Applied Environmental Science and Engineering for a Sustainable Future

Seema Patel

Emerging Bioresources with Nutraceutical and Pharmaceutical Prospects

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Applied Environmental Science and Engineering for a Sustainable Future

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This is for my parents
and
husband Surya

Foreword

"Sustainable Future" is not merely a catch phrase of today but a serious concern for the entire world to be aware of. Our resources are depleting due extensive utilization of them in the pursuit of advancing the standard of living. Our need for various consumer products has increased enormously and thus the demand for resources to produce them also has increased significantly. All three spheres (air, water and soil) of our earth are being polluted due the emission/discharge of various pollutants. The consequences are apparent in the form of global warming, extreme weather events such as flooding, forest fires and droughts. While the above sentences depict a pessimistic picture, it is never late to slow down, reduce and finally to reverse those adverse effects through concerted efforts by all disciplines such as science, engineering, medicine, sociology etc.

It has become obvious that more than 50,000 products are used by consumers and they need many chemicals in the form of elements and compounds. This exhaustive list of consumer products however can broadly be categorized into the following six groups: (i) food, (ii) metals, (iii) wood, leather and paper, (iv) clothing and textiles, (v) petroleum, chemicals and plastics and (vi) electronics, computers and transport. They are used in the welfare of societies such as food, shelter, health, transport, communication and education as well as other needs namely research, security/defense etc. for example, only half of the petroleum oil is converted to gasoline and rest of the petroleum is used in 6000 products.

Further, the resources that are released to the environment mostly degrade the quality of the latter, due to the way they enter the environment. Some of the examples are as follows:

- Increased levels of carbon dioxide from industrial processes as well as transportation has led to greenhouse effects.
- Municipal and industrial wastewater discharges have polluted the receiving natural water bodies.
- Discharge of concentrate and pre-treatment effluents from seawater desalination has damaged the coastal seawater.
- Disposal of solid waste to landfills have caused greenhouse gas emissions and release of toxic leachate to the environment.

Therefore, efforts should be focused on the following in order to have a sustainable future:

- Reducing consumption as well as reuse and recycle of resources
- Recovering raw materials, process chemicals and by-products
- Reducing pollution at source as well as segregating pollutant streams for better treatment and resource recovery; employing process/technology modification
- Improving the treatment technologies to reduce the concentrations and loads of pollutants that enter the environment
- Developing monitoring techniques to analyse
  - the contaminants that have already entered the environment and
  - the existing contaminants that are present in both trace and large quantities as well as in concentrated and dispersed forms
- Developing novel technologies in order to utilise the existing resources in sustainable ways
- Understanding and eliminating the adverse effects of new technologies
- Maintaining Sustainable growth of industries through appropriate resource management using industrial ecological principles.

It is essential that the entire world starts to apply the above steps continuously and consistently in all activities for sustainable use of natural resources and to reduce the rate of increase of the emission of pollutants to the environment.

For example, one of the best ways to have sustainable growth of industries is to employ industrial ecological principles where resources are utilised among several industries. The term resource includes by-products and wastes, since one industry's by-products and wastes could be another's raw materials. The industrial ecological principle should be practiced within an industry, among industries in an industrial park, industrial parks in a country and finally among countries. Developing such co-ordination would take enormous effort and time but eventually would pave way to global sustainability which is inevitable if human race to exist on the earth.

This book series on "Applied Environmental Science and Engineering for a Sustainable Future" will bring edited books on *Biodiversity, Biofuels, Bioremediation, Cleaner production and green synthesis, Climate change adaptation and mitigation, Desalination, Environmental Technology and Engineering, Nuclear waste, Soils and sediments, Solid and hazardous waste management, Waste gas treatment, Wastewater, Water, etc.* This approach will provide broader prospective on sustainability by looking at air, soil and water. Further, this book series will attract *Academics, Researchers, Postgraduates, Consultants and Government Employees* in the area of environment and industrial production. The series will also help to form a background to all readers who seek to develop their own solutions to overcome the environmental problems that they encounter.

The first book of the series on "Emerging Bioresources with Nutraceutical and Pharmaceutical Prospects" belongs to *Biodiversity* and provides a great insight to

the value and usefulness of underutilized food sources. Phytochemical profile and nutritional benefits of ten neglected nutritional sources such as strawberry guava, opuntia (prickly pear), carissa (type of shrub), purslane (hogweed or miner's lettuce), grape seed extract, chia, prosopis, quinoa, milk thistle and chaga have been evaluated thoroughly. Large number of references has been included at the end of each chapter allowing readers to gain additional information and data for their use.

Preface

Mankind has fallen susceptible to an army of ailments, be it for nutritional deficiency, pathogenic or metabolic causes. The detrimental effects necessitate mitigation in order to ensure robust health and longevity. Excess reliance on chemical drugs has its risks like microbial resistance and irreparable side effects. If nutrition and immunity could be derived from the diet, it would be the best solution to the nagging issues. In fact, the discovery of functional foods and their enrichment with bioactive components has emerged as a fast-track research area. Several promising plant-derived components have been recognized in this regard. For their phytochemical abundance *viz*. dietary fibre, essential amino acids, poly unsaturated fatty acids, minerals, vitamins and sterols, they are touted to be ideal nourishing agents and safeguard against all kinds of physical threats. The term 'superfood' has been devised for this league of foods with plentiful phytochemicals and few anti-nutrients. More or less, we are familiar with blueberry, ginkgo, green tea, cereal bran, ginseng, spirulina, seaweeds, mushrooms, acai berry, fish oil and propolis as some of the most popular nutraceuticals.

However, apart from these familiar nutrition sources, there exist scores of other candidates with potential to be developed as health foods. For certain reasons they have hardly been exploited. Endemic distribution, low yield and post harvest loss have been identified as the key reasons for their low consumer approval. This book attempts to introduce the current status and future prospects of these obscure as well as up-and-coming food sources. However, it is a vast area and is beyond the scope of this compilation to discuss the entire emergent candidates. So, the author selects ten random resources (the author has taken the liberty of deciding the chapters as per own interest and perspective) from different living kingdom, family and geographical reasons for elaboration. A chapter has been dedicated to each candidate with unique nutritional profile, presenting crucial overviews and visions of their nutraceutical and pharmaceutical implications. The author has strived to project various aspects, namely current status, nutritional constituents, therapeutic spectrum, pitfalls encountered, innovative processing, future prospects and conclusion. The looming food insecurity, agricultural waste management, biodiversity conservation, threats of invasive plants, value-addition of food and importance of dietary intervention against metabolic diseases are the motivation behind this work. This

manuscript is believed to contribute to the significance of bioprospecting for potent functional food sources and guarantee food security. Most of these resources discussed are scantily explored or at least their application in food is in nascent phase. To be more specific, Chap. 1 is about an invasive tropical plant strawberry guava, Chap. 2 is about xerophyte opuntia fruits, Chap. 3 is about Apocyanaceae genus carissa, Chap. 4 is about weed purslane, Chap. 5 is about agro-waste grape seeds, Chap. 6 is about mint family member chia seed, Chap. 7 is about drought-resistant prosopis genus, Chap. 8 is about pseudocereal quinoa, Chap. 9 is about Asteraceae family member milk thistle and the final, Chap. 10 is about medicinal mushroom chaga. This volume is strongly believed to make people cognizant of the nutritional potential of under-utilized resources and prompting further work.

I thank Prof. Piet Lens, the Editor-in-Chief of 'Reviews in Environmental Science and Biotechnology' for suggesting me to write this book. Also, Springer deserves my gratitude for providing me this opportunity to publish this manuscript.

Dr. Seema Patel, PhD

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About the Author

Dr. Seema Patel is currently a researcher at the Center of bioinformatics and medical informatics at San Diego State University, USA. She has earned her doctorate from Indian Institute of Technology Guwahati, India by conducting research on food and pharmaceutical-grade polysaccharide production from natural isolates of lactic acid bacteria. Also, she had held position as an Assistant Professor in biotechnology at Lovely Professional University, India. She has published about 35 scientific papers in peer-reviewed journals and a book chapter. She serves as reviewer for about 30 international journals. Though she has a microbiology background and is presently involved in bioinformatics research, she is also interested in nutraceutical development. This is her maiden endeavour in writing an entire book and she seeks constructive suggestions from experts in this area as well as general readers.

CA, USA San Diego

Abbreviations

A-549	Human alveolar basal epithelial carcinoma
ABTS	2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid
AGE	Advanced glycation end products
AGS	Stomach adenocarcinoma
ALA	α-linolenic acid
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AMPK	5′-AMP activated protein kinase
AST	Serum aspartate transaminase
BALF	Bronchoalveolar lavage fluid
BCRP	Breast cancer resistance protein
Caco-2	Human colon adenocarcinoma
CAT	Catalase
COX-2	Cyclooxygenase-2
CT-26	Murine colon carcinoma
DHA	Docosahexaenoic acid
DNMT	DNA methyltransferase
DPP-4	Dipeptidyl peptidase-4
DPPH	1,1-diphenyl-2-picryl hydrazyl
DSS	Dextran sodium sulfate
ECG	Epicatechin-3-gallate
EGCG	Epigallocatechin-3-gallate
EGF	Epidermal growth factor
EMT	Epithelial-mesenchymal transition
eNOS	Endothelial nitric oxide synthase
EPA	Eicosapentaenoic acid
ERK	Extracellular signal-regulated kinase
FRAP	Ferric-reducing antioxidant power
GABA	Gamma-aminobutyric acid
GC	Gas Chromatography
GC/MS	Gas chromatography-mass spectrometry
GI	Glycemic index

GSH-Px	Glutathione peroxidase
HCC	Hepatocellular carcinoma
HDAC	Histone deacetylase
HDL	High-density lipoprotein
HeLa	Human cervical cancer
HepG2	Human hepatocellular carcinoma
HIF-1	Hypoxia-inducible factor 1
HL-60	Human leukemia
HO-1	Heme oxygenase-1
HPLC	High-performance liquid chromatography
HSV	Herpes simplex virus
HT-29	Human adenocarcinoma
HUVECs	Human umbilical vein endothelial cells
IFNγ	Interferon gamma
Ig	Immunoglobulin
IL	Interleukin
iNOS	Inducible nitric oxide synthase
KLF2	Krüpple like factor 2
LC-MS	Liquid chromatography-mass spectrometry
LDH	Lactate dehydrogenase
LDL	Low-density lipoprotein
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MCD	Mast cell density
MCF-7	Breast adenocarcinoma
MDA	Malondialdehyde
MDA-MB-231	Metastatic human breast carcinoma
MMP	Matrix metalloproteinase
mTOR	Mammalian target of rapamycin
MTT	3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide
NF-κB	Nuclear factor-kappaB
NO	Nitric oxide
NSCLC	Non-small cell lung cancer
ORAC	Oxygen radical absorbance capacity
PA-1	Human ovarian teratocarcinoma
PC-12	Pheochromocytoma of the rat adrenal medulla
PGE2	Prostaglandin E2
Ppm	Parts per million
PUFA	Polyunsaturated fatty acids
RANKL	Receptor activator of nuclear factor kappa-B ligand
ROS	Reactive oxygen species
RSM	Response surface methodology
SNU-1	Human gastric carcinoma
SNU-16	Human gastric carcinoma
SOD	Superoxide dismutase

STAT	Signal transducers and activators of transcription
SW80	Human coloncarcinoma
TBARS	Thiobarbituric acid-reactive substances
TCM	Traditional chinese medicine
TEAC	Trolox equivalent antioxidant capacity
TGF-β1	Transforming growth factor-β
TNF-α	Tumor necrosis factor-alpha
TRAMP	Transgenic adenocarcinoma of the mouse prostate
TRAP	Total reactive antioxidant potential
TUNEL	Transferase-mediated dUTP nick end labeling
USDA	United States department of agriculture
VEGF	Vascular endothelial growth factor

Chapter 1
Introduction

Abstract While food insecurity is looming large, the abundance of underutilized sources of nutrition strikes as a contrast. Further, the large quantity of discarded agro-and industrial processing wastes occurs as a seer squandering of resources. It motivated the author to scour the literature and explore biodiversity to discover and bring the underutilized food sources to limelight. In this volume, ten of such unappreciated candidates have been discussed *viz*. strawberry guava, opuntia, carissa, purslane, grape seed extract, chia, prosopis, quinoa, milk thistle and chaga. Their phytochemical profile and nutritional benefits have been emphasized. There exist a legion of such natural products languishing is obscurity, waiting to be tapped. This chapter presents a nutshell description of the ten neglected nutritional sources.

Keywords Food insecurity · Under-utilized crop · Bioprospecting · Agro-wastes · Industrial refuge

1.1 Introduction

Lack of food security is one of the major issues in current times. Feeding a growing world asks for expanded and intensive agricultural production, which is not in the best interest of the environmental health. The doubling in global food demand poses huge challenges for the sustainability of food production (Tilman et al. 2002). While, more and more statistical studies project this grim picture that scanty intake of nutritious food, low fruit and vegetable intake is causing malnutrition (kwashiorkor, marasmus, night blindness, anemia). Also, increasing the risk of metabolic syndromes (diabetes, cancer, hypertension) and chronic diseases. Global supply of these nutritious foods is insufficient to meet the recommended needs of current and growing population. The global nutrition and agricultural communities are expected to to find innovative ways to increase food and vegetables production and consumption to meet health needs, particularly in low-income countries (Siegel et al. 2014).

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Staple crops are few and sole reliance on them is not a wise practice. Expanding the food sources is critical for achieving food security (Mayes et al. 2012). To reduce the existing pressure on these major crops there is an urgent need to search for other alternative crops having the potential to replace, or at least supplement the available food demand (Rastogi and Shukla 2013). While finding a solution to the above conflicts with simultaneous retention of environmental sustainability, underutilized crops appear very promising. These are crops that are rich sources of vital nutrients, yet have not been exploited optimally. They are recognized, cultivated, traded and consumed only locally (Nandal and Bhardwaj 2014). These crops are potentially valuable resources for meeting increasing food requirement (Pearl and Burke 2014). Dansi et al. (2012) reviewed that most of the neglected and underutilized species are rich in nutrients and have some proven medicinal values and the promotion of their use would help in combating malnutrition and improving the health status of the local populations.

In the quest to find the ideal food sources a variety of ecosystems are being investigated. Jackson et al. (2010) investigated nutritional profile of an obscure legume, morama bean growing in Kalahari Desert and neighboring sandy regions of Botswana, Namibia, and South Africa. The species proved to have good quality protein, mono- and di-unsaturated fatty acids, useful micronutrients, phenolic compounds and oligosaccahrides. The findings report that the bean can be developed as a cash crop. Gordon et al. (2011) evaluated polyphenolic constituents of four underutilized fruits from the Amazon region. Araçá, jambolão, muruci and cutite emerged as good sources of hydrolyzable tannins and/or flavonols. The authors report that the luxuriant forest harbours many other nutritionally-eligible plant species. Adekunle and Oyerinde (2012) came across eight indigenous edible fruit species from tropical rainforest ecosystem and recommended them as potential cheap sources of protein and other essential nutrients. Singh et al. (2012) determined the antioxidant activity and phytochemical contents in 10 underutilized fruits of Andaman Islands. The bioprospecting effort generated some valuable information and projected *Malpighia glabra* L. as an antioxidant-rich species. Conserving the genetic diversity of underutilized species is pivotal to future improvement of the plants, so it should be taken care of (Pearl and Burke 2014). Further, many minor crops are hardy species, with capability to grow well in adverse conditions.

Dhyani et al. (2011) reported based on their study that seabuckthorn (*Hippophae salicifolia*) can offer food and nutrition to rural people of Uttarakhand. Vadivel et al. (2011) determined antioxidant activity of *Cassia hirsuta* L. seed methanolic extract. A decent content of phenolic components (15.82 g catechin equivalent/100 g extract) were reported. Maikhuri et al. (2012) performed biochemical and nutritional analysis on *Viburnum mullaha* and reported that its fruits with high vitamin B2, C, E content can be utilized for developing various edible products. Katoch (2013) profiled phytochemicals of a lesser-known pulse, rice bean. The result showed that some genotypes of this legume having high protein digestibility, good ratio of unsaturated fatty acids and low level of antinutrients are as much nutritionally-acceptable as other members of Vigna family. Rastogi and Shukla (2013) have reviewed the excellent phytochemical profile of amaranth. With an appreciable content of

proteins, minerals, vitamin A and C, it possesses potential to be the 'future staple crop'. Despite the above reports, the conducted studies and the obtained results are just the tip of iceberg and lot remains to be done.

The idea and inspiration for compiling this book stemmed from the observation of inexpensive yet ample natural nutrition sources in the biodiversity. In this book, the author has attempted to furnish the validated information on some of the potential functional foods. The cause of their poor popularity have been explored and key deficiencies as perishability, transportation limitations, unknown nutritional value, lack of consumer awareness have been identified. Suggestions to overcome the hurdles by innovative technologies have been made. The importance of conserving gene pool has been emphasized. I sincerely hope that the readers will be aware about the nutritional wealth and be benefitted by going through this book. Also, the topics might pique the interest of researchers to investigate deeper into the nutritional possibilities.

Though the list of food sources in the 'underutilized list' can be exhaustive, here I have carefully selected ten from various geographical climates, living kingdom, plant family and industrial refuge. The topics covered in the book are on strawberry guava, opuntia, carissa, purslane, grape seed extract, chia, prosopis, quinoa, milk thistle and chaga. Some of them are outright unknown, some forgotten, some misunderstood and some neglected. They have distinct phytochemical profiles and potential to supplement human nutrition and health. The section below will describe them succinctly.

Strawberry guava (*Psidium cattleianum*) a Myrtaceae family member has been identified to bear abundant fruits and contain pectin. Antioxidant, antimicrobial and antiproliferative activities of its various extracts have been validated. Opuntia (prickly pear), a cactus genus is rich in dietary fiber, polyunsaturated fatty acid, minerals, protein and pigments. It's antioxidant, anti-inflammatory, antidiuretic, hypocholesterolemic, antidiabetic, antiproliferation, immunostimulatory and antiulcerogenic activity have been confirmed. *Carissa* genus from Apocyanaceae family has bioactive compounds ranging from polyphenolics, flavonoids, flavanones and lignans to sesquiterpenes. Biological activities as broad as antioxidant, analgesic, anti-inflammatory, hypolipidemic, wound healing, antimicrobial, antidiabetic, antiepileptic, anticancer, diuretic, nephrotoxicity amelioration and hepatoprotection have come forth. *Portulaca oleracea* (purslane) belonging to family Portulacaceae, once dismissed as a weed has been now found to have alkaloids, ω-3 fatty acids, coumarins, flavonoids, cardiac glycosides, anthraquinone, protein, α-linolenic acid and β-carotene, mono terpene glycoside, N-trans-feruloyltyramine to vitamin C. Further, antioxidant, anti-inflammatory, antiatherogenic, antidiabetic, anticancer, hepatoprotective, gynaecological, neuroprotective and antiviral properties have been validated. Grape seed extracts seeds have been discovered to be an excellent source of polyphenol proanthocyanidin. The extract is successfully being used for antioxidant, anti-inflammatory, antilipemic, antihypertensive, hepatoprotective, osteoprotectant, antidiabetic, neuro-protective, anticancer and antimicrobial applications. Chia seed (*Salvia hispanica*), a Lamiaceae family member has been realized to have dietary fiber, proteins, ω-3 fatty acids, antioxidants, vitamins and essential minerals. For its cardiac, hepatic, antidiabetic and anticancer roles, its

popularity is soaring. Prosopis pods from Fabaceae family contain high-protein and low-carbohydrate. It holds promise to be developed as functional food, antioxidant, hypolipemic, antihypertensive, antidiabetic, antinociceptive, estrogenic, anticancer, antimicrobial and neurostimulator to larvicide. Quinoa (*Chenopodium quinoa*), a Amaranthaceae family member is garnering repute in functional food sector. Its seeds have been found to be packed with wholesome protein, essential fatty acids, minerals and vitamins. Their consumption has been validated to confer antioxidant, anti-obesity, hypolipemic and anti-diabetic properties. Milk thistle (*Silybum marianum*) a member of Asteraceae family has been verified to inhibit a broad range of cancers *viz*. skin, larynx, lungs, breast, liver, pancreas, ovary, prostate, colon, kidney, cervix and blood. Silymarin and silibinin (a flavonolignan) in the seed extract have been discovered to be the key active components responsible for the anti-neoplastic, anti-angiogenesis, anti-metastasis and synergy with chemotherapeutics and side-effect amelioration properties. Chaga (*Inonotus obliquus*), a white-rot fungus has been found effective against diabetes, digestive disorders, intestinal worms, liver ailments, cardiovascular diseases, tuberculosis and cancer An arsenal of phenolic compounds, polysaccharides, melanins, lanosterol, inotodiol, ergosterol and trametenolic acid have been identified in the mushroom. Antioxidant, antiinflammation, anticancer, immunomodulation, antiallergy, antilipemic, hypoglycaemic, antiviral, neurostimulation potencies have been validated so far.

Considering their recent recognition in food and pharmaceutical sector, the available data for the above topics is meagre, which the author has carefully gleaned to shape these chapters. This volume will inspire further work in this field, eventually building a rich body of literature as a beacon for future research and development.

1.2 Conclusion

The discussed natural sources might emerge as promising candidates to complement the conventional crops and help meet the ever-increasing global demand for food. Strategies must be devised to leverage the consumption of these resources. Constraints inherent to their incorporation must be sorted, while taking steps to improve their nutrition, promote bio-availability and ensure sustainable production. This book is strongly believed to contribute in the fight against food insecurity.

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Chapter 2
An Underutilized Tropical Plant *Psidium cattleianum* (Strawberry Guava)

Abstract *Psidium cattleianum* Sabine or strawberry guava is an exotic, tropical plant belonging to Myrtaceae family. This shrub bears prolific fruits, yet it is more recognized as an ornamental than an edible plant. Its emergence as an invasive species in Hawaii and defiance towards biological control agents has further lowered its desirability. However, recent findings have shed light on its food potential. It has been identified as a reserve of savory fruit and pectin. Copious phytochemicals have validated its antioxidant, antimicrobial and antiproliferative activities. This chapter presents an overview of its current status and prospect in functional food formulation.

Keywords *Psidium cattleianum* · Phenolic compounds · Antioxidant · Anticancer · Antimicrobial

2.1 Introduction

Psidium cattleianum commonly known as strawberry guava, Chinese guava, cattley guava, Jeju guava, cherry guava, purple guava, waiawi, guayaba or araçá is a member of Myrtaceae family. Native to the Atlantic coast of Brazil, it has adapted to the tropical climates of Hawaii and several Caribbean islands. For its dense contour, hardiness, evergreen glossy foliage, white flowers and bountiful red fruits, it is desired in landscaping (Fig. 2.1a, b). This shrub is grown as ornamental fruit plant in Florida, California, South America and Central America, West Indies, Bermuda and the Bahamas. Since, past two decades, *P. cattleianum* has got a bad name for assuming invasive form and endangering the native flora of Hawaii's rain forests and Seychelles Island (Pino et al. 2001; Gerlach 2004). Brazilian scale insect *Tectococcus ovatus* was determined as a suitable candidate for biological control of *P. cattleianum* in Florida, yet the threat to biodiversity continues (Wessels et al. 2007). The ripe fruit is eaten fresh or used to flavour beverages, ice creams and desserts. Its leaf extract has traditionally been used in some Oriental countries (for the treatment of diarrhoea and diabetes) and French Polynesia. Recently, the leaf extract has been administered in the therapy of cancer, pathogenic infections and inflammation

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Fig. 2.1 *Psidium cattleianum* plant **a** whole shrub. **b** ripe fruits

in experimental models (Im et al. 2012). Patel (2012) published a holistic review on this plant, discussing its potentials and obstacles in popularity. These reports prompted to explore the nutraceutical potential of *P. cattleianum*.

