing the herbal intervention, including name of the herbal medicine, its characteristics, dosage used, and information regarding qualitative testing (Gagnier, Boon et al. 2006).

Although RCT reporting is improved, with thousands of NHPs available in the marketplace and multiple potential indications for each product, it is simply not feasible to conduct large RCTs for every condition–intervention pair to guide clinical decision making. "N-of-1" trials may offer some assistance, as they allow assessment of specific therapies in an individual patient (Guyatt et al. 1986). N-of-1 trials are prospectively planned multiple crossover trials in an individual participant (Guyatt et al. 1990) and often offer the same methodological advantages of RCTs by being blinded and controlled. An N-of-1 approach can help overcome issues of heterogeneity, in effect allowing clinicians to determine whether a product is achieving a beneficial effect in a specific individual.

Given that some herbal medicines have therapeutic abilities equivalent to (and sometimes superior to) those of conventional medicine, patients have a wider range of treatment options, which allows for greater patient autonomy. For instance, both cisapride and probiotics have been favorably evaluated for their role in gastrointestinal motility (Muller-Lissner 1987; Nurko et al. 2000; Bu et al. 2007). However, there are adverse events associated with cisapride (as discussed in Section 21.4.2) that makes its use less favorable compared to probiotics. St. John's wort is another example of the comparative effectiveness of NHPs, as its ability to treat mild to moderate depression is equivalent

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to that of conventional pharmaceuticals (Whiskey, Werneke, and Taylor 2001; see also Chapter 11 on St. John's wort). Saw palmetto is yet another example, as it relieves prostatic symptoms of the urinary tract that are comparable to the effects of finasteride, a prescription medication used for the same indication (Wilt et al. 1999).

As knowledge about efficacy is a cornerstone of informed consent, it is important to note that basic and clinical research on NHP efficacy is available, although its quantity and quality can still be improved upon. Although it is promising that some NHPs have been evaluated favorably in comparison to their conventional pharmaceutical analogues, it is premature to assume this is always the case. In each instance, the relative efficacy and relative safety of a given product must be considered for a given individual's health state to promote their well-being.

21.4.2 Safety

Herbal medicines may pose harm to patients under a variety of circumstances. The NHP itself may be associated with idiosyncratic or allergic reactions (Doyle and Kargin 1996; Taylor et al. 2003). NHPs may also interact with conventional pharmaceuticals. This issue is examined in more detail in Section 21.7. NHPs may also be harmful if adulterated or contaminated.

Adulteration is the purposeful addition of extraneous substances to a product. Most often, this has been the undeclared addition of pharmaceuticals to enhance the purported effects of an NHP. Common examples of NHPs that have suffered from adulteration include weight-loss remedies and sexual enhancement products. Numerous weight-loss products have been adulterated with prescription medications like sibutramine, cetilistat, furosemide, or chemicals like phenolphthalein, all of which are only available with a prescription or are illegal to add to health products in an undeclared fashion (U.S. Food and Drug Administration 2009b). In a study of 26 herbal medicines taken from a pharmacy in Hong Kong and tested for adulterants, 15 were adulterated with phosphodiesterase V inhibitors, including sildenafil (Poon et al. 2007).

Contamination refers to the inadvertent addition of extraneous substance. For herbal medicines, this can be heavy metals (Centers for Disease Control and Prevention [CDC] 2004), other herbs (Slifman et al. 1998), or bacteria (Stickel et al. 2009). Some NHPs from India and China have been found to have traces of heavy metals such as lead, arsenic, and mercury (Kew et al. 1993; Centers for Disease Control and Prevention [CDC] 2004). A recent case in which an herbal medicine tested positively for the presence of microorganisms has confirmed the seriousness of bacterial contamination. The herbal medicine was also implicated in two cases of liver damage (Stickel et al. 2009).

Thus far, NHPs have not had many reported cases of associated harm. Product safety depends on pharmacovigilance. Around the world, passive surveillance is the usual regulatory standard, including for NHPs. However, passive surveillance, relying on an individual's initiative to report suspected harm, is known to be affected by both under-reporting (Alvarez-Requejo et al. 1998) and poor quality reports (Charrois et al. 2007), making determination of causality extremely challenging for regulators. Moreover, a general preconception that NHPs are safe and a lack of public appetite for considering potential interactions with prescription medications contribute to the lack of reported NHP adverse events (Charrois et al. 2007).

An ethical approach to NHP safety demands comparative assessments with conventional pharmaceuticals for the same indication. For example, cisapride was found to cause long QT syndrome in some individuals (Lewin et al. 1996) and was withdrawn from the market in Canada (Canadian Pharmacists Association 2009). In contrast, evidence has yet to show any significant safety issues when probiotics are taken by people in good health, even for extended periods (Charrois, Sandu, and Vohra 2006). Using another example mentioned in Section 21.4.1, selective serotonin reuptake inhibitors for depression can be associated with serious side effects, including serotonin syndrome (Ozdemir, Yalug, and Aker 2008). In contrast, the herb St. John's wort is relatively benign as a monotherapy (Whiskey, Werneke, and Taylor 2001), although it can have serious side effects when taken with some other medications (Mathijssen et al. 2002; see also Chapter 11 on St. John's wort).

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21.5 HERBAL MEDICINES AS PRIMARY TREATMENT

Using herbal medicines as primary treatment is certainly feasible, and even recommended, if its risk-benefit ratio compares favorably to conventional alternatives. This does not depend only on the herbal medicine being more effective. Herbal therapy may be the preferred choice if it is less harmful than its conventional counterpart. The degree to which a clinician might feel comfortable to support the use of herbal medicine as primary treatment often depends on the seriousness of the situation (Adams et al. 2002). For mild or self-limiting illnesses, a clinician might be more willing to tolerate herbal medicine as primary treatment, if it is believed to be safe and it is consistently preferred by patients, even in the absence of compelling efficacy data. A corollary is that the patient must be informed if the herb is not well studied, because the benefit of treating a minor illness may not be worth the potential harm of an unstudied therapy (Adams et al. 2002). Risk must be evaluated in a relative fashion, in light of the known safety profile of the herbal medicine and its conventional counterpart, so that the patient can make an informed choice. For example, if a patient is suffering from mild nausea due to pregnancy, it may be acceptable to let the patient use an NHP such as ginger, which is found to be both safe and effective (Borrelli et al. 2005; see also Chapter 7 on ginger). In cases of moderately severe illness, a clinician might be willing to promote the use of herbals as primary treatment (i.e., as the sole therapy rather than as an adjunct) if conventional treatment was not working as anticipated or if the patient had experienced side effects that limited its use. In contrast, if a patient had a serious or life-threatening illness, the use of herbal medicines as primary treatment would be limited to conditions for which there were no known effective conventional therapies or for which herbal medicines had compelling efficacy data.

In each instance, evidence and patient preference should guide decision making in conjunction with clinical judgment. If evidence suggests that the herbal medicine may be effective and without harm, then it is reasonable for the clinicians to discuss this therapeutic approach with patients (Cohen and Eisenberg 2002). In cases where the NHP is relatively unknown to the clinician, he or she should be encouraged to seek advice on its use and to develop experience in therapies of relevance and interest to his or her patient population.

21.6 HERBAL MEDICINES AS ADJUNCT TREATMENT

Many individuals use NHPs to increase the effect of pharmaceutical medication or to minimize its side effects (Yeh et al. 2000; Boullata 2005). Under these circumstances, when herbal medicine is an adjunct, a clinician may feel comfortable with a lower standard for proof of efficacy than the herbal medicine being used as primary treatment. Examples of adjunct therapy include the following: using butterbur for migraine prophylaxis (Lipton et al. 2004) along with propranolol (Pradalier et al. 1989), traditional Chinese medicine (TCM) herbals and corticosteroid for eczema (Sheehan and Atherton 1992), and plant sterols (de Jongh et al. 2003) and atorvastatin to treat hypercholesterolemia (Nawrocki et al. 1995). To minimize sleeplessness associated with the use of methylphenidate (Adesman and Morgan 1999), patients with attention-deficit hyperactivity disorder (ADHD) may use melatonin to help them sleep (Van der Heijden et al. 2007). Of course, when using NHPs as adjuncts to drug therapy, precaution should be taken to avoid patient harm through NHP–drug interactions.

21.7 ISSUES OF DRUG–HERB INTERACTION

An important demographic of NHP users is serious, chronic, or recurrent medical conditions (Roy-Byrne et al. 2005). These individuals are also most likely to take prescription medications, and as the likelihood of an adverse event increases with the number of products taken concurrently, they are at an increased risk for experiencing drug–herb interactions. Although it seems that few reports of NHP adverse events exist, studies have shown that adverse events due to NHP–drug

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interactions do occur but are underreported (Charrois et al. 2007). In some cases, NHP–drug interactions can cause serious harm or even death (Ruschitzka et al. 2000; Kupiec and Raj 2005).

Of note, many NHP users report taking more than one NHP simultaneously (Martin et al. 2002; Glover et al. 2003; Lanski et al. 2003), which puts them at risk for herb–herb interactions (Goldman et al. 2008). Some commonly used NHPs include the following: fish oil, glucosamine, and ginseng (Barnes, Bloom, and Nahin 2009). These are of note as each has been implicated in NHP–drug interactions (Singer et al. 1990; Janetzky and Morreale 1997; Knudsen and Sokol 2008). Ethical use of NHPs demands caution regarding concurrent drug use or use of multiple NHPs.

NHP–drug interactions can be pharmacodynamic or pharmacokinetic and may result in additive (synergistic) or opposing (antagonistic) effects. In a pharmacodynamic interaction between chamomile and warfarin, the effects of one product are changed by the presence of the other (Baxter and Stockley 2005), and a stable index of blood clotting, the INR, was raised (Segal and Pilote 2006). The authors described the effect as a result of the combined anticoagulatory effects of the two products. In a pharmacokinetic interaction, the absorption, distribution, metabolism, or excretion of one product is affected by another (Baxter and Stockley 2005). St. John's wort is implicated in pharmacokinetic interactions with irinotecan, as it will decrease the maximum plasma concentration of this chemotherapeutic agent when taken concomitantly (Mathijssen et al. 2002).

Unfortunately, much remains unknown about herb–drug interactions. Many herbs have been little documented about their pharmacological properties and even less about their potential interactions with pharmaceutical medications. One challenge that exists is determining which theoretical interactions will be important clinically (Brulotte and Vohra 2008). Recently, a rapid guide for clinicians to assess the likelihood of a known herb–drug interaction was made available (Cvijovic et al. 2009). Importantly, this distinguishes between NHP–drug interactions that have only been reported in theory and those that have been documented in clinical cases (Figure 21.1).

21.8 RECOMMENDATIONS

Improvements to clinical care, research, and education may promote the ethical use of herbal medicines, and therefore these areas are the focus of the Recommendations section.

21.8.1 Recommendations for Clinical Care

Given the widespread use of NHPs, good patient care demands that clinicians routinely inquire about all product use—conventional, complementary, and alternative—to promote patient safety and ethical care. While research is actively underway to expand the knowledge base regarding herbal medicine safety and efficacy, clinicians are obligated to work with the best available evidence at the time. Cohen and Eisenberg (2002) suggested a very usable model to evaluate safety and efficacy to guide a clinician's response. If safety and efficacy are known, then use of an NHP can be promoted, while maintaining a watchful eye on the patient's condition. In cases where the NHP is proven to be safe but its effectiveness in treating a condition is inconclusive, the clinician should allow for NHP use but also warn patients to be prudent. Clinicians should also watch for improvement in the patient's condition as a result of the NHP (Cohen and Eisenberg 2002). When there is evidence that the NHP is efficacious, but its safety is uncertain, the clinician may accept the therapy but again must suggest prudence and watch for adverse events in the patient (Cohen and Eisenberg 2002). In contrast, when neither safety nor efficacy has been demonstrated, clinicians should actively discourage the use of the NHP.