2.2 Phytochemical Analysis

P. cattleianum boasts of a repertoire of phytochemicals. Volatile compounds have been isolated from the fruits by simultaneous steam distillation-solvent extraction followed by capillary GC-MS. Two hundred and four compounds have been identified in the aroma concentrate of which ethanol, α-pinene, (Z)-3-hexenol, (E)-beta-caryophyllene and hexadecanoic acids have been identified as the major constituents. The aliphatic esters and terpenic compounds impart the unique flavour of the fruit. Medina et al. (2011) studied the constituents of aqueous and acetone extracts of the fruits. High level of phenolic compounds (768 mg/100 g), with the predominance of epicatechin was observed. Carotenes, ascorbic acid and anthocyanins were present as minor constituents. Several immunoreactive isoflavones, namely glycitein, glycitin, ononin, sissotrin were detected in water-ethanolic extracts of *P. cattleianum* leaves (Lapčík et al. 2005). Vriesmann et al. (2009) studied the monosaccharide composition of its mesocarp fraction and reported uronic acid, galactose, arabinose, glucose, mannose, methyl fucose and xylose to be the major components. The leaf oil constituents were identified to be β-caryophyllene, α-pinene, myrcene α-thujene, 1, 8-cineole, epi-α-muurolol, α-cadinol, epi-α-cadinol and caryophyllene oxide. Jun et al. (2011) also isolated the sesquiterpene β-caryophyllene oxide from the leaves and identified the chemical structure by various spectroscopic analyses. Recently, Adam et al. (2011) examined its leaf oils by GC/MS. The oil was rich in sesquiterpene hydrocarbons (48.8%) and monoterpene hydrocarbons (10.1%). The main sesquiterpene in the oil was confirmed to be β-caryophyllene (31.5%), followed by α-humulene (4.4%). Also, a small amount of the phenylpropanoid, methyl-isoeugenol as both isomeric forms E (0.6%) and Z (0.1%) were detected in the oil (Fig. 2.2).

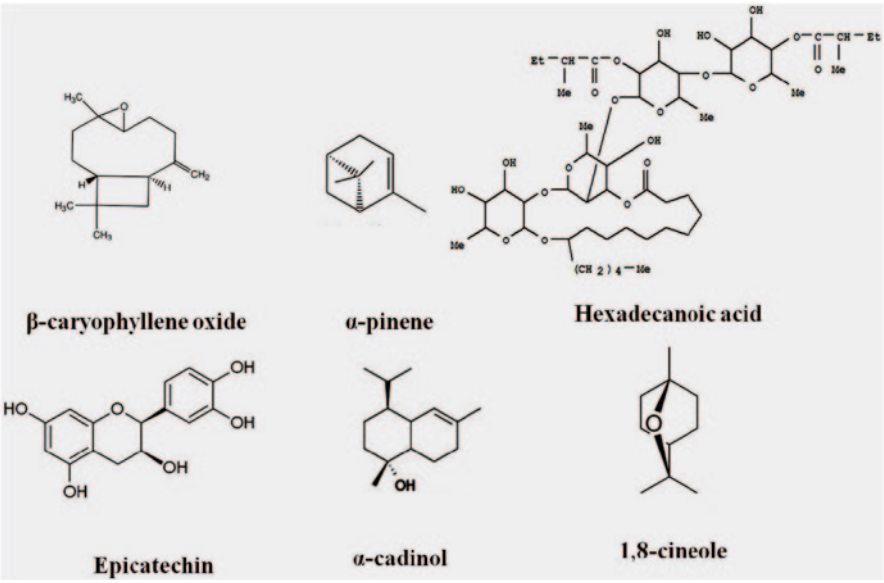

Fig. 2.2 The active compounds isolated from *Psidium cattleianum*

2.3 Uses of *P. cattleianum*

Despite the looming dangers, there are several advantages of growing a *P. cattleianum* plant in home garden. The benefits and potential uses are presented below.

2.3.1 As Food Source

The aromatic fruits with sweet-tart taste are consumed when ripe. Also, the fruits are processed into fillings, jams, jellies, puree, sauce or punch. In a Latin dessert "cascos guayaba", the fruit is stewed and served with cream cheese. Haminiuk et al. (2006) investigated the effect of temperature on rheological behaviour of *P. cattleianum* pulp. Shear thinning behaviour was reported, in which the viscosity decreased with an increase in temperature. Pectins have myriad applications in food formulations and new sources of these polysaccharides are constantly searched for. In this regard, *P. cattleianum* pulp holds promise as a rich source. These fruits might contribute cheap raw materials for the industrial production of pectin in Brazil and Hawaii (Vriesmann et al. 2009). The seed oil content was determined to be 12% and was rich in linoleic acid (81.38%). Also, it proved fit for human consumption (Kobelnik et al. 2012). Chalannavar et al. (2013) analyzed the phytochemicals of the oil extracted dried leaves of *P. cattleianum* and reported the food preservative potential of the principal component caryophyllene oxide.

2.3.2 Antioxidant and Anti-Inflammatory Activity

Luximon-Ramma et al. (2003) studied the possible antioxidant capacity of *P. cattleianum* Sabine fruits, in terms of total phenolics, proanthocyanidins, flavonoids and vitamin C content. High antioxidant content with potential beneficial effects on health was reported. Medina et al. (2011) also confirmed that the fruit extract possesses considerable antioxidant activity, tested through yeast assay and DPPH radical scavenging assay. Biegelmeyer et al. (2011) conducted the chemical characterization of the fruit extract by HPLC-DAD and investigated its antioxidant activity by TRAP method. High polyphenolic content was reported (501.33 mg/100 g), where hyperoside was identified to be one of the major flavonoids. In addition to flavonoids, the extract presented the anthocyanin, cyanidin. Ho et al. (2012) isolated seven flavonoids along with a benzoic acid from the leaves of *P. cattleianum*. The antioxidant potencies of these compounds were validated by ALP, DPPH, ABTS and ORAC assays. McCook-Russell et al. (2012) compared the total phenolics, proximate contents, antioxidant and anti-inflammatory properties of *P. cattleianum* with that of *P. guajava*. The former scored higher in the above properties, also possessed more vitamin C and fibre content. The hexane and ethyl acetate extracts of *P. cattleianum* fruits (250 μg/ml) showed COX-2 enzyme inhibitory activities of 18.3 and 26.5%, respectively. The anti-inflammatory activity proves its beneficial effect on health.

2.3.3 Antiproliferative Activity

Moon et al. (2011) evaluated the antiproliferative activity of the chloroform fraction of *P. cattleianum* Sabine leaf extract against several cancer cell lines. Maximum cytotoxicity was observed in human gastric carcinoma SNU-16 cells, at concentrations of 50–100 μg/ml. The induction of apoptosis was confirmed by immunological and molecular tools. The phytochemicals in the fractions of the leaf extract are assumed to be the bioactive ingredients. Medina et al. (2011) also reported antiproliferative effects of the fruit extract against breast cancer MCF-7 and colorectal Caco-2 cells. Jun et al. (2011) conducted an MTT assay against several cancer cell lines to study the cytotoxic effects of β-caryophyllene oxide. Its potent cytotoxic activity against HepG2, HeLa, SNU-1 and SNU-16 cells were evidenced. Im et al. (2012) investigated the molecular mechanism behind the antimetastatic effects of the butanol fraction of its leaf extract. The extract suppressed MMP-9 as well as MMP-2 expression and activity in part through the downregulation of the ERK1/2 activation in lung cancer cells. Also, the major components of the fraction were identified as glucuronic acid, quercetin 3-glucuronide, loganin and xanthyletin. Collectively, the findings indicate that the leaf and fruit extracts of *P. cattleianum* could restrain various forms of malignant tumours.

2.3.4 Antimicrobial Activity

Brighenti et al. (2008) assessed the effects of *P. cattleianum* leaf water extract on pathogenic *Streptococcus mutans*. The biofilms exposed to 1.6% extract for 2 h showed significant change in protein expression of the pathogens and inhibited their acid production. It was concluded that the leaf extract kills most of the *S. mutans* when applied at high concentrations i.e. 25, 50 or 100%. Crivelaro de Menezes et al. (2010) evaluated the influence of *P. cattleianum* Sabine aqueous extracts on *S. mutans* counts and dental enamel micro-hardness of rats with caries. The extracts decreased *S. mutans* accumulation and enamel demineralization. De Menezes et al. (2010) also studied and corroborated that *P. cattleianum* aqueous extract significantly reduces the *S. mutans* counts and decreases the enamel demineralization rate. The leaf extract was also shown to exert anti-caries effects in rats (Jun et al. 2011). Medina et al. (2011) reported the antimicrobial effect of fruit extract against *Salmonella enteritidis*, which was attributed to the abundance of phenolic compounds. Brighenti et al. (2012) evaluated the effect of its leaf extract on enamel demineralisation, extracellular polysaccharide formation, and the microbial composition of dental biofilms. The volunteers wore enamel blocks that were dripped with 20% sucrose 8 times a day. Twice a day, *P. cattleianum* extract was trickled on the sugar-soaked enamel blocks. After 2 weeks of this treatment, the various parameters were measured. The subjects with extract-applied enamels showed lower total streptococci, *S. mutans* and exopolysaccharides, indicating the anticariogenic effect of the extract.

2.4 Hurdles in Popularity and Future Scopes

Despite plentiful production of fruits and therapeutic values of various parts, *P. cattleianum* is yet to attract attention of consumers, horticulturists and food technologists. Out of the many likely reasons, the notoriety as invasive and perishability of fruits are most critical. Development of better cultivars may tackle these demerits and help consolidate its status as a nutrition source.

2.5 Conclusion

As the findings testify, *P. cattleianum* has immense potential to be developed as a popular fruit tree. The investigations conducted till now are too scanty to appreciate its full importance. A lot needs to be explored, and given due attention, it can certainly nourish, boost antioxidant status and keep cancer at bay. Also, there is urgent need to resove its invasiveness. The family Myrtaceae is rich in species with food and medicinal values *viz*. Acca, Callistemon, Eucalyptus, Eugenia, Syzgium, Leptospermum, and this species seems equally promising.

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Chapter 3
Opuntia Fruits as a Source of Inexpensive Functional Food

Abstract Opuntia (prickly pear) is a genus of xerophyte belonging to the Cactaceae family, growing abundantly in the arid parts of the world. Owing to high nutritional value in terms of dietary fiber, polyunsaturated fatty acid, minerals, protein and an assortment of other phytochemicals, Opuntia fruits are gaining popularity. Validation of health benefits *viz.* antioxidant, anti-inflammatory, antidiuretic, hypocholesterolemic, antidiabetic, antiproliferation, immunostimulatory and antiulcerogenic activity have further consolidated their position in emerging nutraceutical sector. Though at infancy, the Opuntia fruit-based foods are garnering consumer approval and are expected to contribute significantly to functional food processing. This chapter explores the proven biological potentials and suggests future scopes.

Keywords Opuntia · Functional food · Antioxidant · Anticancer · Antidiabetic

3.1 Introduction

Opuntia (prickly pear) is a cactus genus growing luxuriantly in the dry belts of the world. Its fruits also known as Indian fig, tuna, nopal fruit, cactus apple, xoconostle *etc.* have been discovered to be rich in fibres, minerals and antioxidants (Fig. 3.1a). The consumption of these succulent fruits by Native Indian tribes (Pima Indians) is well-documented. Apart from dietary uses, the fruits had ethno-medicinal importance. Also, Traditional Chinese Medicine (TCM) deemed it beneficial in tumour suppression and immunity reinforcement (Liang et al. 2008). Indigenous communities across the globe derived sustenance from this xerophytic fruit *i.e.* Tunisia (Ennouri et al. 2006) and Latin America (Garcia-Solis et al. 2009; Marin et al. 2012).

The genus Opuntia consists of about 200 species and thrives in South Western USA, Mexico, Africa, Chile, Korea and Mediterranean countries. Parish and Felker (1997) reported that the Chilean varieties of the fruits are the most desirable due to their high sugar and low seed contents. Mexican varieties with high yields do not contain much sugar. Sicilian cultivars of prickly pear (*Opuntia ficus indica*) produce yellow, red, and white fruits, due to various combinations of two betalain pigments, the purple-red betanin and the yellow-orange indicaxanthin (Butera et al. 2002). Stintzing et al. (2005) reported that the ratio and concentration of the

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Fig. 3.1 **a** Opuntia plant and its fruits. **b** Bioactive compounds extracted from the Opuntia fruits

pigments are solely responsible for the colour of the fruits ranging from yellow, orange and red to purple. Out of the numerous cultivated varieties, *O. ficus indica* is the most common, while *O. megacantha* is regarded to be the most delicious. De Wit et al. (2010) reported on the basis of their studies that Meyers is the most suitable cultivar of *O. ficus indica* for economical purposes in South Africa. El-Guizani et al. (2012) studied the Tunisian cultivars of *O. ficus indica* fruits namely, Thorny, Gialla and Rossa. The Thorny cultivar produced pears with smaller size and more seeds, whereas the latter two produced bigger, juicier fruits with fewer seeds. Feugang et al. (2006) have summarized the current knowledge on the chemical composition of Opuntia fruits with particular emphasis on their efficacy as food and medicine. The encouraging biological results necessitate the commercialization of this so-far obscure food source. This chapter presents the recent findings of biological importance, technical advances, bottlenecks and future scopes, in an effort to promote inclusion of Opuntia fruits in regular diet.

3.2 Phytochemical Constituents

Opuntia fruits possess a wealth of nutrition. El Kossori et al. (1998) investigated the composition of *O. ficus indica* fruits and reported the abundance of ethanol-soluble carbohydrates in the skin (glucose) and pulp (glucose and fructose). The seeds contained the highest amount of protein. The skin had high cellulose content along with remarkably high calcium (2.09%) and potassium (3.4%). Galati et al. (2003) investigated the fruit juice of Sicilian cultivars of *O. ficus indica* and determined the contents of ascorbic acid, total polyphenols, and flavonoids. Ferulic acid measuring at 746 μg/mL, emerged as the chief phenolic compound. Rutin and isorhamnetin were the principal flavonoid derivatives, imparting antioxidant activity. Phenolic compounds like anthocyanins, phenolic acids, stilbens and tannins were reported. Ramadan and Morsel (2003) compared the seeds and pulp of *O. ficus indica* L. fruits in terms of fatty acids, lipids, sterols, fat-soluble vitamins and β-carotene.

High amounts of neutral lipids were quantified in seed oil, while glycolipids and phospholipids scored high in pulp oil. However, linoleic acid was the major fatty acid followed by palmitic and oleic acids in both the oils. Vitamin E (α-tocopherol) level was higher in the pulp oil, whereas γ-tocopherol was the major component in seed oil. Also, the pulp oil contained higher β-carotene compared to seed oil. Tesoriere et al. (2005) assessed biothiols, taurine and flavonols, as well as tocopherols and carotenoids in the fruit pulps of Sicilian red yellow and white cultivars of Opuntia. The yellow cultivar had high level of reduced glutathione, whereas the white cultivar had the highest amount of cysteine content. Maximum taurine content was measured in the yellow cultivar. Ishurd et al. (2010) isolated a water-soluble polysaccharide (PS-1) from the *O. ficus indica* fruit by hot water extraction, anion-exchange and gel-permeation chromatography. A battery of structural investigations characterized the 360 kDa polysaccharide to be α-D-glucan with a (1→4)-linked α-d-Glc*p* backbone, with (1→6)-linked (1→4)-α-d-Glc*p* side chains. Ozcan and Al Jhaimi (2011) determined the mineral composition of *O. ficus indica* seeds, which showed the chief components to be calcium (471.2 mg/kg), potassium (532.7 mg/kg), magnesium (117.3 mg/kg), phosphorous (1627.5 mg/kg) and sodium (71.3 mg/kg). Banuelos et al. (2011) investigated the selenium content and their localization in *O. ficus indica* using inductively coupled plasma mass spectrometry (ICP-MS), microfocused X-ray fluorescence and liquid chromatography-mass spectrometry (LC-MS). The trace element reputed to be a crucial dietary supplement was found in physiologically-important free selenocystathionine form. Al-Juhaimi et al. (2013) too assessed the seed mineral profile of *O. ficus indica*, collected from various locations with the aid of ICP-MS, which showed substantial variation in calcium, potassium, magnesium and phosphorous content. The biochemical analyses substantiate the high nutritive value of these fruits and suitability for consumption. Patel (2013) has published review on nutraceutical value of various species of Opuntia fruits. Some crucial phytochemicals identified in Opuntia fruits are illustrated in Fig. 3.1b.

3.3 Health Benefits of Opuntia Fruits

Multiple nutritional attributes of Opuntia pears have emerged in the last decade. The pronounced nourishing and therapeutic effects have been presented in Table 3.1.

3.3.1 As Functional Food and Additives

The Opuntia fruits can be eaten raw or made into an array of dehydrated, frozen, canned, fermented delicacies *viz.* candy, jam, puree, marmalade, salad dressings, syrup, sauce, pies, fruit leather, smoothie, shakes, beverages (margarita) and health drinks (Sarkar et al. 2011). Armenta and Pena-Valdivia (2009) purified and quantified the mucilage, pectin and cellulose content in the fruits of *O. matudae* and indicated their richness in soluble and insoluble dietary fibers. Bensadon et al. (2010) reported that about 45% fresh weight of *O. ficus indica* fruits is discarded

Table 3.1 The Opuntia species studied so far and their biological effects

Opuntia varieties	Biological effect	Fruit parts	References
O. ficus indica (L.) Mill.	Antiulcerogenic Immunostimulatory Anticancer Hypocholesterolaemic Antioxidant Hepatoprotective Diuretic Colorant Sweetener	Fruit juice Betalain pigment Seed oil Pear extract Pulp	Galati et al. 2003; Aires et al. 2004; Sreekant et al. 2007; Ennouri et al. 2006; Stintzing et al. 2005; Tesoriere et al. 2004; Butera et al. 2002; Saénz et al. 2009; Fernandez-Lopez et al.2010; Moßhammer et al. 2005; Sanez et al. 1998; Alimi et al. 2012a, 2012b, 2012c
O. dillenii Haw	Antioxidant	Seed oil	Liu et al. 2009
O. robusta	Antidiabetic Hypocholesterolaemic Antioxidant	Soluble fibre Pectin Pear extract	Wolfram et al. 2002; Stintzing et al. 2005; Castellanos-Santiago and Yahia 2008
O. dillenii Haw	Antioxidant	Seed oil, pulp, peel	Chang et al. 2008; Liu et al. 2009
O. fuliginosa	Antidiabetic	Pear extract	Trejo-Gonzalez et al.1996
O. undulata	Antioxidant	Pear extract	Fernandez-Lopez et al. 2010
O. stricta	Antioxidant Colorant	Pear extract	Fernandez-Lopez et al. 2010; Castellar et al. 2008
O. vulgaris	Antioxidant	Fruit extract	Saoudi et al. 2011
O. lindheimeri	Antioxidant	Fruit extract	Kuti 2004
O. macrorhiza	Colorant	Peel and pulp	Moussa-Ayoub et al. 2011a
O. megacantha	Functional food	Fruit	Ndhlala et al. 2007
O. joconostle	Antioxidant		Morales et al. 2012
O. matudae	Antioxidant Functional food		Morales et al. 2012 Armenta and Pena-Valdivia 2009

as by-products which contain significant amount of dietary fibers and antioxidants. Guzman-Maldonado et al. (2010) also carried out nutrient evaluation of the wastes of *O. matudae* fruits and reported that the total fiber content in the peel was 2-fold higher to that of edible mesocarp. The finding shed light on the food potential of the peels. Also, the iron content of the peel was higher than that of the mesocarp. Pena-Valdivia et al. (2012) determined the soluble and insoluble dietary fiber content of the fruits of *O. spp*. The acidic fruits ('xoconostle') had significantly higher

unavailable polysaccharides content than sweet fruit, either way the fruits proved to be excellent reserves of of dietary fibers. Shetty et al. (2012) suggested the use of immature fruits as mock-gherkins (pickled cucumbers). Ozcan and Al Jhaimi (2011) reported that *O. ficus indica* seeds are an important source of natural fiber and linoleic acid, so the oil is suitable to be used as a nutraceutic agent. El-Guizani et al. (2012) studied the Tunisian varieties of prickly *O. ficus indica* and reported that about 7.3 % oil with significant nutraceutical value can be extracted from them. Rodriguez-Lerma et al. (2011) produced Opuntia fruit wine by employing a mixed starter inoculum of *Pichia fermentans* and *Saccharomyces cerevisiae.* The product contained 8.37 % alcohol and nine volatile compounds *viz*. isobutanol, isopentanol, ethyl acetate, isoamyl acetate, ethyl octanoate, ethyl decanoate, ethyl 9-decanoate, β-Phenylethyl acetate, and phenylethyl alcohol. The wine was graded to be of high-quality with fruity flavor and palatability.

These fruits may also be incorporated into processed foods as valuable additives. Their application as sweetener and colorant for edible purposes deserves discussion. Saenz et al. (1998) evaluated *O. ficus indica* fruits as a source of natural liquid sweetener. The juice of the fruit (16.5 Brix) was clarified with enzymes, treated with activated carbon and vacuum concentrated to obtain a 60 Brix syrup. The acidity (pH of 4.31) was greater than that of high fructose corn syrup of 0.035 %, and the ash values (1.4 %) were reported higher to glucose syrup (1.0 %). Sensory evaluation revealed that this syrup had comparable sweetness with glucose. Stintzing et al. (2005) reported that the yellow betaxanthins and red-purple betacyanins may be of great use as food colorants. Moßhammer et al. (2005) developed a betalain-based food colorant from *O. ficus indica* cv. 'Gialla' and cv. 'Rossa' fruits, which showed promise as a substitute of *Beta vulgaris* (red beet). Castellar et al. (2008) extracted betanin from the fruits of *O. stricta*, after yeast fermentation of the fruit juice, followed by centrifugation and vacuum drying of the supernatant. Characteristics of the final product obtained were pH 3.41, 5.2 degrees Brix, 9.65 g betalain/L juice, color strength of 10.8, and viscosity of 52.5 centipoise. Castellanos-Santiago and Yahia (2008) conducted the qualitative and quantitative analyses of betalain pigments in 10 cultivars of *Opuntia spp.* fruits grown in Mexico. Betacyanins and betaxanthins were identified by comparison with the UV/vis and mass spectrometric characteristics as well as the retention times of semi-synthesized reference betaxanthins. HPLC-DAD and ESI-MS data revealed that the ratio and concentration of betalain pigments are responsible for the color in the different cultivars. The highest betalain content was measured in the fruit of purple colored *O. robusta* Wendl. (8.1 mg/g dry fruit), which is comparable to that found in *B. vulgaris* L. ssp. Var. Pablo) (8.6 mg/g dry tissue). Saénz et al. (2009) encapsulated the bioactive compounds of pulp and ethanolic extracts of the *O. ficus indica* fruit with maltodextrin or inulin coat for developing an antioxidant-rich red colorant. A 2^2 statistical factorial design revealed that the 3:1 ratio of core/coating material at 140 °C inlet air temperature were the optimal conditions for stable microcapsules preparation. An increase in phenolic compounds was observed during storage at 60 °C. Indicaxanthins showed higher stability than betacyanins during the storage. Moussa-Ayoub et al. (2011) reported that the peel and pulp of *O. macrorhiza* fruits rich in betacyanins

provides a deep red-purple color, with higher intensity than *B. vulgaris* spp. and about 8-fold higher than red fruits of *O. ficus indica*. Obon et al. (2009) prepared a red-purple food colorant from *O. stricta* fruit juice by co-current spray. The glucose-syrup dried colorant retained stability for a month. The organic colorant was incorporated in yoghurt and soft drink which found favor with the consumers. Opuntia fruits could be developed as alternatives to synthetic dyes. Ennouri et al. (2012) extracted an exo-type amylase from seeds of *O. ficus indica* which exhibited high thermal stability and wide range of pH stability, making it a promising candidate for food applications.

3.3.2 Antioxidant and Antinflammatory Activity

Opuntia fruits have been shown to possess an interesting corpus of antioxidants. Butera et al. (2002) investigated the antioxidant activities of the methanolic extracts *O. ficus indica* pulp*s*. Spectrophotometric analysis revealed that the yellow cultivar had the highest amount of betalains, followed by the red and white ones. When measured as Trolox equivalents, the extracts rich in indicaxanthin-type betalains exerted remarkable antioxidant activity. All the extracts dose-dependently inhibited of the organic hydroperoxide-stimulated red cell membrane lipid oxidation, the white extract offering the maximum protection. Tesoriere et al. (2004) also investigated the antioxidant activity of *O. ficus indica* through a randomized, double-blind study on 18 healthy volunteers by administering them with either 250 g fresh fruit pulp or 75 mg vitamin C twice daily for 2 weeks. Consumption of the fruit augmented the redox balance and antioxidant status, while diminishing the oxidative damage to lipids. Kuti (2004) investigated the antioxidant makeup of four varieties of Opuntia fruit extracts *viz. O. ficus indica* (green-skinned), *O. lindheimeri* (purple-skinned), *O. streptacantha* (red-skinned) and *O. stricta var. stricta* (yellow-skinned). Quercetin was the most abundant component in all varieties. Kaempferol was found in green, purple and red-skinned varieties and isorhamnetin in green and purple-skinned varieties. The red-skinned fruits contained the most ascorbic acid (815 μg/g) and the yellow-skinned fruits the most carotenoids (23.7 μg/g). The antioxidant activity was stronger in the purple-skinned *O. lindheimeri*, compared to other varieties owing to the higher total flavonoid content. Ndhlala et al. (2007) assessed the phenolic acid composition of the peel and pulp of the fruits of *O. megacantha* (L.) Mill and found ferulic acid, caffeic acid and vanillic acid to be the dominant antioxidants. Liu et al. (2009) analyzed the fatty acid makeup of supercritical carbon dioxide-extracted seed oil from *O. dillenii* to be linolenic acid (66.56%), palmitic acid (19.78%), stearic acid (9.01%) and linoleic acid (2.65%). Assessment of the seed oil by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay and β-carotene bleaching test showed strong antioxidant activity comparable to ascorbic acid and butylated hydroxytoluene (BHT). Fernandez-Lopez et al. (2010) determined the *in vitro* antioxidant capacity of Spanish red-skinned *O. ficus indica*, *O. undulata* and *O. stricta* by Trolox as well as DPPH method. *O. ficus indica* fruit

extract exerted the strongest antioxidant capacity and contained the highest taurine content. *O. stricta* fruits were the richest in ascorbic acid and total phenolics, whereas *O. undulata* fruits showed the highest carotenoid content. Quercetin and isorhamnetin were the main flavonoids detected. Dried powder of *O. ficus indica* fruit 'Cacti-Nea', was evaluated for its chronic diuretic and antioxidant effects in rats. Oral administration of Cacti-Nea for seven days at a dose of 240 mg/kg/day significantly promoted urine production (at par with the standard drug hydrochlorothiazide), while reducing the body weight gain. Also, the long term consumption of Cacti-Nea considerably increased the levels of the crucial metabolic enzyme, glutathione peroxidase in blood (Bisson et al. 2010). Sumaya-Martínez et al. (2011) assessed the protective ability of 18 cultivars of Opuntia fruits collected from Mexico, by β-carotene-linoleic acid emulsion and ferrous chelating assay. The results indicated that the antiradical potency of the yellow and white pear cultivars was less than that of red and purple cultivars. The superior antioxidant profile of the red pears was correlated to the concentration of total phenolic compounds and ascorbic acid. Saoudi et al. (2011) studied the ameliorative effects of *O. vulgaris* fruit extract against methanol-induced haematological and biochemical toxicity in rats. The treatment of rats with methanol has adverse effects on lipid peroxidation as well as activities of metabolic enzymes *viz.* SOD, CAT and GSH-Px. The fruit extract restored normalcy by increasing the levels of red blood cell, haemoglobin, haematocrit, serum total protein and decreasing glucose, cholesterol and triglyceride levels in serum. Further, significant increase in the activities of the metabolic enzymes in erythrocytes was registered. Spleen histopathology showed that the extract alleviates the induced lesion. Cardador-Martínez et al. (2011) assessed the total phenolics, flavonoids and tannins of peel and seeds of four cultivars and reported the better antioxidant capacity of the former. Fruits with light-green or yellow-brown peel exerted more pronounced antiradical activity and TEAC values compared to those with red-purple peel. The promising result warrants the exploitation of Opuntia fruit residues as natural antioxidants. Chang et al. (2008) evaluated the antioxidant content of *O. dillenii* Haw seed, peel and pulp extract. The seed extract was rich in polyphenols and flavonoids, such as gallic acid, catechin, sinapinic acid, epicatechin, *p*-coumaric acid, quercetin and ferulic acid; whereas the peel and pulp contained high amounts of betanin, isobetanin and ascorbic acid, but with lower contents of phenolics and flavonoids. Morales et al. (2012) evaluated the antioxidant properties of pulp and seeds of *O. joconostle* and *O. matudae.* The pulps contained ample ascorbic acid, while the seeds contained fiber, phenolics, flavonoids, PUFAs and tocopherols in appreciable amounts. Alimi et al. (2012a) investigated the efficacy of *O. ficus indica f. Inermis* fruit juice in reversing oxidative damage of rat RBCs induced by chronic ethanol intake. Pre-administration of 2–4 ml/100 g juice to ethanol-intoxicated rats significantly restored the decrease in metabolic enzymes *viz*. SOD, CAT, GSH-Px. *O. ficus indica* (L.) Mill. juice has been credited to cancel the ulcerogenic effect of ethanol by promoting mucus production and the restoration of the normal mucosal structure (Galati et al. 2003). Osorio-Esquivel et al. (2011) reported richness of *O. joconostle* fruit pericarp in phenolic compounds and betacyanins. Abd El-Razek et al. (2012) demonstrated that Opuntia fruit juice with its

high total phenolics, total flavonoids, vitamin C, vitamin E and β-carotene content can keep oxidative stress under check and minimize cataract risk in diabetes patients. Oral administration of 3 ml juice daily to alloxan-induced hypoglycaemic rats for 8 weeks remarkably increased the concentration of GSH-Px, SOD and decreased NO. Alimi et al. (2012b) investigated the effect of *O. ficus indica f.* Inermis juice against ethanol-induced liver injury in rats. Heptaoprotection was due to the free radicals inhibitions and enhancement of the endogenous antioxidants activities. Alimi et al. (2012c) further proved the defending role of *O. ficus indica f.* Inermis juice against ethanol caused damage to RBC membranes. Dose-dependent amelioration was exerted by the polyphenols, flavonoids, ascorbic acid, carotenoids and betalains in the extract. Saoudi et al. (2012) evaluated the potential of *O. vulgaris* fruit extract in offsetting the toxicity induced by methanol. The pretreatment with the extract protected from the hepatic and renal histopathological and oxidative damage, as manifested in the lowered TBARS and enhanced SOD, CAT and GSH-Px level. Kim et al. (2012) evaluated the protective activity of *O. ficus indica* var. saboten fruit juice and its constituent betanin against stress-induced acute gastric lesions in rats. Pretreatment of the lyophilized powder of the fruit and betalain significantly reduced the stress lesions. Both components prevented the fall in gastric mucus content and attenuated the increase in the level of gastric mucosal TMF-α and myeloperoxidase. Betalain contributed in preventing the myeloperoxidase mediated mucosal damage and proinflammatory cytokine production. Cha et al. (2013) reported that the ethyl acetate fraction of the *O. humifusa* fruit ethanol extract is a significant source of antioxidant, the key phenolic component being ferulic acid and protocatechuic acid. The most abundant flavonoid was taxifolin, followed by myricetin.

3.3.3 Hypocholesterolemic Activity

Dietary intervention for bringing down blood cholesterol level has emerged as the safest of all antilipemic approaches. Opuntia fruits have been investigated for bioactive components to address this objective. Fernandez et al. (1992) determined the effects of Opuntia fruit pectin on plasma low-density lipoprotein (LDL) metabolism, by feeding guinea pigs a low cholesterol diet in which cellulose was partially substituted with pectin. The pectin-fortified diet lowered LDL level by 33%. Further, Fernandez et al. (1994) correlated the decrease in LDL concentrations to the mechanisms altering hepatic cholesterol homeostasis. Wolfram et al. (2002) also tested the efficacy of soluble fibres and pectin in *O. robusta* pears for lowering cholesterol levels in hyperlipidemic volunteers. After an 8 week consumption of the fruit, a decrease of total cholesterol (12%), LDL-cholesterol (15%), apolipoprotein B (9%), triglycerides (12%), fibrinogen (11%), blood glucose (11%), insulin (11%) and uric acid (10%) was recorded. Ennouri et al. (2006) reported fall in blood total cholesterol and LDL level in the rats fed with PUFA-rich *O. ficus indica* seed oil, for a period of nine weeks.

3.3.4 Antidiabetic Activity

Opuntia fruits contain fiber and pectin with an ability to lower blood glucose level. Trejo-Gonzalez et al. (1996) evaluated the hypoglycaemic activity of a purified extract of *O. fuliginosa* pears on streptozotocin-induced diabetic rats. Blood glucose and glycated haemoglobin levels were reduced to normal values by a combined treatment of insulin and the extract. In absence of insulin, the extract alone could maintain the normal glycaemic state. Further, it was observed that the rats receiving the combination treatment for 7 weeks followed by the extract alone were capable of attaining blood glucose level as that of non-diabetic rats. The extract at a dose of 1 mg/kg daily could elicit anti-diabetic effect. Wolfram et al. (2002) reported that a soluble fibre in *O. robusta* fruits can retard carbohydrate absorption and reduce the postprandial rise in blood glucose and serum insulin in patients with type-II diabetes. Liu et al. (2010) also investigated the hypoglycaemic effect of the extracts of Opuntia fruit polysaccharide in streptozotocin-induced diabetic rats. After 8 weeks administration of the extract, the pathology of beta and alpha cells in the pancreas of experimental rats were examined. Compared to control group, the blood glucose, total cholesterol and triglyceride were remarkably low in administered test groups. Histochemical tests demonstrated the enhancement in the number of beta cells in islets of Langerhans. Van Proeyen et al. (2012) investigated the effect of *O. ficus indica* fruit-skin extract on blood glucose and plasma insulin increments due to high-dose carbohydrate ingestion, before and after exercise. Administration of 1000 mg fruit extract 30 min before and immediately after could increase the plasma insulin and lower blood glucose level, as determined by the double-blind placebo-controlled crossover study.

3.3.5 Antiproliferative Activity

As the quest for mining plant-based anticancer compounds gets momentum, Opuntia fruit extracts opens up new realms of possibilities. Zou et al. (2005) tested aqueous extracts of *O. ficus indica* fruit gathered from Arizona, for their anti-cancer effects on cultured cells and on an animal model. Cells exposed to the fruit extracts significantly succumbed to apoptosis and growth inhibition occurred in a dose- and time-dependent manner. The fruit extracts significantly suppressed tumour growth in nude mice, increased annexin IV expression and decreased VEGF by modulating the expression of tumour-related genes. Sreekant et al. (2007) evaluated the antiproliferative effects of betanin from *O. ficus indica* fruits, on human chronic myeloid leukemia K562 cell line. A dose and time dependent decrease in the proliferation of the treated K562 cells occurred with an IC_{50} of 40 μM. Further studies involving scanning and transmission electron microscopy revealed the apoptotic characteristics *viz*. chromatin condensation, cell shrinkage and membrane blebbing. Betanin treatment induced the release of cytochrome *c* into the cytosol, poly (ADP) ribose polymerase (PARP) cleavage and down-regulation of Bcl-2 (intrinsic

pathway). Liang et al. (2008) studied the possibility of polysaccharides extracted from Opuntia fruit in inhibiting the proliferation of cervix, ovary and bladder cancer cells and suppressing ovarian cancer in S180-bearing mice. Under electron microscopy, polysaccharide-treated tumour cells showed typical morphology of early apoptosis with condensed chromatin at the margins of nuclei, disintegrated nucleolus and vacuoles in the cytoplasm. The thymus index was significantly higher in middle and high dose polysaccharide groups than control group, indicating the immunity promotion ability of Opuntia fruit. Chavez-Santoscoy et al. (2009) studied the anticancer properties of the juices of nine *O. spp.* fruits. The 'Moradillo' variety contained the highest flavonoids and diminished both prostate and colon cancer cell viability without affecting mammary or hepatic cancer cells. The 'Rastrero' variety reduced the growth of all four cancer cell lines without affecting normal fibroblast viability. Inter-varietal differences in terms of phytochemicals anticancer attributes surfaced. Garcia-Solis et al. (2009) evaluated the antiproliferative effect of aqueous extracts of Opuntia fruit on the breast cancer cell line MCF-7 though no substantial inhibitory effect was recorded. Feuganag et al. (2010) reported the anticancer effect of Arizona grown *O. ficus indica* mixture aqueous extract which reduced the gynaecologic cancer cells growth by inducting apoptosis. When treated 5–10 % extract dose for 2 days, the ovarian cancer cells exhibited a dramatic increase in reactive oxygen species (ROS). The intracellular ROS pool activated a cascade of reactions leading to the apoptosis, evident from high levels of DNA fragmentation and enhanced expression of Bax, Bad, caspase 3, Bcl2, p53, and p21.

3.3.6 Immunestimulatory Activity

Wolfram et al. (2003) tested the effect of daily consumption of 250 g Opuntia fruit on various parameters of platelet function in human volunteers. Ingestion of the fruit significantly reduced the platelet proteins (platelet factor 4 and β-thromboglobulin), adenosine diphosphate (ADP)-induced platelet aggregation and improved platelet sensitivity against prostaglandin. Aires et al. (2004) evaluated the immune response elicited by *O. ficus indica* polyphenolic compounds in human Jurkat T-cells (the immortalized T cell line). The results suggested that the phytochemicals induce increment in $[Ca^{2+}]$ i via endoplasmic reticulum pool, open Ca^{2+} channels and exert immunosuppressive effects in Jurkat T-cells. The available evidences are scanty yet suffice to steer further investigations on immunomodulatory activity of Opuntia fruits.

3.4 Role in Food Processing and Functional Food Development

Effective processing can lead to development of nutritious and appetizing Opuntia fruit-based food products. Clarification and distillation of juice, efficient extraction of seed oil, preservation of extracts and retention of nutrients, reduction of

microbial load are some of the vital steps. Membrane-based filtration technologies are emerging to be the most effective methods. Moßhammer et al. (2006) applied flow microfiltration for preservation of the Opuntia fruit juice as concentrates and powders. The juice concentrates and fruit powders were produced by rotary evaporation and freeze drying at laboratory scale and compared to products obtained at pilot plant-scale applying a three-stage column evaporator and spray drying, respectively. Retention of colour and selected quality parameters without any adverse reactions (apart from the non-enzymatic browning and hydroxymethylfurfural formation after concentration), makes it a feasible processing technique. Cassano et al. (2007) clarified the fresh juice, with a total soluble solids (TSS) content of about 11 Brix by ultrafiltration followed osmotic distillation up to a TSS content of 61 Brix at 28 °C. Cassano et al. (2010) investigated the effect of microfiltration and ultrafiltration processes on the physicochemical composition of the fruit juice. Moussa-Ayoub et al. (2011b) assessed the applicability of pectinases and cellulases as soft hydrolysing agents on flavonol glycosides for identification and quantification of flavonol aglycons in the fruits. Liu et al. (2009) investigated the antioxidant activity of supercritical carbon dioxide-extracted seed oil from *O.dillenii* Haw. The maximum extraction yield of 6.65 % was achieved at a pressure of 46.96 MPa, a temperature of 46.51 °C, a time of 2.79 h and a CO_2 flow rate of 10 kg/h. Tesoriere et al. (2005) reported that as a consequence of industrial processing, a total loss of GSH and β-carotene and a net decrease of vitamin C and cysteine were observed in the fruit juice; whereas betalains, taurine and vitamin E appear to be less susceptible to degradation. Mobhammer et al. (2007) determined the impact of stabilizing additives as ascorbic, isoascorbic and citric acid on heat stability of betalains. Pigment stability and colour characteristics depended on type and concentration of the additives and pH conditions. Maximum pigment retention amounting to 79 % was obtained when 0.1 % citric acid was added to the juice at pH 6. Gandia-Herrero et al. (2010) characterized the purified indicaxanthin from the Opuntia fruit and enhanced its stability by encapsulating in a maltodextrin matrix. The spray-dried encapsulation yielded a bright yellow powder with stability at 20 °C for months. Gharras (2011) assessed the stability of the pigment betalain in juice of *O. ficus indica* as the function of temperature and pH. The degree of pigment retention decreased when the temperature was increased. Ascorbic acid proved an effective protective agent at pH 3.5, increasing the stability by 40 %. Sarkar et al. (2011) reported that the blending of Opuntia fruit solids in flour enhances the density and breaking strength of the extrudates, desirable criteria for incorporation in foods. Zafra-Rojas et al. (2013) evaluated the effect of ultrasound conditions at amplitude levels on the characteristics of purple *O. ficus indica* juice. The ultrasound treatment for time period of 15 and 25 min significantly reduced the microbial count without affecting the juice quality and its antioxidant properties.

The broad array health-promoting attributes necessitates integration of Opuntia fruits in diet. Fortified foods and supplements with these fruits as chief ingredients have started trickling into markets. Health supplement' Cacti-Nea™' is a indicaxanthin-rich soluble powder, possessing diuretic and weight management properties. 'TriVita Nopalea', is an anti-inflammatory drink. 'Meratol' is a weight management supplement processed from the fruit extract. Sisel International, a dietary supplement manufacturer, has formulated a weight loss agent 'SlenderPOPs' from the

extracts of *O. ficus indica* fruits. Institute of cellular pharmacology manufactures *O. ficus indica*-fortified nutraceutical 'Tex-OE' that is claimed to protect body against a host of assaults. Coming time is sure to witness surge in similar products with diverse biological potencies.

3.5 Strategies to Reduce Postharvest Loss and Role In Food Security

Minimization of Opuntia fruit postharvest loss is of considerable economic importance. Packaging and coating with hydrogels and polymeric materials have shown promise in this regard. The efficacy of preservation method can be assessed by the monitoring of headspace gas concentrations, spoilage microorganisms, sensory characteristics, weight loss and the quality deterioration. Piga et al. (2003) tested the potency of gas permeable film in retaining the chemical, physical, microbiological and sensorial parameters of peeled fruits. Vitamin C and antioxidant capacity remained unchanged, while polyphenol content showed reduction after 6 days storage. Sensorial properties were not affected, despite the change in pH and acidity. Moreover, the film could check microbiological growth. Cefola et al. (2014) characterized the optimal parameters for peeling and processing of these fruits as ready-to-eat products. The storage of the fruits at 4 °C in both passive (air) and semi-active (10 kPa O_2 and 10 kPa CO_2) modified atmosphere improved the marketability by 30%.

During the last decade the Opuntia pear has become an important fruit crop in the semi-arid lands of Mexico, for its minimal water requirement, hardiness and adaptability to extreme temperatures (Pimienta-Barrios 1994). These plants are grown at domestic level as well as organized plantations, as a source of food and therapeutics. Opuntia crop could be established in drought regions of many countries, including Africa and India (Ennouri et al. 2006). Germplasm conservation, genetic engineering and tissue culture are key strategies in successful implementation of this vision (Shedbalkar et al. 2010; Zoghlami et al. (2012). Axillary-branching micropropagation technique for high degree of clonal fidelity and conservation of interesting genetic resources was recommended.

3.6 Conclusion

The findings furnished above say loud for the nutraceutical potential of Opuntia fruits. Despite being rich in nutrients, they are yet to get credit as a major food source. Localized production, prickly fruits and perishability are the impediments in popularity. Recognition of other disadvantages and efforts to eliminate them will catapult these fruits to regular fruit platter. Quality improvement by genetic intervention, blocking post-harvest loss and incorporation in processed foods will boost

consumer acceptability and evoke research interest on this low-profile fruits. This chapter with the updated overview is believed to spur further work in the suggested direction.

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Chapter 4
Food and Pharmaceutical Potential of Carissa Genus

Abstract Shrubs belonging to *Carissa* genus (Apocyanaceae family) are potential sources of food and medicine, yet they have an obscure status and rarely been exploited. Since antiquity, the stem, root bark, leaves, fruit and seed extracts have been used in folk medication. Now, the emerging scientific investigations are validating the ethno-medicinal uses of this genus. Bioactive compounds ranging from polyphenolics, flavonoids, flavanones and lignans to sesquiterpenes have been isolated from them. *In vitro* and *in vivo* studies have revealed the antioxidant, analgesic, anti-inflammatory, hypolipidemic, wound healing, antimicrobial, antidiabetic, antiepileptic, anticancer, diuretic, nephrotoxicity amelioration and hepatoprotective activities. This chapter summarizes the recent findings for promoting the nutraceutical applications of this genus.

Keywords Carissa · Polyphenolics · Antioxidant · Anticancer · Food

4.1 Introduction

The genus Carissa belonging to family Apocynaceae comprises of 20–30 species. The shrubs of this genus are native to tropical and subtropical regions of Asia, Africa and Australia. They are grown as ornamental plants in the USA, ranging from Florida to California. The evergreen shrubs are 2–10 m tall, have branches with thorns, glossy leaves and white flowers. The shrubs are very attractive as hedge plants for the evergreen, dense branches and star-like fragrant flowers (Fig. 4.1a). The ripe fruits are crimson red, edible with tart taste, rich in Vitamin C, calcium, magnesium and phosphorus (Fig. 4.1b). Common species belonging to this genus are *Carissa carandas, C. macrocarpa, C. grandiflora*, *C. edulis, C. spinarum, C. lanceolata, C. opaca, C. congesta* and *C. bispinosa*.

C. edulis fruits are consumed by Maasai, Sonjo, Gogo, Kurya, Barbaigs Zulu tribes. Several species of the genus Carissa have a long history of use in traditional medication. *C. edulis* is used traditionally for the treatment of headaches, chest complaints, rheumatism, gonorrhoea, syphilis, rabies and as a diuretic (Nedi et al. 2004). Folkloric uses of this species include the treatment of fever, sickle cell anae-

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Fig. 4.1 **a Carissa macrocarpa** bush **b** Ripe fruits

mia and hernia. *C. carandas* (Karanda) is used as stomachic, antidiarrheal, anthelmintic and cooling agent in Ayurvedic medicine formulations in India (Meena et al. 2009). Stems of *C. carandas* are used to strengthen tendons, fruits are used in skin infections and leaves are deemed remedy for fever, earache and syphilitic pain. The tribal healers of Western Ghat region of Karnataka, India use this species as a hepatoprotective and antihyperglycemic agent (Itankar et al. 2011). An ethnobotanical survey conducted in Sariska and Siliserh regions in Alwar District of Rajasthan, India, recommended that *C. carandas* can be used as an insect repellent, hypotensive and dropsy therapeutic (Jain et al. 2009). Rahmatullah et al. (2009) have reported that this species may alleviate a broad range of ailments ranging from malaria, epilepsy, nerve disorders, pain, fever, insanity, myopathic spasm, dog bite, coughs, colds, itches leprosy and anorexia. *C. spinarum* (Jungle karunda) has traditionally been used as wound healing agent (Sanwal and Chaudhary 2011). Abbasi et al. (2009) documented the medicinal relevance of many plants, including *C. opaca* in the amelioration of jaundice and hepatitis. Ahmad et al. (2009) reported that the stems, leaves and fruits of this species are used as an antipyretic by communities residing in Margalla Hills National Park, Islamabad. Fruits of this plant mixed with roots of the *Mimosa pudica* are taken as an aphrodisiac. But no scientific data is available to validate this folkloric claim. Scientific validity of the pharmacological importance of Carissa genus is increasingly being proven. Patel (2012) has published a review on the food and pharmaceutical potential of Carissa gebnus. This chapter assembles the discrete information with an aim to project its holistic importance in food and pharmaceutical development.