In clinical circumstances when patients are adamant on using NHPs that lack supporting evidence, it may be difficult to offer therapeutic options that are medically sound and respect the patient's desires (Adams et al. 2002). Respect for patient autonomy demands that health care professionals be well informed about herbal products in order to provide information (Sugarman and Burk 1998). Ethical practice requires that clinicians anticipate and prevent harm whenever possible,

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CPJRPC

A tool for rapid identification of potential herbal medicine–drug interactions

Kosta Cvijovic, Heather Boon, Joanne Barnes, Walter Jaeger, Mano Murty, Duc Vu, Susanne Reid, Sunita Vohra

	Gastro intestinal system				Cardiovascular system							Respiratory system		Central nervous system						Endocrine system					Anti-infectives				
	Laxative agents	Antiulcer agents	Antacid agents	Antidiarrheal agents	Anticoagulant agents	Lipid-lowering agents	Antihypertensive agents	Beta-blockers	Diuretic agents	Cardiac glycosides	Antiarrhythmic agents	Antiasthmatic agents	Antiallergy agents	Antiparkinson agents	Analgesic agents	Antipsychotic agents	Antiepileptic agents	Antidepressant agents	Sedative agents	Sex hormones	Corticosteroids	Hyperthyroid agents	Hypothyroid agents	Antidiabetic agents	Antifungal agents	Antibiotic agents	Anti(retro)viral agents	Nephrotoxic*	Hepatotoxic*
Alfalfa																								1					
Aloe vera																						2	3	4					
Angelica					5																								
Black cohosh							6																						
German chamomile																													
Chaste tree																													
Cranberry					7																								
Devil's claw					8																								
Echinacea																													
Ephedra						9	10	11		12	13							14			15	16	17	18					
Evening primrose							19									20													
Feverfew																													
Garlic					21		22																				23		
Ginger					24																								
Ginkgo					25		26		27															28					
Asian ginseng					29		30	31	32	33	31							34						35					
Hawthorn							36																						
Horse chestnut																													
Kava																			37										
Licorice							38		39	40											41								
Milk thistle																								42					
Peppermint																													
Saw palmetto					43		44																						
St. John's wort				45	46	47	48			49								50	51	52		53	54		55				
Tea tree oil																													
Thyme																													
Valerian																		56											
Wild yam																													
Willow					57		58																						

No reported or theoretical interactions (Caveat: Interactions are nonetheless always possible!)

Theoretical interactions based on animal or in vitro data.

Theoretical interactions extrapolated from clinical data.

Interactions supported by clinical evidence (reported in human case reports or clinical trials).

Numbers on this chart refer to interaction details described on reverse page.

*Nephrotoxic/hepatotoxic: Herbs that potentially influence liver and/or kidneys also potentially influence drug metabolism and can therefore cause general interactions.

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FIGURE 21.1 **(See color insert.)** Potential interactions associated with concomitant use of commonly used prescription medications and herbal medicines. (Reproduced from Cvijovic et al. 2009. *CPJ* 141:5. With permission.)

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including those due to NHP–drug interactions. Routine inquiry will help promote clinician awareness of patients' use of herbal medicines, and a proactive approach is required for nonmalfeasance.

21.8.2 Recommendations for Research into Herbal Medicine

There have been significant gains in NHP research, but much is still unknown. Ultimately, research should provide information on efficacy and safety that can be translated into the clinical setting and become useful to patients. Research should aim to fill the current gaps in knowledge about the potential harm and effectiveness of NHPs (Matthews, Lucier, and Fisher 1999) such that consumers may have enough information to make a well-informed decision. Additional data are needed on NHP–drug interactions as well as on NHP safety in specific populations, as some patient groups may be at more risk than others (Matthews, Lucier, and Fisher 1999). An example of a group at increased risk for NHP–drug interactions are those with liver or kidney dysfunction.

Attempts should be made to characterize the active chemical constituents in NHPs (Matthews, Lucier, and Fisher 1999). Once these constituents are known, NHP research should try to use well-characterized products with minimal heterogeneity. The relevance of future research will be enhanced if comparative effectiveness data are obtained for NHPs with their conventional pharmaceutical competitors. Another research need involves finding appropriate ways to combine data in order to conduct meta-analyses, given the current heterogeneity in product traits and quality, and when reporting on NHPs that are safe and effective, distinctions between companies and products should be made to reduce the potential for commercial interests to bias the research (Institute of Medicine (U.S.) Committee on the Use of Complementary and Alternative Medicine 2005). Finally, given the under-reporting associated with passive surveillance, active surveillance to document NHP safety is recommended, including scrutiny for NHP–drug interactions. Such a study is now underway (Cvijovic et al. 2009), but much more of this type of study is needed to provide clinicians with the evidence they need to provide ethical care using herbal therapies as primary or adjunct treatments.

21.8.3 Recommendations for Education in Herbal Medicine

Clinicians should take advantage of continuing medical education courses to update their knowledge on NHPs, which would increase their ability to answer questions on NHPs and anticipate potential herb–drug interactions. No clinician is expected to know all of the possible interactions, but there should be some effort to at least know of common interactions based on frequently used products in their patient population.

Universities with health science faculties (medicine, nursing, pharmacy, etc.) should be encouraged to develop and implement a curriculum regarding NHPs, so that future health care providers can be more comfortable and more knowledgeable when discussing NHPs with their patients. Fortunately, some universities have already adopted this approach, those belonging to the Consortium of Academic Health Centers for Integrative Medicine. The major focus of the Consortium is to promote integrative medicine-related principles in educational settings; this may be achieved by supporting the formation of integrative medicine curricula, research, and potential therapeutic modalities (Consortium of Academic Health Centers for Integrative Medicine 2009a). There are 46 institutions that belong to the Consortium, including Yale University, Johns Hopkins University, and Harvard Medical School (Consortium of Academic Health Centers for Integrative Medicine 2009b).

Greater education about NHPs should help clinicians handle some of the challenges associated with the use of NHPs. Clinicians will become more aware of therapies that are effective and will be able to offer alternatives to patients, which will help improve patient autonomy and beneficence. Also, health care professionals will be more informed as to what therapies may cause harm for patients, and thus, they can guide patients to avoid these deleterious practices. Good manufacturing processes represent a way in which manufacturers can improve the quality of their products, help

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decrease the chance of contamination, and produce more reliable NHPs (Institute of Medicine (U.S.) Committee on the Use of Complementary and Alternative Medicine 2005).

21.9 CONCLUSIONS

Beneficence, nonmalfeasance, and patient autonomy are important bioethical principles of conventional medicine that are equally applicable to CAM, including herbal medicines.