4.2 Phytochemical Constituents

Phytochemical constitution of several Carissa species has been assessed in recent times. The prevalence of polyphenolics, flavonoids and flavanones in the extracts has been evidenced. The methanolic extract from the root of *C. edulis* contained

5% lignans (nortrachelogenin, carinol and carissanol) and 5% sesquiterpenes (carissone, cryptomeridiol and β-eudesmol). Lignans and sesquiterpene glucosides have also been isolated from *C. carandas* stems (Wangteeraprasert and Likhitwitayawuid 2009). A new germacrene derivative, carenone and a new ester, 3′-(4″-methoxyphenyl)-3′-oxo-propionyl hexadecanoate were isolated from the stems of *C. spinarum* L. (Rao et al. 2005). Lupeol, oleuropein, carissol and β-amyrin were isolated from *C. edulis* (Festus et al. 2009). From the methanolic extract of the roots of *C. congesta*, des-N-methylnoracronycine (an acridone alkaloid), carissone, carindone, lupeol, stigmasterol, ursolic acid and its methyl ester were derived (Ganapaty et al. 2010). The volatile oil obtained by hydrodistillation of the roots of *C. opaca* yielded 2-hydroxyacetophenone (Mallavarupu et al. 2009). Eudesmanes carissone, dehydrocarissone and carindone were isolated from the dichloromethane extract of the wood of *C. lanceolata* (Lindsay et al. 2000). Isoquercetin, hyperoside, vitexin, myricetin and kaempherol were isolated from the methanol extract of *C. opaca* leaves (Sahreen et al. 2011a, b). Carissone, benzyl salicylate, benzyl benzoate and α-farnesene were isolated from *C. opaca* (Rai et al. 2005). Carissa fruits have been reported to be a rich source of vitamin A and C. *C. macrocarpa* fruits contained Ca, Mg, Fe, Mn, Cu, Pb, Se, Ni and Zn in decreasing order. The Pb content is low enough to be recommended for dietary purposes. Lipid profiling revealed the appreciable monounsaturated and essential fatty acid contents in the fruits (Moodley et al. 2012).

4.3 Nutraceutical Potential of Carissa Genus

Consumers are waking up to the benefits of functional foods and the importance of phytotherapeutics is being recognized. Carissa genus holds ample possibilities in the regard. The sections below embody the recent findings with key consequences.

4.3.1 As Food and Delicacy

The ripe fruits of some Carissa can be consumed or prepared into jams, jellies, marmalades, squash, sauces, syrups and chutneys. *C. carandas* produces berry-sized fruits which are very often made into pickles in India. The *C. spinarum* plums are relished as fruit salads, topping for cakes, puddings and ice cream. Carissa fruits are prepared into soup, pies and bread. Consumption of these fruits is known to alleviate malnutrition in Uganda. *C. edulis* fruits have been analyzed to possess comparable nutritional value as mangoes and paw-paws. Rahmatullah et al. (2009) surveyed the potential sources of plant-derived functional foods in Bangaldesh and reported that *C. carandas* merits consideration as part of the regular diet in impoverished communities. In Dinajpur district leaves of this plant are boiled in water and

the resultant syrup is consumed as an appetite stimulant, apart from the edible fruits. Moodley et al. (2012) reported that the fruits of *C. macrocarpa* are consumed by the local people of KwaZulu-Natal, South Africa. These findings advocate usage of this fruit for functional food preparation, for broader sections of consumers.

4.3.2 Antioxidant Activity

Extracts of various parts of Carissa demonstrate antioxidant activities as determined by DPPH, superoxides, hydroxyl, hydrogen peroxide, ABTS radicals and exhibiting strong iron chelating assays.

Rao et al. (2005) reported antioxidant activity of *C. spinarum* L. chloroform extract. The chloroform and aqueous fractions of *C. opaca* fruits were rich in phenolic and flavonoid compounds, responsible for strong antioxidant activity (Sahreen et al. 2010). Patil et al. (2012) evaluated the functional food prospect of *Carissa carandus* by evaluating its antioxidant quotient. The LC-MS/MS profiling revealed the berries to be rich sources of polyphenolics.

4.3.3 Analgesic, Anti-Inflammatory and Antipyretic Activities

Though, the ethnobotanical uses of Carissa extracts for pain and fever remedy have been here since antiquity, experimental justifications are accumulating now only. Ibrahim et al. (2007) evaluated the analgesic effect of the water extracts of the root bark, stem bark, leaves, fruits and seeds of *C. edulis* in mice using both mechanical as well as chemical methods. The mechanical method showed highest activity of fruits followed by leaves, seeds, root bark and stem bark; whereas the chemical method showed that the maximum efficacy of seeds followed by fruits, leaves, root bark and stem bark. Chromatographic analyses revealed the presence of salicylates, which explained the usage of the plant in curing toothache, lumbago, edema and chest complaints. The 50% ethanolic extract of *C. carandas* fruits exerted a dose-dependent inhibition of carrageenan-induced swelling and squirming episodes in cotton pellet-induced granuloma in rats (Sharma et al. 2007). Bhaskar and Balakrishnan (2009) examined the analgesic, anti-inflammatory and antipyretic activities of *C. carandas* root ethanol and aqueous extracts on murine model. Analgesic (by hot plate and acetic acid induced writhing methods), anti-inflammatory (by carrageenan induced paw edema method) and the antipyretic (by Brewer's yeast-induced pyrexia method) activities were confirmed at the doses of 100–200 mg/kg. *C. spinarum* was documented to be instrumental in chronic joint pain management (Wambugu et al. 2011).

4.3.4 Wound Healing Property

In literature search of wound management attributes of Carissa genus, several promising results were found. Ayyanar and Ignacimuthu (2009) reported that the Kani tribals residing in Tirunelveli Hills in South India depend on indigenous medicinal plants which include the Carissa genus for nursing cuts, burns, bruises, boils and blisters encountered in day-to-day life. The wound healing properties of the methanolic extract of *C. spinarum* were investigated *in vivo.* The root extracts obtained by cold maceration were applied on burn wound mice models. The wound healing activity of 1–2.5 % of the liquid extract was assessed in terms of the rate of wound contraction, period of epithelisation and hydroxyproline content. At the administered dose, significant wound amelioration was witnessed (Sanwal and Chaudhary 2011).

4.3.5 Antimicrobial and Antiprotozoal Activities

A convincing number of findings exist authenticating the antimicrobial roles of Carissa extracts. The antibacterial activities of the dichloromethane extract of the wood of *C. lanceolata* R. Br were tested against the pathogens *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. The inhibitory effect was attributed to carissone, dehydrocarissone and carindone (Lindsay et al. 2000). The ethanolic extract of *C. congesta* also exerted antibacterial action against *Bacillus subtillis, Staphylococcus aureus, Streptococcus faecalis, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhimurium* (Devmurari et al. 2009). Bibi et al. (2011) reported moderate activity of chloroform and ethyl acetate fractions of *C. opaca* against *Bacillus subtilis.* It was assumed that the plant extract rich in hydroxyacetophenone is responsible for the antibacterial activity. Singh and Sangwan (2011) evaluated the antimicrobial potential of the methanolic extract of *C. carandas* against bacterial cultures of *Bacillus macerans*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and obtained positive results. Saklani et al. (2011) reported the inhibitory activity of the ethanolic extract of *C. opaca* fruits against *Streptococcus pyogenes*, *Streptococcus aureus* and *Bacillus subtilis*. The phytochemical 2'-hydroxyacetophenone and carinol isolated from *C. lanceolata* roots were found to possess significant antibacterial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* (Hettiarrachchi et al. 2011). Israr et al. 2012 tested the ethanolic extracts of several medicinal plants of folkloric importance, including *C. carandas* against a range of pathogenic Gram positive and Gram negative bacteria.

Tolo et al. (2006) reported the *in vitro* as well as *in vivo* antiviral potential of aqueous extract of the *C. edulis* roots. The extract demonstrated inhibition of plaque formation in Vero E6 cells infected with wild type herpes simplex virus (HSV). The mortality rate of the extract-treated infected mice was significantly reduced by 70–90 % as compared to the 100 % mortality in the untreated group. Further,

the therapeutic dose of 250 mg/kg did not induce any toxicity. Festus et al. (2009) also evaluated the anti-HSV potency of *C. edulis*. A triterpenoid lupeol was identified as the component stimulating the antiviral action. At a concentration 10 µg/ml the compound reduced the viral yields in Vero E6 cells by 98.3 %. On oral administration to infected mice at a dose 20 µg/ml, a delayed onset of infections and slow progression of the characteristic lesions were observed. Moodley et al. (2011) isolated four immunoactive pentacyclic oleanane triterpenes from the fruits of *C. macrocarpa* which held promise to combat HIV and hepatitis virus in South Africa. Nagata et al. (2011) interviewed herbal practitioners in Mfangano Island, Kenya and learned the reliance of HIV/AIDS patients on *C. edulis*. Wangteeraprasert et al. (2012) evaluated anti-HSV potency of *C. spinarum* stem extract and obtained affirmative result. The cardiac glycoside evomonoside in the extract exerted moderate activity against HSV.

The ethanolic extract of *C. congesta* displayed considerable anticandidal action (Devmurari et al. 2009). Siddiqi et al. (2011) also demonstrated that the crude methanolic extract of *C. carandas* substantially inhibits certain fungal species.

Kebenei et al. (2011) evaluated the *in vitro* activity of root bark methanolic extract of *C. edulis* against *Plasmodium falciparum*. The extract inhibited the chloroquin-sensitive strains of the pathogenic protozoa. A lignan component nortrachelogenin was held responsible for the anti-plasmodium activity. Development of Carissa-based, anti-malaria drugs could be anticipated in the future.

4.3.6 Antidiabetic, Antilipidemic, Hypotensive and Antileptic Activities

The antidiabetic activity of *C. carandas* unripe fruit methanolic extract was evaluated against alloxan-induced diabetic rats. The polyphenolic, flavonoid and flavanone contents of the extract were determined and correlated to the antidiabetic effect. The ethyl acetate fraction of the extract significantly lowered the elevated blood glucose levels by 48–64.5 % at a dose level of 400 mg/kg (Itankar et al. 2011).

Sumbul and Ahmed (2012) examined the lipid lowering potential of an ethanol extract of *C. carandas* in egg yolk- induced hyperlipidemic rats. The extract significantly reduced the body weights, cholesterol and triglycerides levels in the tested rats. Histopathological alterations induced by high cholesterol diet were also significantly subdued by the extract. The activity of the extract could be compared to that of atorvastatin (a common cholesterol-lowering medicine).

Shamim and Ahmad (2012) evaluated the effect of *C. carandas* ethanol extract on cardiovascular function of normal rats. Intravenous bolus injection of this extract at 5 to 45 mg/kg caused dose-dependent reduction in arterial blood pressure. Significant reduction in heart rate frequency following the injection was recorded, with efficacy comparable to acetylcholine. The hypotensive effect was correlated to the stimulation of the muscarinic receptors on the endothelial cells of the vasculature,

which resulted in the release of relaxing factors or nitric oxide inducing further relaxation.

The anticonvulsant activity of the root bark extract of *C. edulis* was investigated in pentylenetetrazole -induced convulsion in mice and maximal electroshock test in chicks. *C. edulis* offered 40 and 20% protection against convulsion at 5 and 20 mg/kg, respectively, compared to the 100% protection imparted by standard drug benzodiazepine. These results suggest that *C. edulis* possesses biologically active constituents that confers anticonvulsant activity and might be useful in epilepsy management (Ya'u et al. 2008). The ethanolic extract of *C. congesta* also showed powerful anticonvulsant action on seizures (Devmurari et al. 2009).

4.3.7 Anticancer Activity

The stem extract of *C. spinarum* was evaluated for its possible anticancer potential. The n-butanol fraction of aqueous extract was assessed for its cytotoxic and pro-apoptotic activity which inhibited cell proliferation of various human cancer cell lines including leukaemia HL-60 cells (Sehar et al. 2011). Wangteeraprasert et al. (2012) reported that *C. spinarum* stem extract containing lignans (−)-carinol, (−)-carissanol and (−)-nortrachelogenin exhibits cytotoxicity against breast MCF7 and lung A549 cancer cells, most likely by promoting antioxidant content.

4.3.8 Diuretic, Nephroprotective and Hepatoprotective Effect

The diuretic activity of the different extracts of *C. edulis* in a dose range of 50–1000 mg/kg was assessed orally in rats. The Soxhlet extract of root bark increased the urine output significantly at the highest dose. The Urinary electrolyte excretion was also affected by the extracts, as sodium, potassium and chloride ions were detected in urine. These findings support the traditional use of *C. edulis* as a diuretic agent (Nedi et al. 2004).

Sahreen et al. (2011a, b) evaluated the possible nephroprotective activity of the methanolic extract of *C. opaca* fruits and its *n*-hexane, ethyl acetate, chloroform, butanol and aqueous fractions against CCl_4-induced nephrotoxicity. Various fractions of the extracts (200 mg/kg) could reverse the damages *viz*. depletion of antioxidant enzymes, alarming levels of H_2O_2, DNA injuries and histopathological lesions. The fractions with higher flavonoids offered better proection towards kidneys against the chemical abuse.

Hegde and Joshi (2009) investigated the hepatoprotective effect of *C. carandas* root extract against CCl_4 and paracetamol-induced hepatic oxidative stress. Therapeutic action mediated by declined activities of serum marker enzymes, bilirubin and lipid peroxidation, and concomitant increase in uric acid, GSH-Px, SOD, CAT and protein level came forth. Abbasi et al. (2009) reported that the aqueous extract of *C. opaca* leaves taken orally twice a day for few weeks can sooth hepatitis.

Sahreen et al. (2011a, b) investigated the possible hepatoprotective effect of *C. opaca* leaf extract on CCl_4-induced liver damage in rats. The methanol extract offered significant protection against the hepatic assault, owing to its flavonoid, tannin, alkaloid, terpenoids, coumarins, anthraquinones and cardiac glycosides.

4.3.9 Management of Hormonal Dysfunction

Hormonal dysfunction has undesirable outcomes and must be balanced before it triggers further threats. In this regard, Sahreen et al. (2013) assessed the protective effects of methanolic extract of *C. opaca* leaves on CCl_4-induced reproductive stress in male rats. The chemical assault led to alteration of antioxidant enzymes, DNA fragmentation and lipid peroxidation caused testicular fibrosis. The alteration in testis anatomy hampered the secretion of reproductive hormones. The co-administration of 100–200 mg/kg extract effectively ameliorated the detrimental effects of the biochemical markers; hormonal and molecular levels. The protective effects of the extract was attributed to the bioactive compound present in the extract *viz*. isoquercitin, hyperoside, vitexin, myricetin and kaempherol.

Future Scope Saklani et al. (2011) recommend the consumption of *C. opaca* fruits for promotion of health. Sumbul and Ahmed (2012) advocate the efficacy of *C. carandas* leaf extracts in ameliorating cardiovascular disorders. Ya'u et al. (2013) evaluated the safety of oral administration of *C. edulis* root bark ethanol extract for 28 days in rats. Short-term administration of the standardized ethanol extract at doses lower than 1000 mg/kg had no toxicity. It did not affect organ weight or hormone profile. The myriad health benefits coupled with non-toxicity suggests wider utilization of Carissa genus. The plausibility of Carissa-based food processing should be explored. Several other members of the family Apocyanaceae viz. Wrightia tinctoria, Picralima nitida, Aspidosperma tomentosum, Strophanthus hispidus, Apocynum venetum, to name a few, have been certified to possess desirable biological activities. Taking cue from these studies, the mining of bioactives from Carissa genus ought to be hastened for providing primary healthcare at nominal cost.

4.4 Conclusion

Carissa genus has been a major source of food and medication in South Africa, Kenya, India, Pakistan and Bangladesh, providing the poor population with food and medication. Due to the lack of updated, organized literature and deficient research on this genus, people are unacquainted about its versatility. Food and pharmaceuticals extraction from Carissa genus must gain momentum, in order to provide cheap sources of sustenance and drug to underprivileged population. Bioactive profiling of hitherto unfamiliar Carissa species and industrial food formulation scopes should

be emphasized. This chapter is expected to play decisive role in promotion of this obscure genus for nutraceutical purposes.

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Chapter 5
Portulaca oleracea: An Untapped Bioactive Repository for Health Amelioration

Abstract *Portulaca oleracea* (puslane) belonging to family Portulacaceae is often vilified as an agricultural weed. However, foraging and impetus on food innovation has highlighted its nutritional aspects. It has been recognized as an excellent source of bioactive phytochemicals, ranging from alkaloids, ω-3 fatty acids, coumarins, flavonoids, cardiac glycosides, anthraquinone, protein, α-linolenic acid and β-carotene, mono terpene glycoside, N-trans-feruloyltyramine to vitamin C. So far, antioxidant, anti-inflammatory, antiatherogenic, antidiabetic, anticancer, hepatoprotective, gynaecological, neuroprotective and antiviral properties have been validated. This chapter is compiled with recently published papers of high relevance, with emphasis on mechanisms of action. Given due research attention, it can be developed into an wholesome food source and effective CAM for many ailments afflicting mankind.

Keywords *Portulaca oleracea* · Antioxidant · Antiinflammation · Antidiabetic · Anticancer

5.1 Introduction

Portulaca oleracea, or commonly known as purslane, little hogweed, miner's lettuce or moss rose, is a succulent plant belonging to family Portulacaceae. This tiny, succulent plant has reddish prostate stems and alternate leaves (Fig. 5.1a). The flowers bloom in profusion in sunny weather and fade by the noon (Fig. 5.1b). On maturity, the capsules dehisce, releasing the seeds (Fig. 5.1c). This cosmopolitan plant is often regarded as an invasive weed in fields and gardens. On the other hand, this drought-tolerant plant is increasingly being deemed suitable for detoxification, bioremediation and retention of soil moisture as groundcover. Also, it has been consumed in fried, boiled, salad, soup, pickle and pasta sauce form, since ages. Apart from the environmental restorative roles and culinary uses, several therapeutic prospects have been reported. Many ethnic uses as herbal treatments have come forth. Several native communities feed on it to treat arthritis, anemia, vitamin C deficiency and fever. In folk medicine, the crushed juice is used to relieve all kinds of inflammations of eyes, gum and gout. Quinlan et al. (2002) reported its use in treatment of intestinal worms.

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Fig. 5.1 **a** Whole plant of *P. oleracea* L. **b** Flowers. **c** Seed pod

Cakilcioglu and Turkoglu (2010) reviewed the medicinal plants from Anatolia Region of Turkey, including *P. oleracea* L. Laitiff et al. (2010) published a review on the diabetic wound healing attributes of *P. oleracea*. In traditional Chinese medication, it is used to detoxify body and to arrest bleeding. In Western culture, this plant has found use in dysentery; boils and sores; eczema; hemorrhoidal bleeding; and abnormal uterine bleeding treatment. For its versatile remedial uses, it's loosely called 'global panacea'. The World Health Organization (WHO) has accorded it medicinal plant status. This chapter endevours to present the nutraceutical aspects of this much-reviled weed.

5.2 Phytochemical Profile

P. oleracea contains many compounds, including alkaloids (oleracein), polysaccharides, ω-3 fatty acids, vitamins (A, C and B complex), minerals (magnesium, calcium, potassium, iron, zinc). Elkhayat et al. (2008) conducted chromatographic fractionation of the chloroform extract of *P. oleracea* L. and reported the presence of diterpene portulene, lupeol, β-sitosterol and daucosterol. Dong et al. (2010) isolated three polysaccharides including a neutral, an acidic and a pectic polysaccharide from the aerial part of *P. oleracea* L. Naeem and Khan (2013) reported the presence of steroids and saponins, among other nutrients. Ora and Anekwe (2013) analyzed the lipids of *P. oleracea*, chief components of which are quanitified to be linoleic acid (66.7%) and triolene (26.7%).

5.3 Nutritional and Therapeutic Spectrum

The biological roles of *P. oleracia* have ramified into antioxidant, anti-inflammatory, antiatherogenic, antidiabetic, anticancer, hepatoprotective, gynaecological, neuroprotective and antiviral. The major findings reported in the recent times have been discussed below.

5.3.1 As Antioxidant and Mineral Source

P. oleracia as an antioxidant cache is much appreciated. Aberoumand (2009) examined some vegetables sourced from South Iran and South India. Among them, *P. oleracia* stood out in terms of nutritive value. Its plant dry weight contained 2 % mineral, which is higher than that of conventional vegetables. High levels of heat-stable, antioxidant compounds were noticed in *P. oleracia*, which proved suitable for high-temperature food processes. Yang et al. (2009) determined the antioxidant activities of three phenolic alkaloids, *i.e.* oleracein A, B and E isolated from this plant. The DPPH radical scavenging activities of these alkaloids were lower than caffeic acid but higher than ascorbic acid and α-tocopherol. Oleracein E was most potent in preventing formation of MDA, the marker of oxidative stress. Dkhil et al. (2011) also determined the antioxidant activity of *P. oleracea* by measuring the GSH-Px, SOD and CAT, as well as the inhibition in lipid peroxidation and nitric oxide formation in liver, kidney and testis of rats. The extract-administered rats showed marked improvement in all the studied parameters. The extract may be used for the prevention of cardiovascular, neurodegenerative and other chronic diseases triggered by oxidative stress. Karimi et al. (2011) evaluated the cytoprotective effect of its ethanolic and aqueous extracts against hemolytic damages, induced by free radical initiator, 2, 2′-azobis(2-amidinopropane) dihydrochloride (AAPH). AAPH-preincubated RBCs were exposed to the extract and concentration-dependent cytoprotective effect was observed. Singh et al. (2011) analyzed the nutritional profile of ten preferred traditional vegetables from Andaman and Nicobar Islands, India. Maximum ash, sodium and iron content were recorded in *P. oleracea*. Also the antioxidant activity was highest in it, suggesting its integration in mainstream vegetable consumption. Behravan et al. (2011) evaluated the protective effects of *P. oleracea* ethanolic and aqueous extracts on human lymphocyte DNA lesions. The lymphocytes were incubated in H_2O_2, water and ethnaolic extract. Alkaline single cell gel electrophoresis approach assay showed that the aqueous extract significantly inhibits DNA damage owing to the antioxidants. Handique et al. (2012) evaluated the antioxidant activities of the n-hexane, ethyl acetate and methanol extracts of three indigenous leafy vegetables of north east India, including *P. oleracea*. A strong correlation between the antioxidant activities and the total phenolic content was observed. Uddin et al. (2012) observed that the concentrations of Ca, Mg, K, Fe and Zn increased with plant maturity. Anastacio and Carvalho (2012) evaluated the effect of salinity on total amounts of fatty acids and omega-3/omega-6 fatty acids ratio in leaves of *P. oleracea* and reported no adverse effect of the fatty acid profile. Chen et al. (2012) evaluated the oxidative injury inhibition effect of *P. oleracea* aqueous extract on high-fat-diet-induced mice. The extract dose-dependently reduced the levels of blood and liver lipid peroxidation and increased the activities of antioxidant enzyme activities. Also, increase in liver leptin/β-actin and PPARα/β-actin, while decrease in liver, spleen FAS mRNA, p-PERK and p-PERK/PERK protein expression levels, in concentration-dependent manner was observed.

5.3.2 Anti-Inflammatory Activity

Persuasive evidences on *P. oleracea* antiinflammatory effects exist. Agha-Hosseini et al. (2010) evaluated its efficacy in the treatment of oral lichen planus (an auto-immune disease causing inflammation of oral mucosa). A randomized double-blind, placebo-controlled study was conducted, which showed partial to complete clinical improvement of the lesions in 83 % of *P. oleracea*-treated patients. Huang and Dong (2011) evaluated the protective effect of *P. oleracea* on the acute injury to rats caused by intra-colonic administration of trinitrobenzenesulfonic acid (TNBS). When treated with 5 and 10 g/kg of the plant extract daily for 10 days, the rats showed better food intake and reduced diarrhea. It exerts protective effect in experimental colitis by relieving inflammatory reactions and repairing the lesions. Jagan Rao et al. (2012) investigated the antinociceptive and the antiinflammatory activities of the petroleum-ether extract of *P. oleracea* in mice. The extract proved non-toxic upto a maximum dose of 2000 mg/kg body weight and exhibited significant inhibition of the acetic acid induced writhing, reduced the paw-licking response time in the formalin test and increased the withdrawal latency time in the tail immersion test.

5.3.3 Anti-atherosclerotic Activity

Atherosclerosis remains one of the leading causes of death all over the world. Barakat and Mahmoud (2011) examined the thearpeutic efficiency of *P. oleracea*/pumpkin seed mixture on hyperlipidemia, kidney function and as immunomodulators in high cholesterol diet-fed rats. The mixtures exerted all the above effects, mediated by unsaturated fatty acids (including α-linolenic acid). Lee et al. (2012c) reported that *P. oleracea* extract dose-dependently reduces an increase of the adhesion of HL-60 cells to TNF-α-induced HUVEC. The extract inhibits the translocation of p65 NF-κB to the nucleus. Also, the extract suppresses the TNF-α-induced degradation of IκB-α and NF-κB binding. The extract also effectively reduces TNF-α-induced mRNA expressions of monocyte chemoattractant protein (MCP)-1 and interleukin (IL)-8 in a dose-dependent manner, building hope for atherosclerosis therapy. Changizi-Ashtiyani et al. (2013) evaluated the effects of oral administration of alcoholic extract of *P. oleracea* on blood fat profiles of rats. Continued feeding for 21 days led to the drop in cholesterol and triglyceride concentrations. The antioxidants and ω-3 abundance in the plant and cholesterol synthesis inhibition was accredited for the biological modulation.

5.3.4 Antidiabetic Activity

P. oleracea has exerted notable hypoglycaemic effect and proved its potential in adjuvant therapy. El-Sayed (2011) investigated the antidiabetic activity of its seeds on type-2 diabetic subjects. When administered with 5 g of seeds twice daily,

significant decline in serum levels of triglycerides, total cholesterol, low density lipoprotein cholesterol, liver alanine-, aspartate- and gamma glutamyl transaminase, total and direct bilirubin, fasting and postprandial blood glucose, insulin was reported. The beneficial effects were attributed to the polyunsaturated fatty acids, flavonoids and polysaccharides content. Lee et al. (2012b) investigated the protective effect of the aqueous extract of *P. oleracea* L. on diabetic vascular complications in mice. When treated with the extract (300 mg/kg) daily for 10 weeks, significant lowering of blood glucose, plasma triglyceride, plasma level of LDL-cholesterol and systolic blood pressure was recorded. Further, it increased the plasma level of HDL-cholesterol and insulin level. The insulin immunoreactivity of the pancreatic islets remarkably increased as a result of the treatment. The extract prevented the development of diabetic endothelial dysfunction. Diabetic nephropathy is a fatal side effect of diabetes, leading to end-stage renal disease. Lee et al. (2012a) investigated the protective effect of the aqueous extract of *P. oleracea* on diabetic nephropathy accelerated by renal fibrosis and inflammation in type 2 diabetic mice. The extract treatment at a dose 300 mg/kg daily for 10 weeks, markedly lowered blood glucose and plasma creatinine level, decreased water intake and urine volume. The extract treatment significantly reduced the expressions of transforming growth factor-β1 (TGF-β1), advanced glycation end products (AGE), and intercellular adhesion molecule (ICAM)-1. NF-κB p65 activation in renal tissues was suppressed. Ahmed et al. (2013) investigated the antihyperglycemic effect of a traditional unani formulation 'Qurs Tabasheer', of which *P. oleracea* seed is a constituent. Streptozotocin-induced rats when administered with the herbal blend for 28 days reduced the level of serum glucose, total cholesterol, triglycerides and glucose-6-phosphatase while increasing fructose-1-6-biphosphatase.

5.3.5 Anticancer Activity

Anticancer potential of the extracts of *P. oleracea* are increasingly being validated. Chen et al. (2010) isolated a water-soluble polysaccharide from *P. oleracea* and subjected them to modification by chlorosulfonic acid method. The sulfated derivatives significantly inhibited the growth of HepG2 cells and Hela cells *in vitro*. Flow cytometric studies revealed that the modified polysaccharides arrest the cell-cycle in S phase. Yan et al. (2012) isolated four portulacanones which selectively exerted *in vitro* cytotoxic activities towards four human cancer cell lines. Especially, 2, 2'-dihydroxy-4', 6'-dimethoxychalcone showed potent cytotoxicity towards human gastric adenocarcinoma SGC-7901 cells. Shen et al. (2013) purified a polysaccharide from *P. oleracea* and evaluated its antitumor activity. It significantly inhibited the growth of transplantable sarcoma 180 and augmented the animal's immune responses, manifested in increased white blood cell and CD4(+) T-lymphocytes. Further, the serum aspartate transanimase (AST), alanine transaminase (ALT), urea nitrogen and creatinine levels in S180-bearing mice were significantly reversed. Zakaria and Hazha (2013) evaluated the cytotoxicity of aqueous and ethanol crude extracts of its leaves on murine mammary adenocarcinoma (AMN3) and human

rhabdomyosarcoma (RD) cells. Both the cell lines showed sensitivity towards the extracts, at a time-dependent manner. Also, it is revealed that the reduction in mitotic index in both cancer cell lines (AMN3 and RD) were highest at 72 h of exposure.

5.3.6 Heptaoprotective Activity

Several liver protective role of *P. oleracea* has come forth. The co-treatment of CCl_4-hepatic injured rats with 70% alcohol extract restored the hepatic marker enzymes and total bilirubin to normalcy (Elkhayat et al. 2008). Hepatic fibrosis is a common pathological process of chronic liver injury caused by oxidative stress and inflammation. Ali et al. (2011) examined the prophylactic and curative effects of oral administration of hydro-ethanolic extract of *P. oleracea* on bile duct ligation-induced liver fibrosis in rats. The administration reversed all the oxidative changes in biochemical parameters. Anusha et al. (2011) investigated the hepatoprotective activity of the aqueous extract of the plant aerial parts in combination with lycopene against CCl_4- induced toxicity in rats. Oral administration for 14 days normalized the liver function marker enzymes in the serum *i.e*. aspartate transaminases (AST), alanine transaminases (ALT), alkaline phosphatase, total bilirubin, total protein and total cholesterol.

5.3.7 Gastroprotective Activity

Zhao et al. (2013) investigated the effects of *P. oleracea* extracts on growth performance and microbial populations in the ceca of broilers. On day 28 and 42, the cecal contents were collected and assayed for microbial populations. E. coli number had dwindled, whereas the population of Lactobacillus and Bifidobacterium had increased. Cecal bacterial community modulation capacity of *P. oleracea* could be exploited.

5.3.8 Neuroprotective Activity

P. oleracea extract have exhibited considerable neuroprotective effect. Wang et al. (2007) investigated whether it possesses hypoxic neuroprotective effects by administering the extract to mice grown in low oxygen environment. Also, PC-12 cells and primarily cultured nerve cells were used for MTT assay. The extract enhanced the erythropoietin mRNA and protein expression in the mouse cortices. Histological analysis indicated that the extracts lessened the inflammation damage of the mouse brain. MTT assay results showed the extracts raised the viability of the tested cells under the tested hypoxic conditions and decreased the degree of LDH in the culture medium in a dose-dependent manner. Hongxing et al. (2007) assessed neuroprotective effects of *P. oleracea* herb aqueous extracts at doses of 2.5, 5 and 10 mg/kg

daily on mice injected with D-galactose. The extract fed-mice showed higher activity on being stimulated, lower anxiety and higher novelty-seeking behavior in the open field tasks, and significantly improved learning and memory ability in step-through. The extract significantly increased SOD activity, while decreasing MDA level. The neuroprotective effect of the extract was assumed to be carried out through a p21(waf1) (the cyclin-dependent kinase inhibitor 1)-dependent pathway. Wang and Yang (2010) assessed the protective effect of betacyanins from *P. oleracea* L. against the D-galactose -induced neurotoxicity in mice. The pigments markedly reversed the learning and memory impairments as measured by behavioral tests. The activities of SOD, CAT, GSH-Px were enhanced, while the MDA content was decreased following betacyanin administration. Wanyin et al. (2012) investigated whether *P. oleracea* ethanolic extract exerts its neuroprotective effects against hypoxia injury through regulation of endogenous erythropoietin expression. Results showed that the extract decreases the serum neuron specific enolase level in hypoxia mice and the activity of caspase-3 in neuron; also increases the neuron viability and attenuates the pathological damages caused by the hypoxia condition. The neuroprotective effect of the extract against hypoxia injury was mediated through the stimulation of endogenous erythropoietin expression by stabilization of HIF-1α. Abdel Moneim (2013) assessed its neuroprotective effects on rotenone-induced biochemical changes and apoptosis in striatum of rats. The ameliorative effect on neurons builds hope for development of *P. oleracea* extract-based prophylactic against brain damage and Parkinson's disease.

5.3.9 Management of Gynaecological Problems

This plant has been regarded effective in addressing gynaecological complications. *P. oleracea* is used in Iranian folk medicine to treat abnormal uterine bleeding. Shobeiri et al. (2009) included ten premenopausal women with menorrhagia, metrorrhagia, polymenorrhea and intermenstrual bleeding who had not responded to standard drugs and were candidates for hysterectomy. After 48h of menstrual onset, the subjects were given 5 g of *P. oleracea* seeds powder in a glass of water every 4 h for 3 days. 80% patients reported that the duration and volume of bleeding had reduced and their patterns of periods had normalized post drinking. No adverse effects were reported and the uteraine bleeding did not recur in the patients responding to treatment for a 3 month follow-up.

5.3.10 Antimicrobial Activity

Elkhayat et al. (2008) reported antibacterial and antifungal activities of *P. oleracea* extract. Bakkiyaraj and Pandiyaraj (2011) reported strong inhibitory potential of *P. oleracea* methanol extract against *Bacillus subtilis*, *Staphylococcus aureus* as well as *Pseudomonas aeruginosa*. The bioactive compounds coumarins,

flavonoids and saponins were accredited to confer the antibacterial effect. Londonkar and Nayaka (2012) isolated total flavanoids and investigated their antibacterial activity against a panel of pathogenic bacteria. Strong inhibition was exerted towards *Salmonella typhimurium* and *Proteus mirabilis*. Dong et al. (2010) showed that *P. oleracea* pectic polysaccharide had anti-herpes simplex virus type 2 (anti-HSV-2) activity. It was was elucidated to check the virus penetration into host cells. Potent components might be extracted from *P. oleracea* for developing antimicrobial agents.

5.4 Future Prospect

Foraging for ensuring sustainable future is a revolution brewing in several parts of the world. *P. oleracea* certainly is an important ingredient in this regard, owing to its world-wide distribution and nutritional opulence. U.S. Department of Agriculture (USDA) is now paying attention to this under-appreciated plant for food uses. Its incluison in the diet can afford plentiful nutrition at nominal cost. Old references support the antiulcerogenic, wound healing, muscle relaxant and bronchodilatory effects which have not been validated in recent times. These could be thrust area of research. Literature search did not furnish any substantial information on the toxicity of *P. oleracea.* However, the plant does contain cardiac glycosides and oxalic acids which might be toxic at excess doses. Research in this direction could repel these ambiguities.

5.5 Conclusion

As the findings testify, *P. oleracea* is a storehouse of bioactive components. Time is ripe for waking up to the biological virtues of this herb. With adequate interest from the food industry, it can be developed as a healthful vegetable. Also, complementary and alternative medicine (CAM) may be formulated.

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Chapter 6
Grape Seeds: Agro-Industrial Waste with Vast Functional Food Potential

Abstract Grapes (*Vitis vinifera*) are universally appreciated fruits for their delicacy and nutrition. All the more fame stems from their fermentation products wines. The by-product pomace comprising of skin and seeds often ends up as wastes. Lately, the seeds have been discovered to contain an ample amount of diverse bioactive compounds. Polyphenols are found in abundance in the seeds, proanthocyanidin being the most prominent class. The seed extracts are increasingly being recognized as human food supplement for disease prevention and overall health promotion. Their uses have ramified to antioxidant, anti-inflammatory, antilipemic, antihypertensive, hepatoprotective, osteoprotectant, antidiabetic, neuroprotective, anticancer and antimicrobial agents. Now that nutrient recovery from food by-products and residues as well as fortification has emerged as thrust areas, the relevance of grape seed extract deserves exploration. This chapter presents a complete account of the current state of knowledge and future directions.

Keywords Grape seed extract · Proanthocyanidin · Anticancer · Hepatoprotective · Functional food

6.1 Introduction

Grapes (*Vitis vinifera*) as palatable fruits and the substrate of much-loved wine are world-renowned. Consumption of the whole fruits and their derivatives exert many health-promoting properties, apart from their delicacy. It's fermentation processes generate enormous waste which ends up in land-fill. The grape seed extract, a source of great nutraceutical and pharmaceutical value is poorly explored. It is now coming forth that the seeds are excellent source of Vitamin E (tocopherol), flavonoids, fatty acids (linoleic acid) and oligomeric proanthocyanidin complexes (OPCs) (Liu and White 2012). Figure 6.1a, b show grape seeds and their chief component OPC. Grape seed oil is used for culinary practices and the seed flour has begun its foray into baking. Its biological role against cancer, blood pressure, Alzheimer, diabetic retinopathy and vascular fragility has been discovered in recent times. It has been validated to prevent the growth of breast, stomach, colon, prostate and lung cancer.

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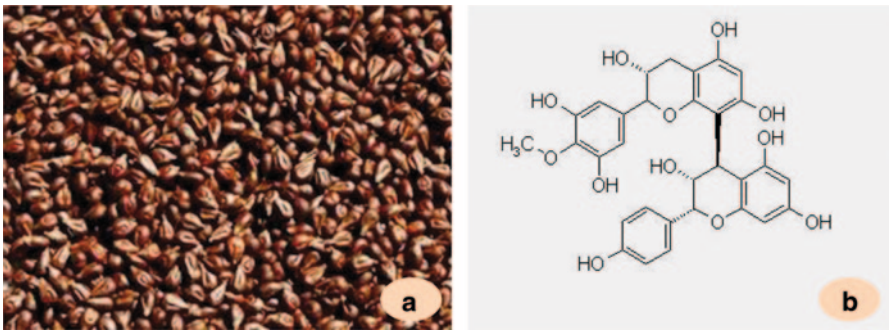

Fig. 6.1 **a** Grape seeds. **b** Oligomeric proanthocyanidin complexes (*OPCs*)

Also, the cancer side effects as liver damage and oedema have been ameliorated. The seed oil is also valued as a skin emollient and cosmetic.

Now, grape seed is available as a dietary supplement in capsules, tablets and liquid extract forms in health stores. Swanson ultra MegaNatural BP, Now, Nature plus, Puritan's pride are some of the popular brands of grape seed extracts. This review furnishes a latest account of the key findings on the nutritional value of grape seed derivatives and how they can revolutionize the functional food sector and soothe debilitating ailments.

6.2 Nutritional and Therapeutic Applications

In recent times, grape seed derivatives have made a niche for them in food processing and pharmaceutical sector. The section underneath summarize the key developments in this regard.

6.2.1 Food Applications

The grape seed oil and flour has found many nutritional uses. Food and Drug Administration (FDA) has approved its addition in various food products considering its GRAS status. The flour is being used in patties, and breads for their antioxidant abundance and color. The heat stability of grape seed flour has been evaluated for its possible use in baking. Thermal treatment (above 180 °C) of the Merlot grape seed flour caused significant decreases in the total phenolic content (TPC), antioxidant power, and specific polyphenolic compounds (catechin and epicatechin). The feasibility of using the flour at lesser temperature for optimal retention of the antioxidants could be assessed. Policosanols are aliphatic alcohols with an efficacy to reduce the LDL-cholesterol level in humans (Ross et al. 2011). A gas chromatography-tandem mass spectrometry (GC-MS/MS) with a low limit of detection quan-

tified 245.15 mg policosanols/kg in the seed oil. Hexacosanol was found to be the most abundant policosanol, followed by octacosanol, tetracosanol and triacontanol (Jung et al. 2011). The consumer acceptance and physical properties of bread, including total phenolic content prepared with varying levels of grape seed flour was evaluated. Dough and breads were prepared using different levels of replacement of hard red spring wheat flour with the grape seed flour (0–10% substitution) and stored for 0, 2, or 6 wk at −20 °C. Replacement of 10% wheat flour increased the bread total phenol content (TPC) from 0.064 to 4.25 mg tannic acid/g dry weight. The replacement of 5% flour is recommended for the production of fortified breads with acceptable physical and sensory properties and high TPC compared to refined bread (Hoye and Ross 2011). Grape pomace extracts were incorporated into beef patties at various concentrations to test their antimicrobial effects during different storage periods. The numbers of microorganisms were decreased by the 10% extract concentration during the 48h storage period. The food-borne pathogens including Enterobacteriaceae and coliform bacteria, and the spoilage microorganisms including yeasts, molds and lipolytic bacteria were inhibited (Sagdic et al. 2011). The effectiveness of grape seed extract compared to butylated hydroxytoluene (BHT) in improving the oxidative stability of sunflower oil during thermal applications was evaluated. After convection and microwave heating up to 240 min under simulated frying conditions, the progress of lipid oxidation was assessed through a battery of assays. The extract showed a significant inhibitory effect on lipid break down. A 600–800 ppm extract inhibited the lipid oxidation as strongly as BHT (Poiana 2012). The effect of grape seed extract and *Cistrus ladanifer* (rockrose)-based diets on lamb carcass was investigated. During a 7 days storage period, the additive protected the meat against lipid oxidation without changing its color or sensory properties (Jeronimo et al. 2012). The effect of marinades (containing grape seed extract, formulated in a water/oil emulsion) on the formation of heterocyclic amines in fried beef patties was evaluated. After application of the fortified-marinades and frying, four heterocyclic amines were found at significantly low levels in all fried patties. The antioxidant content in the extract was credited for the detoxifying properties. Further, it improved the acceptance of flavor and color (Gibis and Weiss 2012). It was determined whether the inclusion of grape seed can enhance the functionality of bread. The dough stability increased to almost double with the increase of grape seed content from 0 to 7.5%. The antioxidant activities of the bread in terms of gallic acid and catechin content increased significantly in direct proportion to seed substitution (Meral and Dogan 2012). It was reported that the seed extracts could be used as antioxidants in meat and poultry products (Karre et al. 2013). The effect of seed extract and tea polyphenols combined with chitosan was evaluated as a preservative for red drum fish fillets, during refrigerated storage. When kept at 4 °C for 20 days, the additives extended shelf life by 6–8 days compared to the control (Li et al. 2013). The effect of fortifying cookies with grape seed extract and its impact on consumer acceptance was determined. Addition of the extract resulted in brown cookies. Microencapsulation of the extract led to partial masking of the darker color of the cookies. Also, the cookies with microencapsulated extract showed a significant higher antioxidant activity, which was related to the lower heat degradation. The enriched cookies were found to be more astringent, with aromas and flavors

similar to whole grains flours. Nearly 60% of consumers showed interest to purchase cookies with enhanced antioxidants (Davidov-Pardo et al. 2012).

6.2.2 Antioxidant Potential

Antioxidants are antagonists of free radicals and hence a safeguard against a host of degenerative and fatal diseases. Contrast-induced nephropathy is a common cause of hospital-acquired acute renal failure. The effect of grape seed proanthocyanidin extract on thwarting the ailment was determined in rat models. After 9 days of supplementation, the blood sample was analyzed for the measurement of renal function parameters. Renal histopathology and transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) was performed to determine oxidative changes and apoptosis, respectively. The adverse effects *viz*. blood urea nitrogen, creatinine, malondialdehyde (MDA) levels, apoptotic index and histopathological alteration were significantly decreased in the group administered with proanthocyanidin (Ozkan et al. 2012). Hepatic ischemia and reperfusion injury cause major complications during liver surgery and transplantation. Ischemic pre-conditioning and post-conditioning are expected to ward off the harmful effects. The outcome of proanthocyanidin conditioning, individually or along with other technical aids was examined on ROS generation, pro-inflammatory cytokines release and hypoxia responses. The proanthocyanidin alone could reduce liver ischemia/reperfusion injury more effectively by increasing the activity of ROS scavengers and antioxidants. Also, it increased the liver HIF-1 alpha protein level (Song et al. 2012a). The hepatoprotective effect of the HV-P411 complex, a mixture of grape seed, *Schisandra chinensis* and *Taraxacum officinale* was examined against D-galactosamine -induced hepatitis. Elevation in serum aminotransferase activity and lipid peroxidation, and decline in hepatic glutathione content were reversed after 24h of treatment. The complex attenuated the increases in serum TNF-α, IL-6, COX-2 protein production and their mRNA expressions. Further, serum IL-10 and HO-1 protein production along with their mRNA expressions were augmented. The increased translocation of nuclear factor-κB and c-Jun phosphorylation were attenuated by treatment with the complex (Kang et al. 2012). It was reported that catechin-rich grape seed extract modulates gene expression and leads to significant reduction of oxidative stress in obese subjects. Fibrogenic cytokines activate hepatic stellate cells that lead to excess collagen formation and resultant liver fibrosis (De Groote et al. 2012). It was reported that the administration of grape seed proanthocyanidin extract (100 mg/kg) markedly suppresses the lipid peroxidation and prevents the development of liver fibrosis induced by chronic thioacetamide administration in mice. The suppression of mRNA expression of transforming growth factor β1 TGF-β1 and α-smooth muscle actin α-SMA, with decreased collagen accumulation was discovered to be the underlying mechanism (Li et al. 2012a). The inhibitory effect of intragastrically-administered grape seed proanthocyanidin extract (200 mg/kg, once daily for 14 days) on selenite-induced cataract formation was investigated in rats. The administration of the extract could dose-dependently preserve the activities of these antioxidative en-

zymes (SOD, CAT, GSH-PX) accompanied by significant reductions in the levels of MDA, NO, Ca(2+) as well as iNOS, and calpainII protein and mRNA expression. In the extract-treated group, the degree of lens opacity and the diameter of nuclear cataract plaques were significantly reduced owing to the normalization of above parameters (Zhang and Hu 2012). The antioxidant effect of grape seed proanthocyanidins was assessed in mice. The treatment with the flavanols for 4 weeks significantly ameliorated oxidative stress by increasing the SOD activities and decreasing MDA formation. It alleviated the elevation of heart/body weight ratio, kidney/body weight ratio and cross-sectional area of cardiomyocytes, decreased collagen deposition in heart and attenuated histopathology injury. Also, it improved the endothelial-dependent aorta ring relaxation by increasing the serum NO content (Wang et al. 2012). It was reported that the ethyl acetate extract of muscadine (*V. rotundifolia*) seed contains high level of phenolics (19 types) and anthocyanins (5 types) which contributes significantly to their antioxidant potential (You et al. 2012). Garlic is known to be rich in antioxidants, but high dose is often associated with anemia, increased MDA, elevated intracellular H_2O_2 and free iron level. Grape seed and skin extract treatment counteracted these deleterious effects (Hamlaoui et al. 2012). Antioxidant-rich lipid nanocarriers were developed for the transport of active ingredients in food. The carriers loaded with β-sitosterol and green tea extract were prepared by a combination of natural oils including grape seed oil. The assemblies possessed good stability, high entrapment ability towards free-oxygen radicals and better sustained release properties (Lacatusu et al. 2012). A RSM-optimized supercritical fluid extraction method was employed to obtain antioxidants from grape seeds. A 12.32 % extract yield, 2.45 mg GAE/ml total phenols and 7.08 mg AAE/ml antioxidants were reported at optimized conditions of 45° C and 153 ~ 161 bar CO_2 pressure. Also, gallic acid, protocatechuic acid and p-hydroxybenzoic acid were obtained (Ghafoor et al. 2012). The protective role of grape seed proanthocyanidin extract against dysfunction and oxidative stress induced by torsion-detorsion injury in rat testis was determined. The extract (100 mg/kg daily) was administrated as oral gavage over 7 days before torsion. Testicular torsion was followed by detorsion, lasting 2 h each. Tissue analyses of the euthanized rats revealed that the extracts negate the deleterious effects of testicular torsion-detorsion, by preventing the rise in MDA, apoptosis, eNOS expression, and improving testicular morphology (Bayatli et al. 2013).