Patients have a right to choice in treatment, but this right assumes that their clinicians can provide them with enough information about their therapeutic options (conventional, complementary, and alternative) for them to do so. The evidence base regarding NHP safety and efficacy is growing, and clinicians must be aware of the risk-benefit ratio when counseling their patients about NHP use, whether as primary treatment or as adjuncts to care. Insufficient detailed knowledge of NHP pharmacology and NHP–drug interactions pose a major challenge, but new strategies are emerging to help clinicians and scientists grow knowledge while optimizing patient care. Given the widespread use of NHPs, one of the most practical ways to optimize care is to encourage open discussion regarding all products use. To promote full disclosure, clinicians should cultivate respectful relationships in which patients know that their preferences, values, and beliefs will be considered when making treatment decisions.

As NHPs become increasingly popular, there are ethical considerations and challenges that must be addressed. With continued research and careful attention to patient-centered care, issues surrounding the ethical use of herbal medicines will be dealt with in such a way as to promote beneficence, nonmalfeasance, and patient autonomy.

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22 Integration of Herbal Medicine into Evidence-Based Clinical Practice
Current Status and Issues

Anthony Lin Zhang, Charlie Changli Xue, and Harry H. S. Fong

CONTENTS

22.1 Introduction 453
22.2 Current Status and Major Issues of Integration of Herbal Medicine in Evidence-Based Medical Therapy 454
22.3 Factors Relevant to/Affecting Integration of Herbal Medicine into Modern Medical Practices 455
22.3.1 Herb Quality Issues 455
22.3.2 Quality Assurance/Quality Control in Processing and Manufacturing/Preparation of Herbal Medicines (Good Manufacturing Practices Issues) 456
22.3.3 Herbal Mechanisms of Action, Bioavailability, and Herbs' Chemical Constituents 457
22.3.4 Herb–Drug Interactions 457
22.3.5 Herb–Herb Interactions 458
22.3.6 Efficacy Measurements: Objective Quantifiable versus Subjective Quality of Life 458
22.3.7 Other Safety Issues 459
22.4 Research Needs 459
22.4.1 Herbal Medicine Quality and Standardization: Quality Assurance and Quality Control 460
22.4.2 Preclinical Pharmacological Assessments and Action Mechanisms 460
22.4.3 Clinical Efficacy and Safety Assessments 461
22.5 Conclusions 461
Acknowledgments 461
References 462

22.1 INTRODUCTION

The integration of herbal and other forms of traditional medicine (TM) can be done in one of the following three ways: First, it can be incorporated as an integral part of a country's formal health care system, with each being separately recognized as legitimate forms of health care within the same framework. Second, it can be practice integrated with modern medicine by individual health care practitioners. Third, traditional and modern practices can be integrated as two branches of medical science, with the ultimate incorporation of elements of both to form a new branch (World

Health Organization 2000a). The incorporation of traditional and modern evidence-based medicine (EBM) as integral parts of a country's formal health care system is most likely to be achieved and has been demonstrated to be practicable in many countries, particularly in Asian countries such as China, Japan, Korea, and India, among others (World Health Organization 2001). On the other hand, the incorporation of traditional medical modalities such as herbal medicine into modern or EBM by either the second or third method of health care integration is not easily achieved for a host of reasons, including scientific, cultural, educational, and legal.

For decades, the People's Republic of China has touted a system of medical education in which its modern medicine practitioners have been required to receive some formal training in traditional Chinese medicine (TCM), so that they are aware of suitable approaches in TCM during their practice of Western medicines. However, documentation of its successful integration in clinical practice is lacking (Giordano, Garcia, and Strickland 2004). In Western countries, such as the United States, Australia, Canada, and members of the European Union, the popular use of herbal medicine in the form of complementary and alternative medicine (CAM) or phytomedicine in the last two to three decades has led to a multinational, multibillion dollar industry, professional and trade organizations, national and international practice and research conferences, establishment of specialized integrated medicine practices and clinics in pain management and adjunctive cancer therapy, incorporation of CAM courses in conventional medical colleges, introduction of CAM degree-level education programs, and establishment of research funding agencies such as the U.S. National Institutes of Health (NIH) National Center for Complementary and Alternative Medicine (NCCAM; http://nccam.nih.gov/), and the Australian National Institute of Complementary Medicine (NICM; http://www.nicm.edu.au/). As a result of these developments, the issue of integration of CAM medicine, including herbal preparations, into modern medicine has been the subject of ongoing international discussions in the last few years (Fong 2002; Barrett 2003; Ruggle 2005; Boyd 2007; Geffen 2007; Evans 2008; Grimaldi 2008; Shang et al. 2008; Jobst 2009; Joos, Musselmann, and Szecsenyl 2009).

However, proof of efficacy or safety for the vast majority of herbal medicine has not been fully established through an evidence-based approach. Further, other issues, such as scientific, cultural, educational, economical, and legal, need to be addressed. In this chapter, we examine the current status and major scientific issues or factors that affect the integration of herbal medicine into evidence-based medical therapy.

22.2 CURRENT STATUS AND MAJOR ISSUES OF INTEGRATION OF HERBAL MEDICINE IN EVIDENCE-BASED MEDICAL THERAPY

Herbal medicine is becoming increasingly used to enhance general health and well-being, and it is also used alone for specific health problems or with modern medicine (Bruno and Ellis 2005; Kennedy 2005). A recent population study on 2526 adults from the Australian state of Victoria indicated that almost a quarter of the adult population used some form of herbal medicine in 2006–2007 (Zhang et al. 2008). Similarly, about one in five or an estimated 38.2 million adults in the United States used herbs and supplements in 2002, according to the National Health Interview Survey, which interviewed 31,044 representatives of the civilian noninstitutionalized population of the United States (Kennedy 2005). Established in 1999, the Consortium of Academic Health Centers for Integrative Medicine represents 44 academic health centers in the United States and Canada. The Consortium has been working on the inclusion of CAM knowledge, such as herbal therapies, into medical school curricula and the establishment of standards for research in integrative medicine and strategies in integrating alternative treatments into clinical care.