6.2.3 Anti-Inflammatory Activity

Leg swelling is a modern-day affliction of sedentary working people. A double-blind, placebo-controlled, crossover clinical study was employed to evaluate the efficacy of grape seed extract intake on leg swelling in healthy Japanese women. A prolonged sedentary position was maintained for 6 h after the extract administration. After 14 day trials, leg volume distension and increased body extracellular fluid were significantly suppressed (Sano et al. 2012). The effect of grape seed-derived

resveratrol and its natural precursor, polydatin on heat-stressed human keratinocytes was evaluated. Both increased the heat shock protein (Hsp70) gene expression that plays an important role in the cytoprotection and repair of cells and tissues. Also, both components increased the release of human β-defensin 2, an antimicrobial peptide. Their ability to reinforce cytoprotective response in stress conditions hold promise for their use in pharmaceutical formulations (Ravagnan et al. 2012). It was determined whether grape seed proanthocyanidin extract has ameliorative role in acute and chronic murine model of asthma. The extract was administered by either intraperitoneal injection or oral gavage before intraperitoneal sensitization of ovalbumin. The mice treated with the extract showed significantly reduced airway hyper-responsiveness, decreased inflammatory cells in the bronchoalveolar lavage fluid, reduced lung inflammation, and decreased IL-4, IL-5, IL-13, and eotaxin-1 expression in both asthma models. Also, the airway subepithelial fibrosis was abated in the lung tissue. Reduced to oxidized glutathione ratio improved after the extract treatment in acute asthmatic lung tissue (Lee et al. 2012). The protective effect of selenium, grape seed extract and both on Indomethacin-induced gastric mucosal ulcers was investigated in rats. Oral pretreatment with these supplements for 28 days significantly decreased the gastric ulcer index (MDA, TNF-α) and increased the antioxidants. The prevention of lipid peroxidation, increase in GSH, activation of radical scavenging enzymes and PGE_2 generation was credited for the anti-inflammatory activity. The synergistic action resulted in more pronounced effect than the individual supplements (Abbas and Sakr 2013).

6.2.4 Lipid Lowering and Cardioprotective Effect

Grape seed extracts owing to the ample procyanidin contents have been recognized to confer benefits in cardiovascular, atherosclerosis, blood pressure and blood vessel fragility conditions. The therapeutic potency of low dose grape seed procyanidin extract was evaluated on body weight and fat deposition. Hamsters supplemented with the extract at 25 mg/kg per day, showed significant decrease in body weight gain. Also, this administration reversed the increase in plasma phospholipids, induced by the high-fat-diet feeding. The treatment induced heparin-releasable lipoprotein lipase activity in the retroperitoneal and mesenteric white adipose tissue depots. The alterations in the lipid metabolic pathways were accompanied by lower free fatty acid levels in the plasma and decreased lipid and triglyceride accumulation in the mesenteric depot. The mechanism of lipid profile normalization was assumed to be due to the activation of both β-oxidation and the glycerolipid/free fatty acid cycle (Caimari et al. 2012). It was deduced that grape seed proanthocyanidins rapidly and transiently repress the expression of the non-coding RNAs miR-33 and miR-122 (regulator of lipid metabolism) in rat hepatocytes *in vivo* and *in vitro*. Also, the miR-33 target gene ATP-binding cassette A1 and the miR-122 target gene fatty acid synthase were modulated. The ATP-binding cassette A1 mRNA and protein levels were increased, and fatty acid synthase mRNA and protein levels were reduced as a result of the variation. It was inferred that the proanthocyanidin treatment stimulates

production of new HDL particles and impedes lipogenesis by meddling with the mRNAs (Baselga-Escudero et al. 2012). The effect of grape seed and skin extract in curbing the side-effects of obesity was investigated in rats. The extract nullified the high-fat diet induced triglyceride deposition and associated disturbances (Charradi et al. 2013).

The cardio-protective effects of proanthocyanidin extract against doxorubicin-induced heart injury were determined in rats. Proanthocyanidin extract (200 mg/kg) was orally administered daily for 15 consecutive days, starting 10 days prior to intraperitoneal injection of doxorubicin. In the control group, drug-induced cardiotoxicity was evidenced by a significant increase in serum AST, creatine phosphokinase isoenzyme, LDH activities and total cholesterol and triglyceride levels. Increased oxidative damage was expressed by the depletion of cardiac GSH, elevation of cardiac total antioxidant level and accumulation of MDA. Also, significant rise in cardiac TNF-α and caspase-3 levels were noticed. These adverse changes were ameliorated in the proanthocyanidin extract-treated groups (El-Boghdady 2012). It was discovered that the ingestion of grape seed extracts inhibits platelet aggregation by inhibiting tyrosine phosphatase activity. In mice models, the supplement exerted the anti-thrombotic effect without enhancing tail bleeding (Jin et al. 2013).

6.2.5 *Hepatoprotective Effect*

The possible protective effect of grape seed extract on liver toxicity, induced by effective radiation dose was evaluated in rat liver. The irradiated group (8 Gy) was orally supplemented with grape seed extract (100 mg/kg) for 7 days. The extract protected the cellular membrane from oxidative damage and consequently from protein and lipid oxidation. The supplement was evidenced to reduce the MDA levels and promote the SOD as well as CAT activities (Cetin et al. 2008). It was demonstrated that intraperitoneally-injected grape seed proanthocyanidins can significantly reduce hepatic ischemia and reperfusion injury in obese mice by protecting the hepatocyte function and increasing the activity of ROS scavengers, as well as decreasing cytokines levels. Also, it enhanced tolerance towards hypoxia. It is more effective than postconditioning in protecting liver against the discussed damages (Song et al. 2012b).

6.2.6 *Bone-Strengthening Effect*

Osteoarthritis occurs due to imbalance in cartilage degradation and synthesis. Heterotopic ossification occurs when ectopic masses of endochondral bone develop within the soft tissues around the joints and is triggered by inflammation of the soft tissues. The role of procyanidin B3, a procyanidin dimer isolated from grape seeds was evaluated in the maintenance of chondrocytes *in vitro* and *in vivo*. It inhibited

H_2O_2-induced apoptosis in primary chondrocytes, suppressed H_2O_2- or IL-1β-induced iNOS production, and prevented IL-1β-induced inhibition of chondrocyte differentiation marker gene expression in primary chondrocytes. Also, it enhanced the early differentiation of ATDC5 (model of endochondral ossification) cells. Daily oral procyanidin B3 administration protected articular cartilage from osteoarthritis and prevented chondrocyte apoptosis in surgically-induced osteoarthritis joints. Furthermore, B3 administration prevented heterotopic cartilage formation near the surgical region. iNOS protein expression was enhanced in the synovial tissues and the pseudocapsule around the surgical region in osteoarthritic mice fed a control diet, but was reduced in mice that received procyanidin (Aini et al. 2012).

6.2.7 Blood Pressure Lowering Effect

The anti-hypertensive potential of grape seed proanthocyanidin extract (250 mg/kg·daily) was determined in rat models. The effect on systolic blood pressure and vascular remodelling was investigated. In ouabain-treated hypertensive model, the extract significantly decreased the systolic blood pressure. The endothelin-1 (ET-1) content was reduced while NO production was increased, which improved vascular endothelial function (Liu et al. 2012). The mechanism governing the endothelial function of grape seed proanthocyanidin extracts was reckoned. By siRNA knocking down, it was proved that the extract increases the eNOS expression in HUVECs *in vitro*, which was attributed to its transcription factor KLF2 induction. Also, the extract activated AMPK and increased surtuin 1 (SIRT1) protein level, crucial for KLF2 induction. After 5 weeks feeding of the extract to hypertensive rats, significant reversal in the induced blood pressure increase and enhancement of the aortic NO production was recorded (Cui et al. 2012).

6.2.8 Hypoglycaemic Effect

Dipeptidyl-peptidase 4 (DPP4) inhibitors are among the newest treatments against type 2 diabetes. It was evaluated whether grape seed-derived procyanidins can modulate dipeptidyl-peptidase 4 activity and expression. *In vitro* inhibition assays on intestinal human Caco-2 cells showed that the procyanidins inhibit pure DPP4. *In vivo* experiment on induced obese animals revealed that the intestinal DPP4 activity and gene expression were decreased by procyanidins. The inhibition of this key enzymatic activity could help in glucose homeostasis directly or by gene expression (Gonzalez-Abuin et al. 2012). A randomized study was conducted to assess whether the consumption of grape seed extract together with high carbohydrate meal affects postprandial glycaemia in eight healthy participants. The results showed that postprandial plasma glucose concentrations at 15–30 min after ingestion of high carbohydrate meal together with 100–300 mg extract were significantly lower than that of control. The inhibition of intestinal α-glucosidase and

α-pancreatic amylase by the extract was attributed to the delayed carbohydrate digestion and absorption. The moderating effect on hyperglycaemia may render grape seed extract useful for diabetes management (Sapwarobol et al. 2012). The effect of grape seed procyanidins on β-cell functionality under an insulin-resistance condition was evaluated. After 13 weeks of cafeteria diet, rats were treated with 25 mg/kg procyanidins for 30 days. The insulinoma INS-1E cells were separately incubated in high-glucose, high-insulin and high-oleate media to reproduce the conditions the β-cells were subjected to during the cafeteria diet feeding. The procyanidin treatment counteracted the decrease of AMPK protein levels and prevented triglyceride accumulation in β-cells. It clearly restored normalcy under hyperlipidemic conditions (Castell-Auvi et al. 2012). The protective effect of grape seed procyanidin B2 against end-stage renal disease (the deadly side effect of diabetic mellitus) was evaluated. Western blot analysis resulted that the oral administration of the procyanidin significantly attenuates the renal dysfunction and pathological changes in db/db mice. It was revealed that milk fat globule EGF-8 (MFG-E8) is a new therapeutic target to prevent diabetic nephropathy. The treatment significantly decreased protein levels of MFG-E8, phospho-ERK1/2, phospho-Akt, and phospho-GSK-3β (Zhang et al. 2013).

6.2.9 Neuroprotective Effect

Obesity predisposes the patients to elevated risks of dementia. The effect of a high-fat diet on brain steatosis (abnormal deposition of lipids) and oxidative stress, along with the protective capacity of grape seed and skin extract was evaluated. The fatty-diet induced ectopic deposition of cholesterol and phospholipid, inhibited the metabolic enzymes (GSH, SOD) and increased acetylcholinesterase activity. The extract might be used as a safe anti-lipotoxic agent in the prevention and treatment of fat-induced brain injury (Charradi et al. 2012). The ability of grape seed extract to antagonize the activity of the post-synaptic neurotoxic fraction, isolated from the venom of the Egyptian sand viper was evaluated. The toxicity characterized by myonecrosis, myofiber hypercontraction, sarcomere disorganization, mitochondrial damage and alterations in motor neurons and axon terminals were inhibited by administrating grape seed extract. The anti-venom property of the extract warrants further probing (Mahmoud 2012).

6.2.10 Anticancer Effect

Grape seed extract has been proven to exert anticancer effects on different tumours. Exploration of pharmacologically-safe chemopreventive agents from the extract seems promising. In this regard, the cytostatic and apoptotic effects of Italia, Palieri and Red Globe cultivars of grape seed extracts on Caco2 and HCT-8 colon cancer cells were evaluated. All the extracts induced growth inhibition and

apoptosis, through intrinsic pathway. Apoptosis induced by these extracts were found higher than that of epigallocatechin, procyanidins and their association (Dinicola et al. 2012). The *in vitro* effects of a red grape seed hydroethanolic extract along with doxorubicin (30 min before drug administration) on HepG2 cell line were investigated. The tumour cells succumbed to the treatment, manifested by cell death, high MDA and protein carbonyl contents. It was inferred that the grape seed extract treatment prior to the drug administration results in selective toxicity towards cancer cells (Postescu et al. 2012). The anti-skin carcinogenic effects of grape seed proanthocyanidins were studied using both *in vitro* and *in vivo* models. The effects of the extract on DNA methylation, histone modifications and tumour suppressor gene expressions were studied on A431 and SCC13 human squamous carcinoma cell lines using a battery of tests. The treatment decreased the level of global DNA methylation, 5-methylcytosine, DNA methyltransferase activity, mRNA and protein levels (DNMT1, DNMT3a and DNMT3b) in these cells. Further, it decreased histone deacetylase activity, increased levels of acetylated lysines (9 and 14) on histone H3 and acetylated lysines (5, 12 and 16) on histone H4. The treatment caused re-expression of the mRNA and proteins of silenced tumour suppressor genes, RASSF1A, p16(INK4a) and Cip1/p21 (Vaid et al. 2012). The effect of proanthocyanidins on enhancing the radio-sensitivity of human hepatic carcinoma HepG2, human cervical cancer HeLa and human leukemia K562 cell line towards X-ray was evaluated *in vitro*. The inhibitory effect of this extract combined with the raditiation was evaluated by sulforhodamine B and clone formation assay, which showed dose- and time-dependent cytotoxicity. The action against the human leukaemia K562 cells was found to be the maximum. The mechanism of sensitization was attributed to the controlling effect of proanthocyanidins on oxygen balance and cell cycle (Pan et al. 2012). The effects of proanthocyanidins on human microvascular endothelial cell-1 and chick chorioallantoic membrane were examined. The treatment resulted in the inhibition of migration, matrix metalloproteinase-2 and -9 secretion and tube formation of human microvascular endothelial cell-1 *in vitro* in a dose-dependent manner. Signalling pathway modulating effect of the extract was credited with the antiangiogenesis (Huang et al. 2012a). The chemotherapeutic effects of proanthocyanidins from grape seeds (0.5 %, w/w) in the form of supplement AIN76A was investigated against human pancreatic cancer MIA PaCa-2 cells. Reduced cell viability and increased G2/M phase arrest of the cell cycle leading to the induction of apoptosis in a dose- and time-dependent manner was observed. The apoptosis was associated with a decline in the levels of Bcl-2 and Bcl-xl and a boost in the levels of Bax and activated caspase-3. Also, it decreased the levels of phosphatidylinositol-3-kinase (PI3K) and phosphorylation of Akt at ser(473). siRNA knockdown of PI3K from pancreatic cancer cells also reduced the phosphorylation of Akt (Prasad et al. 2012). The cancer-protective effects of major phenolic antioxidants in grape skin and seed extracts were reviewed. The seed extracts along with the skin exerted strong free radical scavenging and showed promise against a broad range of cancer cells by apoptosis and cell cycle arrest. The mechanism was deduced to be targeting the epidermal growth factor receptor and its downstream pathways, inhibiting over-expression of COX-2 and prostaglandin E2 receptors, or modifying

estrogen receptor pathways (Zhou and Raffoul 2012). The chemotherapeutic effect of proanthocyanidins on squamous carcinoma cells derived from oral cavity (SCC1), larynx (SCC5), tongue (OSC19) and pharynx (FaDu)) were investigated using *in vitro* and *in vivo* models. The intake of 0.5% proanthocyanidin with AIN76A control diet inhibited cell proliferation, induced apoptosis, decreased the expression of cyclins and Cdks, also that of epidermal growth factor receptor (EGFR) (Prasad and Katiyar 2012). It was observed that grape seed proanthocyanidins inhibit colon tumor-induced angiogenesis on chick chorioallantoic membranes. The mechanism was attributed to ROS scavenging-mediated inhibition of the expression of VEGF and angiopoietin 1, the initial step of tumour angiogenesis. It raised hope for development of angiopreventive agent against colorectal cancer (Huang et al. 2012b). The seed extract of Burgund Mare variety grapes were investigated in SKH-1 mice, irradiated with UVB for 10 consecutive days. The extract (4 mg total polyphenols/cm^2) was applied topically, 30 min before each UVB exposure. The extract remarkably inhibited the activation of iNOS and therefore, the generation of NO and nitrotyrosine, also suppressed NF-kB activation. In multiple UVB irradiations, the extract increased NO formation and total ERK1/2 activity, while reducing the iNOS activity and nitrotyrosine levels. Eventually, the extract inhibited cell proliferation, diminished p53 and caspase-3 immunoreactivities and increased the percentage of Bcl-2 positive cells (Filip et al. 2013). The anticancer mechanisms of seed extract from several cultivars (Italia, Palieri and Red Globe) were investigated against Caco-2 cells. Upon exposure to the extract, ROS and intracellular Ca2+ levels increased in the cancer cells, concomitantly with ERK inactivation. It was revealed that ERK-based mechanism leads to apoptosis (Dinicola et al. 2013). The effects of procyanidin extract on apoptosis and proliferation in INS-1E β-cells were studied. The extract enhanced the pro-apoptotic effect of high glucose level. Also, it showed clear anti-proliferative effects under high glucose, insulin and palmitate conditions. These effects are likely due to high molecular weight compounds contained in the extract (Cedo et al. 2013).

6.2.11 Antimicrobial Effect

Several convincing reports on antimicrobial effects of grape seed extracts have accumulated in recent times. *Escherichia coli* O157:H7 has been associated with several outbreaks in minimally processed foods. Sodium hypochlorite-sanitized greens were inoculated with *E. coli* for 24 h and then re-washed with sterile water. It was followed by electrostatic spraying of malic and lactic acids and grape seed extract on spinach and lettuce. It eliminated the pathogens, improved the safety and lowered the public health risk (Ganesh et al. 2012). The antibacterial and antifungal activities of Merlot and Syrah grape pomace extracts were evaluated using bacteria pathogens *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa* and three fungal pathogens *Candida albicans*, *Candida parapsilosis* and *Candida krusei*. The CO_2 supercritical fluid extracts presented the highest antimicrobial efficacy due to the presence of bioactives (De

Oliveira et al. 2012). Noroviruses are common causes of food-borne gastroenteritis. The anti-norovirus effect of grape seed extract was determined by a battery of assays *viz*. plaque assay for murine norovirus 1 (MNV-1), cell-binding reverse transcription-PCR for human NoV GII.4 and saliva-binding enzyme-linked immunosorbent assay for human NoV GII.4 P particles. The extract at 0.2 and 2 mg/ml was shown to reduce the infectivity of the virus and the specific binding ability of virus to Caco-2 cells. Human NoV GII.4 virus-like particles showed deformation after treatment with the extract. Also, a 1.5- to 2-log PFU/ml reduction in MNV-1 infectivity was observed when 2 mg/ml was used to sanitize water in the washing bath of fresh-cut lettuce (Li et al. 2012b). The effect of grape seed extract was evaluated against peri-implantitis microflora, found in craniofacial implants. When tested by modified agar dilution Millipore method, the extract showed positive inhibitory effects against *S. aureus*. The extract in combination with polyethylene glycol and propylene glycol also showed dose-dependent inhibitory effect on this pathogen (Shrestha et al. 2012). The application of grape seed extract (commercial Gravinol-S) was evaluated against hepatitis A virus (HAV) and human norovirus surrogates, feline calicivirus (FCV-F9) and murine norovirus (MNV-1), on vegetables (lettuce and pepper). The reduction in viral load varied depending on the titer and the produce. The extract clearly showed potential for foodborne viral reduction on fresh foods as part of hurdle technologies (Su and D'Souza 2013).

6.3 Process Parameter Optimization

The effect of drying parameters on the stability of Pinot Noir and Merlot grape seed and skins were investigated. The freeze-dried samples retained the highest bioactive compounds followed by ambient air drying. Though the bioactive loss was obvious, the drying followed by storage at 15 °C up to 4 months was found optimum. The dietary fiber content of the samples was 57–63 % of total dry matter (Tseng and Zhao 2012). The triglyceride makeup of seed oil samples from 32 hybrid grape varieties was investigated with the aid of ESI-tandem and matrix-assisted-laser-desorption-ionization/time-of-flight mass spectrometry. The hybrid grape seed oils showed higher dilinoleoyl-oleoylglycerol and lower dilinoleoyl-palmitoylglycerol content, respectively. A particularly high content of trilinolein (rich in unsaturated fatty acids) was found in seed oils from two red varieties (De Marchi et al. 2012).

6.4 Future Trends and Side Effects

The broad-spectrum therapeutic benefits must be harnessed for restoration and promotion of consumer health. Development of cost-effective methods to retrieve nutrients from winery wastes should be emphasized. For acquiring better insights, the obscure areas should be explored. Whether climatic and edaphological factors play

determinant role in nutritional composition should be assessed. The phenolic compound profiles of European (*V. vinifera*) and Japanese (*V. coignetiae*) grape seed species were compared. The content of total phenolics was higher in the *V. vinifera* seeds, which also contained more tannins, catechins and phenolic acids, except for caffeic acid. Also, the extracts from *V. vinifera* seeds possessed better radical scavenger properties and stronger reducing power. The solvent used for extraction too played crucial role in determining the phenolics. The total contents of phenolic compounds and tannins in the acetone extracts were higher than that of methanolic extracts (Weidner et al. 2013).

The side-effects of grape seed product consumption are reported to be itchy scalp, dizziness, headache, high blood pressure, difficult breathing, swollen lips, hives, indigestion and nausea. Amendment with grape seed extract often leads to astringent flavour and bitterness in the food product. Microencapsulation is expected to mask the unpleasant taste. Heat instability is another lacuna that needs to be addressed for deriving maximum nutrition. The cancer-combating potential of the extract is particularly very promising. National Cancer Institute (NCI) is sanctioning generous funds to carry out research on breast and colon cancer therapy potency of grape seed extract. Further, its cosmetic implications could be explored. The cosmeceutical potential of a blend of coenzyme Q10, retinyl palmitate, tocopheryl acetate, grape seed oil and linseed oil was explored. The battery of tests revealed that the nanoparticles (140 nm) cause significant reduction in the wrinkles after 21 days of application compared to the control. The compounds were safe for topical use and presented anti-aging activity *in vivo*. Further, they were not irritant, sensitizing and comedogenic (aggravating acne), satisfying all criteria to be a suitable cosmetic ingredient (Felippi et al. 2012).

6.5 Conclusion

Grape seed extract is a fairly new nutraceutical that could keep a host of health issues at bay. Many nutrition-poor foods can be fortified with this phenolic storehouse. It could be developed as a robust complementary and alternative medicine for cancer remediation. Any potential risk inherent to it must be determined and good manufacturing practice needs to be established. A growing body of evidences are accumulating to support its biological roles. More clinical trials are required to address the inconsistent efficacy. This chapter is expected to be an information trove of recent advances and challenges in this context.