Currently, thousands of TM and other CAM herbal products are available as therapeutic agents worldwide. Yet few of these products have been subjected to randomized clinical trials (RCTs) under the International Conference on Harmonization (ICH) Good Clinical Practice Guidelines to determine their efficacy and/or safety (International Conference on Harmonization 2010.). Of the nearly 2000 herbal medicine clinical studies listed on the Cochrane Controlled Trials Register as of June 2009,

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most concern single-plant herbal or phytomedicine (www.thecochranelibrary.com). In recent years, in the case of multicomponent herbal medicines, an increased number of RCTs on traditional herbal medicine has been reported in the literature (World Health Organization 2004). For example, an Australian study on a Chinese herbal medicine prescription for the treatment of allergic rhinitis concluded that level II evidence is available that may substantiate the use of Chinese herbal medicine for both seasonal and perennial allergic rhinitis (Xue et al. 2003). Unfortunately, the quality of the majority of the clinical studies of herbal medicines reported to date is of great concern due to a number of factors that have rendered the data of dubious value. In a review of 206 RCTs on herbal medicine, which was published in Medline from 1966 to 2003, important methodological components of RCTs, particularly allocation concealment, generation of the allocation sequences, and intention-to-treat analyses, were incompletely reported. In these studies, only slightly over a quarter of the trials adequately reported blinding, and one-fifth reported generation of random allocation sequences (Gagnier et al. 2006). Furthermore, an earlier review of 2938 RCTs on TCM reported in 1980–1997 (Tang, Zhan, and Ernst 1999) concluded that the majority of these studies suffered from methodological defects. For example, only 15% of these studies used blinding, the sample size was mostly less than 300 patients, the controls were inadequate, few studies used quantitative outcome measures, and the studies were short term.

There have been many nonclinical in vitro and in vivo studies on herbal medicines that have commonly supported the traditional therapeutic claims. However, systematic reviews of the study protocols or the data interpretation and validation are lacking. Further, the translation of an in vitro and/or in vivo biological/pharmacological effect of a herbal medicine to human therapeutic use may not be successful due to species differences or other mitigating circumstances, including the simple attribute of a biological or clinical outcome by the name of the mother herb, while neglecting the type of plant extract, methods of processing, and pharmaceutical formulation, which invariably contain varying content and proportions of active chemical components (Brinker 2009).

In addition to the preclinical biological or pharmacological issues, the quality of the herbal products can affect the clinical outcomes and thus can impact their successful integration into EBM. Herbal medicine quality can be substantially different due to intrinsic and extrinsic factors. Species differences, organ specificity, and diurnal and seasonal variations are examples of intrinsic factors that can affect the qualitative and quantitative accumulation of the biologically or pharmacologically active chemical constituents produced and/or accumulated in the herb. Extrinsic factors affecting the quality of the herbal medicine include environmental conditions, cultivation and field collection practices, postharvest handling, storage, manufacturing, inadvertent contamination, substitution, and intentional adulteration (Awang 1997; Huang, Wen, and Hsiao 1997; Slifman et al. 1998; Mahady, Fong, and Farnsworth 2001; Cordell 2002; Fong 2002; Chadwick and Fong 2006).

22.3 FACTORS RELEVANT TO/AFFECTING INTEGRATION OF HERBAL MEDICINE INTO MODERN MEDICAL PRACTICES

A range of interrelated quality, safety and efficacy issues could contribute to the rational and successful integration of herbal medicine into modern medical practices.

22.3.1 Herb Quality Issues

Fundamental to assuring the efficacy and reproducibility of any medicinal agent, be it a single chemical or a complex herbal mixture, is the assured quality of the product. In the case of single chemical drugs, the quality and properties are well defined and documented in pharmacopoeias or on file with regulatory agencies or marketing authorities. On the other hand, herbal medicines, be they single herbs or polyherbal products, suffer from a lack of uniformity in their chemical and physical qualities due to various factors as mentioned above. All these factors have contributed to extensive lists of herbal medicines being reported in the scientific and lay media to be of inferior and questionable quality and authenticity.

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In our early postmarket surveillance of selected commercial ginseng products prepared from *Panax ginseng* C.A. Meyer., *P. quinquefolius* L., and *Eleutherococcus senticosus* Max (eleuthero) marketed in North America in 1995–1998, we found that 26% of these products did not meet label claims with respect to the claimed ginsenoside content of the *Panax ginseng* and *Panax quinquefolius* products (Fitzloff, Yat, and Lu 1998). Studies on the quality of St. John's wort products showed the hypericin content ranging from 22% to 165% and silymarin content in milk thistle (*Silybum marianum* L. Gaertn.) products ranging from 58% to 116% of the labeled claims (Schulz, Hubner, and Ploch 1997). Gilroy et al. (2003) reported their investigation of herbal medicines sold as "echinacea" in the United States. A total of 59 products were studied (Gilroy et al. 2003) and of these, seven of nine so-called standardized products contained substantially less of the marker compounds echinacoside or cichoric acid than the stated content, with the other two being totally devoid of either compound.

Another major extrinsic quality problem concerns substitution and/or adulteration. Herbal medicines collected in the wild as well as some cultivated source materials, where more than a single species is grown in a given farm or site, can lead to nontargeted species being harvested by either accidental substitution or intentional adulteration. Substitution of *Periploca sepium* Bunge for *Eleutherococcus senticosus* (eleuthero) had been well documented (Awang 1997), and the U.S. Food and Drug Administration (FDA) had traced the original adverse reactions attributed to plantain (*Plantago ovata* Forsk.) as having actually been caused by *Digitalis lanata* Ehr., a contaminant introduced during harvesting of plantains (Slifman et al. 1998).

Unintentional in-process adulteration with heavy metals, microbial and chemical agents (pesticides, herbicides, and heavy metals), as well as with foreign matter such as insects, animals, animal parts, and animal excreta during any of the stages of source plant material production or procurement can result in unsafe source materials (Fong 2002). Besides unintentional in-process adulteration with heavy metals, it is well established that Ayurvedic medicine and TCM sometimes employ complex mixtures of plant, animal, and minerals such as lead, mercury, cadmium, arsenic, and gold in certain formulations (Ernst and Thompson Coon 2001).