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Chapter 7
Newest and Robust Entrant to the Functional Food Sector: Chia Seeds

Abstract Chia seed (*Salvia hispanica*) is an emerging plant-derived nutraceutical. With a balanced composition of dietary fiber, proteins, ω-3 fatty acids, antioxidants, vitamins and essential minerals, this seed has attracted the attention of nutritionists. Rich in α-linolenic acid, it is credited to be instrumental in cardiac and hepatic protection. Also, the abundance of dietary fibres is recognized to confer digestive benefits, whereas the proteins augment physical endurance. Further, antidiabetic and anticancer properties are being disclosed. Improvement of meat quality by feeding chia diet to the animals is another potential implication. Though a relatively newbie in the functional food sector, this seed is expected to boom in popularity within no time. In this regard, an updated account on the health potentials of this emerging 'superfood' has been furnished. This chapter is expected to play role in revival of this erstwhile source of sustenance into a sustainable food.

Keywords Chia seeds · ω-3 fatty acid · Dietary fibre · Cardiovascular risk · Functional food

7.1 Introduction

Modern diet is loaded with calories and trans-fats, leading the consumers towards cardiovascular and other chronic diseases. The quest for wholesome food sources has catapulted chia seeds to prominence. Chia (*Salvia hispanica*) belongs to Lamiaceae family. The seeds are tiny, oval shaped and black or white in colour (Fig 7.1). It's native to Mexico and Guatemala. Cahuilla tribe residing in Southern California used chia seeds to cure eye inflammation. Nahua peoples of ancient Mexico used to cultivate chia along with corn. Chumash and Tarahumara people of Coastal regions of South California consumed the seeds. The Aztec and Inca people used it as a nutritious food. In fact, these seeds were regarded as the source of vigour for these erstwhile civilizations. With Spanish invasion, chia seeds lost their prominence from food platter.

With revived interest and research undertakings, these ancient seeds have again emerged as a potential superfood. These are collected from the wild as well as commercially grown. The major cultivating countries are Argentina, Peru, Bolivia,

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Fig. 7.1 **a** Chia (*Salvia hispanica* L.) plant. **b** Chia seeds

Colombia, Ecuador, Guatemala, Mexico and Australia (Jamboonsri et al. 2012). The seeds are rich in fibres, protein and ω-3-fatty acids. Apart from that, they contain vitamin B, minerals (Ca, P, K, Zn, Cu, Mg and Fe) and antioxidants. They are highly hygroscopic and form a gel on imbibing water. These are flavourless, with only a mild nutty taste thus can be blended with virtually any food *viz.* salad, cereals, smoothies, berries, sauce, jam, porridge, cakes, muffins, soup, bread, crackers, snack bar, chips and spreads. These seeds are claimed to keep body hydrated, maintain gut health, prevent bloating and constipation, enhance endurance and help in weight shedding. Antiinflammatory, antihypertensive, cardioprotective and hepatoprotective roles of chia seeds are coming forth. Even diabetes and cancer amelioration properties are being revealed (Fig. 7.2). Grocery giants Wal-Mart, Costco, Sprouts and Trader Joes

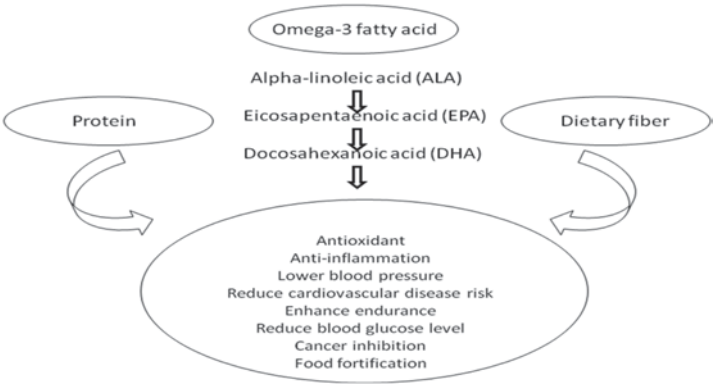

Fig. 7.2 Schematic diagram of the ω-3 fatty acid conversion and the health benefits

sell it. ‘Nutiva’, ‘Spectrum essential’, ‘Trunature’, ‘Health warrior’, ‘Swanson’ are some of the renowned brand of chia seeds. It is making way into the USA households in breakfasts and many other recipes, with special favour from health-conscious lot.

Ulbricht et al. (2009) have reviewed the efficacy of chia seeds as dietary supplements for allergies, athletic performance enhancement, cancer, coronary heart disease, hormonal disorders, hyperlipidemia, and blood pressure management. Mohd Ali et al. (2012) reviewed the active ingredients, oil extraction methods, health benefit updates and market potential of chia seeds. Nutritionists are claiming it to be a phenomenal wholesome food and new research findings are accumulating. This chapter digs the available facts about this topic and aims to disseminate information and awaken consumers about the multifaceted health benefits of this emerging nutraceutical.

7.2 Nutritional Importance

Beneficial roles of chia seeds are gradually being validated. The most promising applications are summarized below.

7.2.1 Food Fortification

Chia seed possesses a host of attributes that makes it an ideal candidate for food fortification. The seeds possess high hygroscopicity and turn into mucilaginous mass on hydration. Studies reveal that they can imbibe 27 times their weight in water (Munoz et al. 2012). When the sensitivity or intolerance towards gluten is on the rise, chia seeds are free of that protein. Also, many people are allergic to fish and ω-3-fatty acids derived from them are unacceptable. Chia seeds are conveniently free of any allergic response towards consumers. Reyes-Caudillo et al. (2008) analyzed the antioxidant content of hydrolyzed and crude extracts of chia seeds. Quercetin, kaempferol and myricetin were quantified in substantial proportions while caffeic and chlorogenic acids were detected in lesser amounts. The antioxdants have been shown to inhibit lipid peroxidation better than vitamin C and E. Alfredo et al. (2009) analysed the nutritional content of fiber-rich fraction of defatted chia flour. The total dietary fiber was quantified to be 56.46 g/100 g out of which 53.45 g/100 g was insoluble dietary fiber. Emulsifying activity was 53.26% and emulsion stability was 94.84%. Antioxidant activity was higher than wine, tea, coffee and orange juice. Ciftci et al. (2012) determined the fatty acids, tocopherols, sterols and triacylglycerols of chia seeds. Predominance of ALA in lipid, 446 mg/kg tocopherol and 4132 mg/kg phytosterol was reported.

Justo et al. (2007) developed bread from whole-wheat flour, chia seeds and flaxseed flour mixed at various ratios. Due to their high levels of protein, unsaturated fatty acids, dietary fiber and folic acid, these breads had high nutritional value.

Glucose dialysis retardation index values were between 89.1–98.1 % and folic acid was in the range 699.44–991.3 mg/100 g dry matter). Also, the taste was approved by the sensory panel. Borneo et al. (2010) determined the overall acceptability, sensory characteristics, functional properties, and nutrient content of cakes baked using chia gel as a substitute of oil or eggs. Sensory panel indicated that the 25 % chia gel cakes were similar to the control in terms of color, taste, texture, and overall acceptability. Olivos-Lugo et al. (2010) determined the nutritional profiles of the protein fractions of chia seeds. The protein isolates demonstrated good emulsifier charcateristics as water-holding and oil-retention capacities. Further, high amounts of glutamic acid, arginine, and aspartic acid were reported. However, essential amino acid lysine was lacking thus supplementation with a suitable protein seemed to be a requisite, for a wholesome protein source. Iglesias-Puig and Haros (2013) investigated the possibility of developing nutritious cereal-based products by incorporating chia and ground chia seeds. The fortification enhanced the levels of proteins, lipids, ash and dietary fibre, without compromising the sensorial quality. Further, the addition of chia inhibited the amylopectin retrogradation of bread delaying its staling during storage.

7.2.2 Endurance Enhancement

High dietary carbohydrate intake by athletes for several days before competition is known to increase muscle glycogen stores resulting in performance improvements in events lasting longer than 90 min. Illian et al. (2011) compared two high dietary carbohydrates, the traditional Gatorade (a sports drink) and a chia seed-based drink for sports performance augmentation. After treadmill and track running trials of six subjects, no difference was observed in the recovery from the intense exercise. From the results, ω-3 chia loading appears a viable option for enhancing performance for endurance events lasting more than 90 min. It slashes the dietary intake of sugar and increases that of ω-3 fatty acids.

7.2.3 Cholesterol Reduction

A skewed ratio of ω-3 and ω-6 fatty acids causes rise in cholesterol level. Chia seeds have been discovered to be an ample source of ALA, known to be the precursor of eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA), the healthy fatty acids in fish. These ω-3 fatty acids are known to reduce inflammation in both the heart and liver. Ayerza and Coates (2007) determined if ALA positively influences plasma composition in murine models. Wistar rats were fed *ad libitum* with whole as well as ground chia seed for 30 days. At the end of the feeding period, the rats were sacrificed, and blood samples were analyzed to determine serum choles-

terol, triacylglycerol content, hemogram and fatty acid composition. Chia decreased serum triacylglycerol content and increased HDL content. It remarkably increased the plasma ALA, EPA and DHA contents compared to the control diet. Gonzalez-Manan et al. (2012) evaluated the hepatic bioconversion of chia oil ALA to EPA and DHA, expression of PPAR-α, acyl-Coenzyme A oxidase 1 (ACOX1) and carnitine acyltransferase I (CAT-I). After feeding chia oil for 21 days, fatty acid composition of total lipids and phospholipids from plasma, hepatic and adipose tissue was assessed by gas-liquid chromatography and TLC. It increased plasma, hepatic and adipose tissue levels of ALA, EPA and DHA and decreased ω-6/ω-3 fatty acid ratio. Also it increased the expression of PPAR-α, ACOX1 and CAT-I. These charactersitics project chia oil as nutritional alternative to conventional ω-3 fatty acid supplement. Rossi et al. (2012) analysed the effect of dietary chia seed on the mechanisms underlying dyslipidaemia and liver steatosis developed in rats fed a sucrose-rich diet. The replacement of maize oil by chia normalised the rise in dyslipidaemia, liver triacylglycerols, fatty acid synthase, acetyl-CoA carboxylase and glucose-6-phosphate dehydrogenase activities, and increased fatty acid oxidase and CAT-I activities. Protein levels of PPARα increased, and the increased mature form of SREBP-1 protein levels in the sucrose-rich diet was restored. Nieman et al. (2012) assessed the effectiveness of milled and whole chia seed in altering disease risk factors in overweight, postmenopausal women. It was observed that the ingestion of 25 g/day milled chia seed compared to whole seed or placebo for 10 weeks by overweight women increased plasma ALA and EPA by 58 and 39%, respectively. Jin et al. (2012) also corroborated that the ingestion of 25 g/day milled chia seeds for 7 weeks by postmenopausal women resulted in significant elevation in plasma ALA and EPA. High-fat diet induced visceral adiposity with reduced lean mass, increased lipid infiltration in the skeletal muscle, impaired glucose and insulin tolerance, cardiac and hepatic deformities, and increased permeation of inflammatory cells in the heart and the liver. Poudyal et al. (2012a) observed that chia seed supplementation for 24 weeks attenuated most structural and functional modifications induced by age or high-fat diet, including increased whole body lean mass and lipid redistribution from the abdominal area, and normalized the chronic low-grade inflammation. These effects may be mediated by increased metabolism of anti-inflammatory ω-3 fatty acids from chia seed. Guevara-Cruz et al. (2012) evaluated the effects of a dietary intervention in checking metabolic syndromes through a randomized trial. A blend of soy protein, nopal, chia seed and oat was given to the test group. The group on this diet had lowered serum triglycerol and C-reactive protein. The group administered with the chia-based diet had a greater decline in body weight and rise in serum adiponectin concentration after 2 month of dietary treatment. Poudyal et al. (2012b) assessed whether chia seeds attenuated high-carbohydrate, high-fat diet-induced cardiovascular and hepatic abnormalities in murine models. Diets of the treatment groups were supplemented with 5% chia seeds for 8 weeks after 8 weeks on fat-rich diet. Chia seed-supplemented rats demonstrated reduced visceral adiposity, decreased hepatic steatosis and reduced cardiac and hepatic inflammation

and fibrosis without changes in plasma lipids or blood pressure. Chia seeds induced lipid redistribution with repression of stearoyl-CoA desaturase-1, the rate-limiting enzyme in tissues fatty acid synthesis. It leads to amelioration of heart and liver in the chia seed-supplemented rats.

7.2.4 Diabetes Manangement

Vuksan et al. (2007) investigated through a single-blind cross-over design whether Salba (a variety of chia) minimizes cardiovascular risk factors in individuals with type 2 diabetes. The subjects were given 37 g/day of Salba for 12 weeks while maintaining their conventional diabetes therapies. Compared with the control treatment, Salba reduced systolic blood pressure, and C-reactive protein with significant decrement in glycated hemoglobin and fibrinogen. Both plasma ALA and EPA levels were increased twofold while consuming Salba. Long-term supplementation with Salba attenuated a major cardiovascular risk factor while maintaining good glycemic and lipid control. Further, Vuksan et al. (2010) determined whether Salba reduces postprandial glycemia in healthy subjects, as a possible explanation for its cardioprotective effects observed in individuals with diabetes. A randomized, double-blind, controlled design was conducted on healthy individuals at different doses. Salba were baked into white bread. Capillary samples and appetite ratings were collected over 2 h after consumption. Decrease in postprandial glycemia provides rationale for improvement in blood pressure, coagulation and inflammatory markers previously observed after 12-week Salba supplementation in type 2 diabetes. Chicco et al. (2009) evaluated the benefit of chia seed in counteracting dyslipidaemia and insulin resistance risks induced by intake of a sucrose-rich diet. The dietary chia seed prevented the onset of dyslipidaemia and insulin resistance in the rats fed the high-sucrose diet for 3 weeks. The above metabolic disorders were normalised when chia seed consumption was continued till 3 months period. Diabetes mellitus begets end-stage renal disease. Pruritus (red, swollen skin) and xerosis (dry, scaly skin) are associated with this kidney complication. Jeong et al. (2010) investigated the effects of topical ω-3 fatty acid extracted from chia seed on volunteers and patients. A topical formulation containing 4% chia seed oils were applied for 8 weeks. After the period of application, significant improvements in skin hydration, lichen simplex chronicus, and prurigo nodularis were observed in all patients as well as healthy volunteers. The study revealed the moisturizing property of chia seed oil in the patients. Poudyal et al. (2012b) also observed that chia seed-supplemented rats had improved insulin sensitivity and glucose tolerance.

7.2.5 Cancer Mitigation

Probing the link between chia seed and cancer has just begun. Espada et al. (2007) investigated the effects of dietary polyunsaturated fatty acids and related eicosanoids on the growth and metastasis formation of a murine mammary gland

adenocarcinoma. In this context, chia oil diet decreased the tumor weight and metastasis number. Apoptosis and T-lymphocyte infiltration were higher and mitosis decreased with respect to other diets. Cancerous growth and metastasis inhibition of chia seeds became evident.

7.2.6 Animal Feed for Carcass Improvement

Apart from the benefits of direct consumption of chia, its nourishment can be derived indirectly. Animals fed with chia offered better meat quality. Peiretti and Meineri (2008) evaluated the effects of three levels viz. 0, 10, or 15% of chia-fortified diet on the growth performance, carcass characteristics and fatty acid profile of rabbit meat and perirenal fat. The polyunsaturated fatty acid concentration in the longissimus dorsi muscle and perirenal fat was significantly increased with increasing chia inclusion, while the saturated fatty acid decreased. The ω-6/ω-3 fatty acid ratio of the rabbit meat decreased from 4.55 in the control group, to 1.03 in the 15% chia-fed group. Coates and Ayerza (2009) studied the effect of feeding chia seeds on fatty acid composition of the meat, internal fats, growth performance, and meat sensory characteristics of pigs. When fed with 10 and 20% chia-amended feed to finishing pigs, it modified the fatty acid composition of the meat fat, significantly less palmitic, stearic, and arachidic acids were found. ALA content increased proportionally with chia content of the diet. Also, the panel members showed preference for the aroma and flavor of the chia-fed meat. Chia seems to be a viable feed that can produce healthier pork for human consumption.

7.3 Cultivation, Extraction and Economy

Ayerza (2010) investigated the chia seeds collected from five locations of Ecuador to determine the effect of the place on the oil content and fatty acid composition. Location seemed to be a crucial determinant in the nutritional profile. Seed yields also vary by edaphic factors, weather, location, and production practices. So, required developments in the respective areas are crucial. Chia grown in Midwestern and Eastern USA flowered late and the seeds succumbed to frosts. To tackle the problem, Jamboonsri et al. (2012) developed several early flowering cultivars with lower photoperiod that can be cultivated in temperate regions.

Ixtaina et al. (2011) investigated the effects of pressure and temperature on the oil solubility and yield by supercritical carbon dioxide (SC-CO_2) extraction method. The yield of chia oil increased with pressure enhancement. The maximum oil recovery using SC-CO_2 was 97%, obtained at 60 °C, 450 bar for 138-min extraction. Martinez et al. (2012) optimized the cold screw press-extracted chia oil yield using Response surface method. The 20 rpm screw press speed and 30 °C barrel temperature were found to be the best processing parameters.

As an excellent source of ω-3 fatty acids for the food and cosmetic industry, chia cultivation is expected to bolster the falling agro-economy of Argentina (Coates and Ayerza 1998). Other countries with ambient climate and edaphological factors should follow suit and cultivate chia crop.

7.4 Future Trend

Chia seeds are gaining unprecedentred recognition and demand from nutritionists as well as consumers. Going by the burgeoning trend, it is assumed that in near future, a range of food products will come fortified with chia. Research focus on chia is rather nascent, that is sure to pick up and reveal myriad other biological roles of this wonder seed. Chia seeds are already touted to provide relief from rheumatoid arthritis, regulate blood pressure, ease menstrual pain, promote gut health, help in loosing weight, though no validations are available. Like other Mesoamerican crops *viz.* maize, beans and squash, that now enjoy global popularity, chia seeds are expected to be a household name in near future. Chia seeds may also be developed into a prebiotic. University of Arizona, Appalachian State University and University of Queensland are among the leading institutes actively involved in the nutritional potential research of the chia seeds. The research trend is likely to accelerate and expand.

7.5 Conclusion

Chia seed is known to be a complete food source since ancient times. As a powerful functional food, it has the capability to prevent many metabolic diseases. Yet, the potential consumers are hardly aware of this nutritious seed. Researchers are optimistic that in near future, chia will occupy spot in the top echeleon of healthy foods. World population has exceeded 7 billion and food security is a major challenge. Developing lesser-known crops into staples are envisioned to salvage mankind from succumbing to starvation. Loaded with vital nutrients, chia merits to be pushed to the forefront in agriculture and global recognition. This review is expected to play its part in this goal of projecting chia seeds as a sustainable food.

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Chapter 8
Prosopis Genus as Food and Drug Repository: Exploring the Literature Databases

Abstract The genus Prosopis, a member of Fabaceae family grows profusely in arid regions of the world. Native tribes have used this plant as a source of subsistence for generations. The high-protein, low-carbohydrate flour of its pod is touted to be a potential food source. Some recently reported bioactive potentials range from functional food, antioxidant, hypolipemic, antihypertensive, antidiabetic, antinociceptive, estrogenic, anticancer, antimicrobial and neurostimulator to larvicide. This chapter furnishes a comprehensive account of the current state of knowledge and proposes future scopes.

Keywords Prosopis · Unconventional food · Value-added product · Ethnobotany · Sustainability

8.1 Introduction

The genus Prosopis encompassing about 45 species belongs to the family Fabaceae. It is quite cosmopolitan in that it is native to the Americas, Africa as well as Asia. It is distributed across arid parts of North America, South America, Africa, Western and South Asia. Some species have also been introduced to Queensland regions of Australia. These medium-size, thorny trees or shrubs are fast-growing, drought-resistant, with nitrogen-fixing capacities. They can thrive in nutrient-poor, saline soils of arid and semi-arid zones. These plants are named differently as per the geographical distribution which often leads to ambiguity. The species growing in Colorado, Chihuahuan and Sonoran deserts of the USA and Mexico are known as mesquite, which include *P. glandulosa*, *P. velutina, P. laevigata*, *P. pubescens* and *P. velutina*. Algarrobos is the group of prosopis generally growing in South America, which includes the species *P. chilensis*, *P. nigra*, *P. alba*, *P. juliflora*, *P. pallida*, *P. flexuos*, *P. alpataco*, *P. denudans* and *P. ruscifolia*. Among African species *P. africana* is the most prominent. Major Asian species are *P. cineraria*, *P. juliflora* and *P. farcta*. This genus has demonstrated multiple utility as a source of timber, firewood, livestock feed, shelter and soil conditioner. Though regarded for its immense utili-

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Fig 8.1 **a** Crown of the tree. **b** Prosopis in blossoms. **c** Pods

tarian value, the food and nutritional potential of this genus has been explored scantily.

The indigenous people used every part of these plants *viz*. the pod, leaves, root, trunk, branches, bark and gum (Fig. 8.1). This genus has been an important source of sustenance for the indigenous communities. The Native American tribe *viz*. Apache, Cahuilla, Chiricahua, Havasupai, Hualapai, Maricopa, Mohave, Paiute, Papago, Pima, Seri and Yavapai depended on these trees for their daily requirements. They used mesquite flour to bake breads. Desert dwellers used the pod meal as a staple food. Mesquite 'atole' was made by boiling the pods in water and pounding them into a pulp. The leaves and pods were brewed into tea. The twigs and leaves were boiled to treat wounds, infections, headaches, dysentry and sore throats. The early settlers also made good use of this desert resource. Pima Indians in Arizona still rely on this bean-based food to a large extent. The pods of *P. cineraria* growing in arid parts of Rajasthan in India are harvested by the desert inhabitants for culinary purposes. Hebbar et al. (2004) conducted an ethnomedicinal survey in Dharwad district of Karnataka in Southern India and found that *P. juliflora* is used to treat dental plaque and caries. *P. africana* is used for menstrual and general body pain alleviation in some parts of Nigeria (Ayanwuyi et al. 2010). *P. strombulifera* fruits are used by several communities of Argentina as astringent, anti-inflammatory and odontalgic agent and anti-diarrheic (Saragusti et al. 2012). In South America, the pods are toasted to make coffee-like beverages. Spurred by the looming food insecurity and keeping with the latest wave of discovering unconventional food sources, the dietary and pharmaceutical aspects of prosopis are being explored. Now, it is being reckoned to emerge as a sustainable food and drugs storehouse. This chapter sheds light on the latest development in this area and associated impediments.

8.2 Phytochemistry

Prosopis seeds are 40% protein and are high in slow-digestible dietary fibers. The sweetness comes from fructose so it is not supposed to interfere with insulin level. The presence of phytosterols, flavonoids, phenolic compounds, tannins, carbohy-

drates, proteins and amino acids were detected in the preliminary investigation of different extracts of stem bark of *P. cineraria* (Manikandar 2009). Retana-Marquez et al. (2012) reported the presence of phytoestrogens with an affinity towards estrogen receptors. Karim and Azlan (2012) reported the richness of *P. cineraria* fruit pods in polyphenolics and xanthones, carotenoids and saponins.

8.3 Food and Medicinal Applications

The sections below embody the recent findings on nutritional uses and versatile biological potential of prosopis genus.