Perhaps the most egregious impediment to the integration of herbal medicine into conventional medicine is the intentional adulteration of herbal medicine products with synthetic pharmaceutical drugs. Multicomponent Chinese or Ayurvedic herbal medicines have long been documented to be adulterated with synthetic anti-inflammatory drugs such as phenylbutazone, indomethacin, and/or corticoid steroids in arthritis remedies (Farnsworth 1993). A Taiwanese study on the chemical adulteration of TM found that about 24% of 2609 herbal remedy samples collected by eight major hospitals were found to contain one or more synthetic therapeutic agents (Huang, Wen, and Hsiao 1997). In more recent years, the most infamous among the documented cases was PC-SPES, a purported Chinese herbal mixture sold in the United States for the promotion of prostate health, and which was used by many prostate cancer patients for its remarkable efficacy. Unfortunately, reports proved the product to have been adulterated with estrogen, warfarin, and other pharmaceuticals (Blumenthal 2002; Cordell 2002). These cited examples are only a few of the quality control (QC) or lack of quality control issues associated with herbal medicines that greatly affect their successful integration into modern EBM.

22.3.2 Quality Assurance/Quality Control in Processing and Manufacturing/Preparation of Herbal Medicines (Good Manufacturing Practices Issues)

The most important extrinsic factor affecting the quality of herbal medicines is the lack of effective policies on quality assurance (QA)/QC in the processing and manufacturing of herbal products under good manufacturing practices (GMP; World Health Organization 2007b). These can vary from country to country (World Health Organization 1998). In some countries, herbal medicines are regulated as medicine and subject to mandated standards, whereas in others very few botanical products are available as prescription or over-the-counter (OTC) drugs.

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The majority of herbal medicines marketed in the United States are sold as dietary supplements under the provisions of the Dietary Supplement Health and Education Act (DSHEA) of 1994, and have only recently been mandated by law to be produced under cGMP (Food and Drug Administration 2007). Unfortunately, the QA/QC requirements are far short of those required in the production of prescription and OTC drugs. For dietary supplements, including herbal medicines, the requirements apply only to the manufacturers of the final product and not to the dietary ingredient suppliers, which have been the source of some of the most high-profile problems of adulterated, substituted, or contaminated ingredients associated with herbal dietary supplements. A study by (Liva 2009), which serves to illustrate this problem, described some cases of poor quality controlled, unfinished herbal materials, including a hops (*Humulus lupulus*) extract, that did not meet the expected chemical profile but instead appeared to contain burned maltodextrin, Asian red ginseng (*Panax ginseng*), and wild yam (*Dioscorea villosa*) extracts containing quintozene, a fungicide that is illegal to use in herbal medicine, and in a solvent residue test, a purported ethanol–water extract of milk thistle (*Silybum marianum*) was found to contain benzene, and a subsequent GC/MS analysis showed 30 different acyclic and cyclic hydrocarbons, including benzene and toluene, which are known carcinogens. Until cGMP requirements are mandated and adhered to in the supply as well as in the manufacturing sides to ensure the availability of quality herbal products, herbal integration into modern medical practice will continue to pose problems.

In several countries, herbal medicines are totally unregulated. Consequently, product quality may differ from country to country, within the same country from product brand to product brand, and even from batch to batch within the same brand.

22.3.3 Herbal Mechanisms of Action, Bioavailability, and Herbs' Chemical Constituents

The underlying mechanisms of action of herbal medicine, whether single herbal or multiple herbal formulations, have generally not been elucidated due to the lack of knowledge of identifying their contained active and/or adjuvant phytochemical constituents. The same problem applies to the study of pharmacokinetics and bioavailability. In the case of single-molecular pharmaceuticals, there is no uncertainty as to which chemical compound is to be used for pharmacokinetic and bioavailability studies. Herbal medicines are constrained by their unknown and/or unidentifiable active chemical constituents (Fong et al. 2006). Nevertheless, some investigators have attempted to conduct such studies. For example, the mechanism of action of a Chinese herbal medicine formula (consisting of seven herbs formulated based on the results of a series of in vitro experiments and a comprehensive literature review) was postulated from a study of its in vitro effect on rat peritoneal mast cells and macrophage cells (Lenon et al. 2009). It was found that the formula significantly inhibited the release of several inflammatory mediators, including histamine and prostaglandins, which led the researchers to conclude that it has multiple mechanisms and that potential synergistic effects of the individual herbal constituents could all have contributed to the actions of the formula. Unfortunately, the potential clinical antiallergic effects of the formula are yet to be tested through adequately powered RCTs, which brings into question the validity of such postulations.

22.3.4 Herb–Drug Interactions

Reports on herb–drug interactions are mainly from case reports that were inadequately documented and/or on the basis of in vitro studies (Awang and Fugh-Berman 2002). A recent review based on extensive literature search suggested that, when herbs are often administered in combination with drugs, there were only limited clinical observations on the interactions among humans (Hu et al. 2005). Nevertheless, the potential of interactions of herbal medicine with prescribed drugs or OTC drugs has been a major safety concern for clinicians as such interactions are difficult to predict and the general lack of available information on the herbs' composition and pharmacological actions (Zhou et al. 2007).

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In recent years, researchers have attempted to identify interactions between commonly used herbs and drugs. Many of them are now well known, such as the interaction between St. John's wort and warfarin (Yue, Bergquist, and Gerden 2000; Henderson et al. 2002) or digoxin (Johne et al. 1999). A recent review concluded that 34 commonly used drugs that interacted with herbal medicines in humans had been identified. These include anticoagulants (e.g., warfarin, aspirin), antidepressants (e.g., midazolam), cardiovascular drugs (e.g., digoxin), and anticancer drugs (e.g., irinotecan; Zhou et al. 2007). If the definition of the herbal medicine extended to botanicals including fungi, algae, and other component matters, nearly 80 herbal medicines would be identified that had clinically significant interactions with drugs. Garlic, ginger, and ginkgo are among the herbs most commonly involved in herb–drug interactions (Ulbricht et al. 2008).

22.3.5 Herb–Herb Interactions

Herb–herb interactions, sometimes referred to as contraindications in the application of herbs or prescription incompatibility, were documented in ancient textbooks on TCM medicinal formulae (i.e., a mixture of herbs). TCM practitioners prescribe herbal formulae based on disease manifestation and characteristics of the herbs. The most well-documented herb–herb interactions were eighteen-incompatible-herbs and nineteen-counteracting-herbs. For example, Wu Tou (*Aconitum rhizome*) cannot be used with Ban Xia (*Pinellia ternata rhizome*; Weng, Nie, and Huang 2004), and Fu Zi (*Radix Aconiti*) is incompatible with Bei Mu (*Bulbus Fritillariae*; Xiao et al. 2005). It should be noted that evidence of the adverse reactions and/or toxicity of the combined use of these herbs was mainly derived from clinical observations in ancient times. Some experienced TCM practitioners may choose to use some combinations for various conditions (Zhang and Li 2009). Researchers have attempted to generate more scientific evidence through modern pharmacological studies, but conclusive recommendations have not yet been possible (Tang et al. 2009).

22.3.6 Efficacy Measurements: Objective Quantifiable versus Subjective Quality of Life

The integration of herbal medicine into evidence-based clinical practice and research also rests on the acceptance of its scientific evidence by the conventional medical profession, including medical practitioners, pharmacists, nurses, and other health care workers. The evidence needs to be verified legitimately and scientifically according to the conventional EBM framework. Studies in other CAM modalities such as acupuncture have been designed with specific details of the experiment (e.g., kind of needle used, location of the points, depth of needle insertion, and techniques for rotating the needles) and the nature of the control method after considering a placebo effect (Sherman et al. 2002). If possible, evidence generated for herbal medicine should be derived from the most powerful method of testing the effect of treatment intervention, the RCT. With a plausible biological basis, herbal products can be evaluated through double-blinded, placebo-controlled, multicenter trials. Reflecting this, the World Health Organization (WHO) has published a number of guidelines for clinical evaluation of the herbal and TMs (World Health Organization 1993, 2000b).

The methodological robustness of outcome measures in 44 CAM trials in oncology had been recently evaluated, and it was concluded that only 37% stated an a priori hypothesis and only 20% addressed the clinical significance of the outcomes (Efficace et al. 2006). Trials with poor outcome measurements can exaggerate the estimates of treatment effects (Schulz et al. 1995). Thus, within the EBM paradigm, RCTs are suggested to be reported in accordance with the 22-item Consolidated Standards of Reporting Trials (CONSORT) checklist (available at www.consort-statement.org), such as a detailed description on patient eligibility criteria, sample size calculation, specific objectives and hypotheses, implementation of the trial, and statistical methods, regardless of whether the intervention is conventional or herbal (Gagnier et al. 2006a, 2006b).

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In parallel with other methodologies necessary to the design of the trials, outcome measurement is central to the development of EBM practice of CAM, including herbal medicine (Long 2002). Thus, item 6 (outcomes) of the CONSORT checklist was recommended to reflect the intervention and indications tested while considering their underlying theories and concepts when reporting RCTs on herbal medicine intervention (Gagnier et al. 2006b). At the request of the NIH, the Institute of Medicine convened a working committee in 2005 and produced a report entitled *Complementary and Alternative Medicine in the United States*. In this report, the core recommendation was that "the same principles and standards of evidence of treatment effectiveness apply to all treatments, whether currently labeled as conventional medicine or CAM" (Institute of Medicine 2005, 2). From this view, for herbal medicine, like any other CAM and pharmaceutical drugs, efficacy measurements used in RCT need to be chosen in accordance with conventional scientific principles (objective and quantifiable) before their results can be generalized and can be made acceptable to the public. Perhaps one obstacle is the holistic concept and approach being emphasized by the unique philosophy of herbal medicine. For this reason, some subjective measurements, including the percentage of patients perceiving benefits and the number of patients "recovering" from the condition, were commonly reported in TCM trials (Ernst 2006). In recent years, the development of the quality of life instrument for herbal medicine research by using an EBM approach has received much attention (Leung et al. 2005; Wu et al. 2009).

22.3.7 Other Safety Issues

Other safety issues influencing herbal medicine integration into modern medicine include cultural and behavioral contexts as well as efficient communication on its use among patients, conventional medical practitioners, and herbal medicine practitioners. Over a few decades of development and with more scientific research data being published, although not all convincing, at least some promising evidence has met the EBM standard. As a result, negative attitudes and doubtful perceptions of herbal medicine may now only be held by a minority of the conventional medical profession. Nevertheless, it is of critical concern to clinicians that many herbal medicine users take herbal remedies and conventional therapies concurrently without informing their medical doctors. Such communication gaps can lead to herb–drug interactions that may be otherwise avoided.

The above-mentioned population study on 2526 Australian adults also indicated that approximately half of herbal medicine users took two forms of therapy on the same day (Zhang et al. 2008). However, only about half of these users had voluntarily informed their medical practitioners about their herbal medicine use. This finding was not striking and, in fact, the situation is similar in the United States, with only one-third of the users having informed their medical providers about their use of herbs or supplements (Kennedy, Wang, and Wu 2008). This study also found that nondisclosure of herb and supplement use was particularly common among racial and ethnic minority groups (Kennedy, Wang, and Wu 2008). Therefore, understanding the reasons for nondisclosure not only can help doctors to provide better clinical care but also to promote safe integration of herbal medicine into evidence-based medical therapy.

22.4 RESEARCH NEEDS

For effective integration of herbal medicine into modern therapeutic practices, the level of research on the preclinical and clinical efficacy of these products and their complementation of or interaction with modern pharmaceuticals must be elevated to much higher levels than is presently the case. Preliminary to these studies, clinical products must be produced by GMP from source materials acquired through good agriculture and collection practices (GACP), be botanically validated, be chemically and/or biologically standardized, and their stability be established. As mentioned previously in Section 22.3.2., few products have been so documented. Hence, the research on herbal medicines for the integrated medical use must begin with the acquisition and QC of source materials

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and processed starting materials. The three most important major areas of research can be defined as (1) herbal medicine quality and standardization, (2) preclinical pharmacological assessments and action mechanisms, and (3) clinical efficacy and safety assessments. Of relevance to conducting these needed studies, the WHO has published a guideline for methodologies on the research and evaluation of TM (World Health Organization 2000b) and the NIH NCCAM has published a policy on biologically active agents used in CAM and placebo materials (available at http://nccam.nih.gov/research/policies/bioactive.htm#), with which its grantees must comply for funding in support of their research projects on the herbal medicine.

22.4.1 Herbal Medicine Quality and Standardization: Quality Assurance and Quality Control

QA of herbal medicine for integrative medical use is a "ground-to-table" process spanning from the acquisition of the source material to the production of the clinical formulation. Therefore, QA/QC research on source materials should begin from the point at which a specific plant part to be used as the "herb" is acquired by cultivation or field collection through GACP. Good agricultural practice guidelines have been established by a number of countries, and the WHO has also published a guideline on GACP (World Health Organization 2003) to assist member states in the production of quality herbs. Further, the WHO has published an example GACP for *Artemisia annua* L. (World Health Organization 2006). A most essential part of botanical QA is that plant materials should be identified by their scientific names (Latin binomial) rather than by common names and should be authenticated botanically according to pharmacopoeial standards employing macroscopic/organoleptic and microscopic methods. Each herb should be subjected to purity as well as contaminant tests for the presence of foreign matters, toxic metals, pesticide residues, mycotoxins, and microorganisms.

Qualitative HPLC-UV or LC-MS analysis leading to the generation of a characteristic profile (fingerprint) and the quantitation of reference marker compound concentrations, including relevant biologically active molecules of an extract of the herb, should be conducted as part of a product standardization study. Chemical standardization studies of the clinical formulation or product can be performed by quantitative HPLC-UV or LC-MS analysis, and stability studies of the final herbal preparation manufactured under GMP conditions (World Health Organization 2007b) can be monitored from the day of production by accelerated and/or real-time analysis. In the process, QC procedures must be implemented from source material acquisition to final product manufacture in order to assure the quality of the product being considered for integration into evidence-based modern medicine.

22.4.2 Preclinical Pharmacological Assessments and Action Mechanisms

Many herbs, such as *Panax ginseng*, possess a wide range of pharmacological activities (Scaglione, Pannacci, and Petrini 2005). Thus, it has been recommended that quality-certified standardization be a prerequisite for future laboratory and clinical investigations (Harkey et al. 2001). As in the case of single-molecule pharmaceutical drugs, herbal medications being considered for integrative therapy must first undergo preclinical pharmacological assessment for safety and efficacy, if possible. However, the biological response to a drug product may not be species transferable, and an active substance in animals may be entirely inactive in humans. On the other hand, acute and/or chronic toxicity manifestations in animal models are reliable indicators of drug safety. In current practice, acute and chronic toxicities are usually determined by experimental studies using animal models. Suitable methods for testing toxicity need to be established so that herbal ingredients and their derived products can be reliably assessed. For herbal medicines, testing for the presence of heavy metals such as lead, mercury, and arsenic should be mandatory, as these toxic substances are environmental contaminants often found accumulated in many herbs. Therefore, preclinical,

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pharmacological, and safety assessments represent a critical step in the scientific integration of herbal medicines into the evidence-based health care paradigm.

In addition, where feasible, the mechanisms of action or bioavailability of herbal medicines should be determined. However, as noted by Brinker (2009), single-dose pharmacokinetic or pharmacodynamic may not yield true data, especially in the cases of herbal preparations exhibiting weak pharmacological effects. As an example, CYP isozyme induction typically requires over a week of repeated dosing to manifest (Brinker 2009). Given the long history of the use of herbal medicine and increasing clinical evidence on its efficacy, extensive investigations of the chemical composition of constituent herbs and of the biological activity of the identified compounds are clearly warranted. In addition to identifying the active compounds and providing information about their mechanisms of action, it seems inevitable that such studies will lead to new and improved therapeutic agents for the treatment of human diseases. In recent years, reviews of the key chemical compounds present in the individual herbs used in the herbal formulae are most useful (Xue et al. 2004; Li and Brown 2009).

22.4.3 Clinical Efficacy and Safety Assessments

Fundamentally, conducting and reporting clinical studies on the efficacy and safety of herbal medicine should follow the context-specific elaborations of the CONSORT statement (Gagnier et al. 2006a,b). In addition to the documentation of all general aspects of RCTs (e.g., randomization, blinding, and analysis) that are known to influence the estimation of treatment effects, specific considerations are needed to attend to the unique obstacles of implementing herbal medicine trials. For many traditional herbal medicine products, the inherent complexities in their organoleptic properties, such as their taste, odor, or appearance, can be distinguished between the clinical preparations and their respective placebos, and thus are more vulnerable as comparison factors for testing their true therapeutic effects. Details of controlling this possible bias must be fully described so that the study can be replicated by other investigators. Backing by pharmacokinetics studies and reporting on adverse events and herb–drug interactions are central to the safety assessment of herbal medicine. On the other hand, systematic review and meta-analysis of the existing clinical evidence should be conducted in line with the intrinsic factors of herbal medicines. Such factors include, but are not limited to, variation in processing, sources (e.g., soil and climate), content, doses, and storage, as well as the diversity of effective ingredients of the herbal medicines.

22.5 CONCLUSIONS

It was said during the WHO Congress on TM in 2008 in Beijing, China, that "the two systems of traditional and Western medicine need not clash" (World Health Organization 2008). During the Congress, WHO member states and other stakeholders were called upon to take steps to integrate TM into national health systems. But integration of herbal medicine into modern clinical practice must be based on an EBM approach. Prior to the clinical evaluation of herbal medicine, be it a single compound, a mixture of herbal ingredients, or a complex herbal formula based on historic evidence of use, the QA/QC in source material acquisition and processing and manufacturing of the products under GMP must be addressed to assure efficacy and reproducibility. In addition to the use of scientifically irrefutable efficacy measurements, clinical studies should monitor and report adverse events, including potential drug–herb interactions. When the safety and efficacy are established in accordance with conventional scientific principles, the integration of herbal medicine into evidence-based clinical practice will likely occur.

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The global popularity of herbal supplements and the promise they hold in treating various disease states have caused an unprecedented interest in understanding the molecular basis of the biological activity of traditional remedies. This volume focuses on presenting current scientific evidence of biomolecular effects of selected herbs and their relation to clinical outcome and promotion of human health. This book also addresses the ethical challenges of using herbal medicine and its integration into modern, evidence-based medicine.

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