8.3.1 Functional Food

This genus played a significant role in subsistence of early civilizations and continues to be valuable for local foragers. Increasing evidences of their implications in functional foods are emerging, yet the edibility is confined to an insignificant group of consumers. Bravo et al. (1994) compared the composition of *P. pallida* pods with that of Mediterranean carob pods (*Ceratonia siliqua*) used in food processing. The prosopis beans showed structural and compositional similarity with the latter, which holds promise for food innovation. Holmquist-Donquis and Ruiz de Rey (1997) prepared a protein concentrate from the *P. juliflora* beans with an aim to develop it as a functional food. The flour demonstrated good water and fat absorption capacity; however, the emulsifying capacity largely hinged on pH and ionic strength conditions. Goycoolea et al. (1997) weighed the possibility of developing prosopis gum as a substitute of gum Arabic (exudates of *Acacia senegal*). Immunological testing revealed that both the gums have almost similar carbohydrate, protein and tannin compositions. Lopez-Franco et al. (2012) also characterized the proteinaceous arabinogalactan gum from prosopis and compared with that of gum Arabic. Low intrinsic viscosity, humidity, inorganic and tannins content as well as high protein content of the former came forth. With orange peel essential oil model, the emulsifying ability of the former was found only slightly less (90%) than that of the latter (100%). Estevez et al. (2000) developed cereal bars with peanut, walnut and *P. chilensis* seed cotyledons. Out of the twelve possible combinations, the cotyledon-fortified bars demonstrated maximum protein content and microwave treatment enhanced the acceptability. Bernardi et al. (2006) prepared cookies from *P. alba* pulp with Fe and Ca additives. The final product was analysed to have a makeup of 8.9/100 g protein, 7.2/100 g dietary fiber, 25/100 g total sugar, 18.5/100 g crude fat, 30 ppm Fe and 340 ppm Ca, respectively. About 78% of the panellists approved of the taste and flavour of the finished product. 'Ogiri-okpei' is a food condiment popular among people of West Africa. Odibo et al. (2008) investigated the biochemical changes involved with the fermentation of *P. africana* seeds

for production of this food. Increment in the total soluble sugar and free amino acid contents were observed. Amylase, protease and lipase activities were conspicuously strong. A varied amino acid profile and mineral (Ca, P, K, Mn and Z) abundance was measured. Galan et al. (2008) quantified the nutritional components of the mature fruits of some South American prosopis species *i.e*. *P. chilensis*, *P. nigra* and *P. alba* from Bolivia and *P. juliflora* from Brazil. Among them, *P. nigra* contained the highest levels of crude protein (11.33/100 g) and ashes (4.12/100 g), whereas *P. juliflora* presented the highest levels of non-reducing sugar (52.51/100 g) and *in vitro* protein digestibility (66.45%). Cereals have higher content of sulphur amino acids whereas legumes score high in lysine. Escobar et al. (2009) blended *P. chilensis* cotyledon flour with cereals to prepare cookies and chips with balanced amino acid profile. Both the snacks were analysed to have high content of protein, lipids, ash and crude fiber, with the optimal fortification at 10% of the flour. Also, the sensory profile was approved by the tasting panel, paving the path for functional food formulation. Bernardi et al. (2010) prepared bread from the seed flour of *P. ruscifolia*. The fortified corn bread was analysed to have higher protein content and antioxidant activity than commercial gluten-free bread. Cerezal Mezquita et al. (2012) formulated a beverage for feeding nutritionally-starved children aged 2–5 years. After 90 days of storage, the potion prepared with *P. chilensis*, lupine, quinoa and raspberry pulp attained a protein content of 1.36%. The drink was approved following sensory evaluation.

8.3.2 Analgesic/Antinociception/Antiinflammatory

Manikandar (2009) compared the analgesic and antipyretic activity of petroleum ether, ethyl acetate and ethanol extracts of stem bark of *P. cineraria* in animal models. Hot plate and yeast-induced hyper pyrexia method were followed for deriving the result. Oral administration of the ethanol extract at the concentration 300 mg/kg exerted dose-dependent analgesic activity, while, the petroleum ether extract at the same dose ameliorated fever. Ayanwuyi et al. (2010) evaluated *P. africana* stem bark methanol extract for analgesic and anti-inflammatory activities using acetic acid-induced writhing and carrageenan-induced inflammation in murine models. The extract attenuated writhing at the dose 250 mg/kg and elicited anti-inflammatory activity after the third hour. Saragusti et al. (2012) investigated the *in vivo* antinociceptive effect of several extracts of *P. strombulifera* in mice. The chloroform extract at a dose of 300 mg/kg demonstrated moderate antinociception. It produced a dose-dependent inhibition of the neurogenic and inflammatory phases of the formalin test. Also, NO production from LPS-treated J774A.1 cells was restrained by the extract.

8.3.3 Estrogenic Potential

Legumes have been known to contain good amount of phytoestrogens with an affinity towards estrogen receptors. Retana-Marquez et al. (2012) evaluated the potential

estrogenic effects of mesquite pod extract on several aspects of behavior and reproductive physiology of the female rats. In ovariectomised rats, mesquite pod extract induced vaginal estrus, increased vaginal epithelium height and induced lordosis, although its intensity was reduced, compared to intact rats.

8.3.4 Antidiabetic, Antihypertensive and Hypolipemic Effect

Diabetes mellitus has assumed epidemic proportions. Obesity and type 2 diabetes result in fatal conditions like hypertension and coronary ailments. Scores of drugs exist to contain the disease and its side effects; however, complete therapy is yet to be achieved. In this regard, a supplement 'Diavite™' prepared from the pods of *P. glandulosa* deserves mention. *P. glandulosa* treatment at a dose of 100 mg/kg/day for 8 weeks moderately lowered the glucose levels in different animal models of Type 1 diabetes. Histological analysis revealed that prosopis diet stimulates insulin secretion, induces formation of small β-cells and improves insulin sensitivity of isolated cardiomyocytes (George et al. 2011). Ranjbar-Heidari et al. (2012) examined the local effect of fruit husk powder and root extract of *P. farcta* on diabetic treatment. Both the husk powder and root aquatic extracct accelerated healing of induced holes on dorsal sides of streptozotocin-injected diabetic rats.

Pinto et al. (2009) explored the possibility of combating the side-effects of type 2 diabetes using Peruvian fruits. Aqueous extracts of *P. pallida* showed high α-glucosidase inhibitory activities. *P. pallida* had significant angiotensin-converting enzyme (ACE) inhibitory activities reflecting antihypertensive potential. It builds prospect of food-based strategies for complementing hypertension therapy. Omidi et al. (2013) investigated the lipid-lowering effect of *P. farcta* bean consumption in ostrich model. After a month of feeding with the bean diet, blood sample was analyzed for lipid level. The HDL cholesterol, total protein, and globulins levels increased significantly whereas LDL cholesterol, inorganic phosphorus, and γ-glutamyltransferase activity decreased significantly. Huisamen et al. (2013) determined the *in vivo* efficacy of *P. glandulosa* pod powder as anti-hypertensive and cardioprotective agent. Diet-induced obese rats were treated with *P. glandulosa* (100 mg/kg) for eight weeks and it was observed that the oral administration of the extract reduces infarct size after ischaemia-reperfusion. Proteins of the PI-3-kinase/PKB/Akt survival pathway were impacted indicating cardioprotection.

8.3.5 Antioxidant Activity

Tapia et al. (2000) assessed the biological activity of extracts from the aerial parts of five Argentinian Prosopis species and the exudates of *P. flexuosa*. At 0.50 mg/ml, the DNA binding activities of alkaloids in the basic fraction of the extract widely varied. It ranged from 28% for tryptamine to 0–27% for the phenethylamine and 47–54% for the piperidine derivatives. Tryptamine and 2-β-methyl-3-β-hydroxy-

6-β-piperidinedodecanol showed a moderate inhibition (27–32%) of the enzyme β-glucosidase at 100 mg/ml. The exudate of *P. flexuosa* displayed a strong free radical scavenger effect in the DPPH decoloration assay. The main active constituent was identified as catechin. Albrecht et al. (2010) reported the oxidative injury-cancelling effect of *P. alba* fruit extract. The viability of leukocytes was enhanced with concomitant drop in ROS level.

8.3.6 Memory Booster

Bithu et al. (2012) investigated the anticholinesterase activity of oral administration of *P. cineraria* stem bark methanol extract to rats. When administered once a day for 7 days, at doses 200–600 mg/kg, the extract improved both spatial reference as well as working memories and increased the time spent in the target quadrant. The extract inhibited the activity of acetylcholinesterase in the hippocampus, prefrontal cortex and amygdale, leading to cognitive enhancement.

8.3.7 Vasorelaxant Activity

Janbaz et al. (2012) evaluated the crude methanolic extract of the stem bark of *P. cineraria* for its possible spasmolytic, bronchodilator, and vasodilator activities. The extract caused relaxation of the spontaneous as well as K(+)-induced contractions at tissue bath concentrations of 3–10 mg/mL in isolated rabbit jejunum preparations, probably mediated through blockade of Ca^{+2} channels.

8.3.8 Antimicrobial/Antiprotozoal/Wound Care Activity

Mazzuca et al. (2003) screened the genera *P. alpataco*, *P. denudans* var. *denudans*, *P. denudans* var. *Patagonica* and *P. denudans* var. *Stenocarpa* to evaluate their antibacterial and antifungal activities. The petroleum ether extracts of all species showed antibacterial activity. The dichloromethane extract of *P. alpataco* showed both antibacterial and antifungal activities. Methanol and aqueous extracts of *P. denudans* var. *Denudans* and *P. denudans* var. *Patagonica* showed antifungal activities. Fatty acids and a group of pentacyclic triterpenes were identified as the antibacterial compounds. Khan et al. (2010) reported that the ethanolic fraction of *P. spicigera* demonstrates a remarkable inhibition of the multidrug-resistant strains of *Candida albicans*, *Candida krusei*, *Candida tropicalis*, *Candida glabrata*, *Escherichia coli, Streptococcus mutans* and *Streptococcus bovis*. The results showed that *P. spicigera* could be developed as potential source of new antimicrobial agents. Ezike et al. (2010) studied the effects of the methanol extract of the stem bark of *P. africana* on bleeding/clotting and coagulation time, excision and dead space

wounds in rats. The extract was subjected to antibacterial, acute toxicity and lethality LD_{50} tests. It significantly reduced coagulation time and epithelialization period of excision wounds. Also, it inhibited the growth of laboratory strains of *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* to varying extents. So, the phytochemical-rich extract holds promise for wound care. Singh et al. (2010) reported that *P. chilensis* stem bark ethanol extracts possesses significant activities against *Streptococcus pneumonia*, *Enterobacter aerogenes*, *Klebsiella pneumonia* and *Candida albicans* as demonstrated by microdilution method. Singh et al. (2011) assessed the antibacterial property of piperidine alkaloid-rich fraction of various parts of *P. juliflora*. Disc diffusion method revealed the strong inhibitory effect of leaf, pod and flower extract comparable to many standard antibiotics. *Klebsiella*, *Acinetobacter*, *Alcaligenes* and *E.coli* were the bacteria succumbing to the extract. Isimi et al. (2011) evaluated the antiplasmodial properties of *Anogeissus leiocarpus* and *P. africana* combined aqueous extract. The extract suppressed parasitaemia in early infection by 50–69 % at 200–400 mg/kg, respectively. Rahman et al. (2011) reported that alkaloid juliprosine isolated from the leaves of *P. glandulosa* exhibits potent antiplasmodial activity against *Plasmodium falciparum* D6 and W2 strains. Also, its potent antifungal activity against *Cryptococcus neoformans* and antibacterial activity against *Mycobacterium intracellulare* was reported. Al-Musayeib et al. (2012) demonstrated the *in vitro* antiprotozoal activity of methanol extracts of *P. juliflora* against erythrocytic schizonts of *Plasmodium falciparum*, intracellular amastigotes of *Leishmania infantum* and *Trypanosoma cruzi* and free trypomastigotes of *T. brucei*. Bansal et al. (2012) evaluated the larvicidal efficacy of aqueous and organic solvent extracts from seeds, leaves and flowers of several plants, including *P. juliflora*. The methanol and acetone extracts were effective against all three studied larvae *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. The prosopis extracts may be developed into suitable antimicrobial and antiprotozoal drugs. Collectively, the above facts furnish evidence of the antimicrobial and antiprotozoal and wound healing potential of prosopis extracts.

8.3.9 Anticancer Effect

Maideen et al. (2011) evaluated the effect of methanol extract of *P. cineraria* against liver tumor in rats. Administration of the extract suppressed the tumor effectively as revealed by the decrease in elevated levels of aryl hydrocarbon hydroxylase, lactate dehydrogenase, g-glutamyl transpeptidase, 5'nucleotidase, DNA and RNA. Sathiya and Muthuchelian (2011) determined the *in vitro* antitumor potential of the alkaloid extract from *P. juliflora* leaves using MTT-based cytotoxicity assay on a human T-cell leukemia cell line MOLT-4. The leaf alkaloids were credited with the genotoxicity-free anticancer effect. Robrtson et al. (2011) investigated the antitumour activity of the hydroalcoholic extract of the leaf and stem bark *P. cineraria* (L.) on Ehrlich ascites carcinoma tumor in mice. A battery of tests led to the finding that the extracts possessed significant cytotoxicity towards the tumour cells.

8.4 Future Trends

Trejo-Espino et al. (2011) established *P. laevigata* cell suspension culture for *in vitro* harvesting of gum productive cell line. Hypocotyl-derived callus was subcultured and the exuded gum-like substance was analyzed. The emulsifying properties of gum-like substance from callus culture and the broth from cell suspension culture were compared to those of mesquite gum and reported similar emulsifying capacity and stability. No concrete reports of side-effects or allergic reactions further substantiate the inclusion of prosopis in food and pharmaceuticals.

8.5 Conclusion

Diverse biological potencies of prosopis genus must be explored. Given due attention, it might contribute towards food sustainability, warding off the perpetual insecutity. Members of this genus are drought-resistant and fit to be grown in arid regions. Developing countries are expected to benefit most from its utilization in diet. Commercialization of prosopis-based functional foods and dietary supplements is anticipated to expand. This chapter is expected to provide insight into this area and augment current knowledge.

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Chapter 9
Resurgence of Interest in Ancient Grain Quinoa (*Chenopodium Quinoa*): An Appraisal

Abstract Quinoa (*Chenopodium quinoa*) is a plant belonging to Amaranthaceae family. Though it is an ancient crop dating back to Inca civilization, recent times have witnessed a revival of popularity owing to its nutritional abundance. The quinoa seeds have been discovered to be nutrient powerhouses packed with wholesome protein, essential fatty acids, minerals and vitamins. Several health-imparting properties as antioxidant, anti-obesity, hypolipemic and anti-diabetic have been validated. The potential to prevent celiac disease attributed to the absence of gluten has grabbed much attention. Quinoa has been effectively incorporated in a wide range of food products. It is expected to tackle looming food insecurity. Global recognition is being emphasized to the extent that 2013 was announced as the 'International year of quinoa'. Considering the human health awareness programme and research impetus, it seems poised to dominate the staple food sector. In this regard, this chapter embodying the latest findings and future scopes holds immense relevance.

Keywords *Chenopodium quinoa* · Pseudo-cereal · Functional food · Celiac disease · Diabetes

9.1 Introduction

Quinoa (*Chenopodium quinoa*) belonging to Amaranthaceae family (Fig. 9.1a) has emerged as a complete food. This seed falling in the group of Swiss chard, spinach, buckwheat, amaranth and chia, is often confused as grain, so given the name 'pseudo-cereal' (Fig. 9.1b). It is claimed as the healthiest vegetable protein for its reserve of essential amino acids. Also, it has appreciable amount of dietary fibre, unsaturated fatty acids, minerals (Mg, Ca, Cu, Mn and Fe) and vitamin B and E (Hager et al. 2012). Also, other bioactive components such as saponins, phytosterols, squalene, fagopyritols and polyphenols have been found (Valcarcel-Yamani and Da Silva Lannes 2012). It's light, fluffy with nutty flavour and comes in an array of colours (black, red, orange, purple and white). It has found versatile culinary uses *viz*. can be consumed sprouted, cooked or used in soup, pasta, cereal, salad, burger, burrito, muffin, biscuit, pancake, pudding, sushi, granola etc. Andean culture

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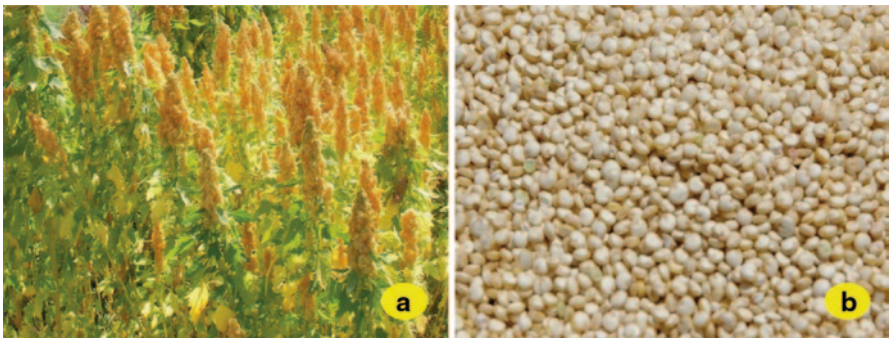

Fig. 9.1 **a** Quinoa plant. **b** Quinoa seeds

(Aztecs and Incas) subsisted on it for thousand years. The Incas made fermented beverage 'chicha' from quinoa (Gonzalez et al. 1989). After Spanish invasion, it lost precedence to wheat and corn. However, recently, its importance has been recognized and integration in regular diets is being given thrust. Several health benefits have been attributed to it *viz*. anti-obesity, hypolipemic, anti-diabetic and most importantly celiac disease management.

The benefits of quinoa consumption were reviewed (Vega-Galvez et al. 2010). Owing to the ample functional components *viz*. minerals, vitamins, fatty acids and antioxidants, inclusion in human diet was recommended. Its minerals work as cofactors in antioxidant enzymes, adding higher value to its rich proteins. The composition of quinoa and its correlation to human health was reviewed (Valcarcel-Yamani and Da Silva Lannes 2012). This chapter attempts to furnish the latest developments in assimilation of quinoa in functional foods. The biological roles, storage and processing, possible hazards, breeding and cultivars, future scopes have been emphasized.

9.2 Nutritional and Therapeutic Potential

Quinoa is steadily rising in popularity and reports of its health benefits are pouring in. The significant findings have been summed up below.

9.2.1 Food and Additives

Quinoa has been incorporated into various food products in an effort to enhance delicacy as well as nutrition. The physicochemical properties *viz*. solubility, water-holding, gelation, emulsification and foaming capacity enable diversified uses. Many quinoa-fortified foods are available in market today, ranging from flour, flakes, puffs, pasta and granola to drinks. The performance of various ratios of

quinoa-wheat flour blends were evaluated in preparation of bakery goods (Lorenz and Coulter 1991). Higher level of quinoa resulted in decreased loaf volume, open crumb grain and harsher texture. A 30% substitution left a bitter aftertaste. However, an acceptable cake and bread quality was achieved with 5 and 10% of quinoa flour. The flavor improved up to 20% quinoa flour in the blend. Ogungbenle (2003) reported that quinoa being rich in D-xylose and maltose with a low content of glucose and fructose could be ideal for malted drink processing. The amino acid composition and the physicochemical as well as functional properties of quinoa protein isolates, obtained by alkaline solubilization followed by isoelectric precipitation and spray drying was evaluated (Abugoch et al. 2008). Some isolates could demonstrate desirable characteristics, fit to be implicated in infants and children nutrition. The isolates might be used in beverages, sauces, sausages, and soups. The rheological properties of doughs prepared from wheat flour fortified with quinoa were investigated (Jancurova et al. 2009). Though dough stability decreased with the increasing content of quinoa, it was concluded that the addition of lower amounts of quinoa (5%) to wheat flour will not significantly impair rheological properties of the dough but augment nutritional value of the prepared bakery products. Amaranth, quinoa and buckwheat were used for the production of pasta with low cooking loss, optimal cooking weight and texture firmness (Schoenlechner et al. 2010). The individual use of grains failed to bring the desired characteristics, but the blend of 60% buckwheat, 20% amaranth and 20% quinoa, improved the dough matrix. The multi-grain pasta was gluten-free unlike that of wheat. A dark chocolate was developed with the addition of 12, 16 or 20% quinoa (Schumacher et al. 2010). The protein concentration of the product increased as the percentage of quinoa was increased. The product containing 20% quinoa showed 9% increase in vitamin E. The amount of essential amino acids was improved in samples containing quinoa. Cysteine, tyrosine and methionine increased by 104, 72, 70%, respectively. The chocolate with quinoa was approved by 92% of the sensory panel.

Soybeans or cereal grains when fermented by the mold *Rhizopus oligosporus* yield tempeh, the cake-like product bound by cottony mycelia. The enzymatic fermentation hydrolyzes the constituents and develops desirable texture, flavour and aroma, apart from improving nutritional attributes (Feng et al. 2007). Quinoa was fermented with *Rhizopus oligosporus* to enhance nutrient quality and palatability (Matsuo 2005). The antioxidant activity of 80% methanol extract of the quinoa tempeh was investigated in rats both *ex vivo* and *in vivo*. In the *ex vivo* experiment, the extract increased the activities of SOD and GSH-Px in the liver, and accelerated the production of 12-hydroxyeicosatetraenoic acid in the lung. In rats fed vitamin E-free diets with 80% methanol extract, the α-tocopherol concentration, TBARS value, and activities of SOD and GSH-Px in serum showed a similar concentration to those of the control rats fed a vitamin E-supplemented diet. Also, the hepatic SOD and GSH-Px activities were higher than those in the control rats. Quinoa tempeh is suggested to be a good source of antioxidants. The fermentation of chickpea, amaranth, quinoa and buckwheat with *Lactobacillus plantarum* C48 and *Lactococcus lactis* subsp. lactis PU1 was evaluated for the biosynthesis of γ-aminobutyric acid (GABA) (Coda et al. 2010). The concentration of phenolic compounds in

non-conventional flour-based sourdough bread was the highest; whereas the rate of *in vitro* starch hydrolysis was the lowest. Texture analysis showed that the sourdough fermentation enhances several characteristics. Sensory analysis showed that sourdough fermentation imparted good palatability and overall taste appreciation. Fermentation by *Lactobacillus plantarum* CRL 778 indirectly stimulated flour protein hydrolysis by endogenous proteases of quinoa slurry. Hydrolysis was faster and peptides and free amino acids yield was higher than that of wheat. Greater concentrations (approx. 2.6-fold) of the antifungal compounds (phenyllactic and hydroxyphenyllactic acids) were synthesized from phenylalanine and tyrosine. Quinoa can be fermented by this strain to formulate sourdough for healthful baked goods (Dallagnol et al. 2012). A 20% quinoa was incorporated into wheat bread formulations, which improved rheological characteristics of dough and enhanced the protein content in bread to around 2%. Further, the organoleptic property was promoted (Stikic et al. 2012). Intact starch granules isolated from quinoa are used to stabilize emulsion drops in so-called Pickering emulsions. It was observed that starch heated for long time (150 min at 120 °C) had a better emulsifying capacity than the unmodified native starch granules (Rayner et al. 2012).

9.2.2 Antioxidant and Anti-Diabetic Effects

For their health restorative potential, antioxidant-rich phytochemicals are constantly sought after. Quinoa as a protective agent against fructose-induced adverse changes was evaluated in rat model (Pasko et al. 2010a). Fructose administration (310 g/kg for 5 weeks) caused oxidative stress that was manifested by the increase in plasma MDA level. The co-administration of same amount of quinoa maintained normal activities of some enzymes. It decreased MDA in plasma and increased the activities of antioxidant enzymes (SOD, CAT and GSH-Px). It came forth that quinoa can offer moderate protection by reducing lipid peroxidation and enhancing the antioxidant capacity of plasma and tissues (heart, kidney, testis, lung and pancreas). Quinoa coats, discarded for their surfactant nature have been discovered to be rich in triterpene saponins. It was evaluated that hydroalcoholic extracts of quinoa seed coat have thiol compounds along with polyphenols. The thiols inhibited microsomal lipid peroxidation (Letelier et al. 2011). The antioxidant potency of quinoa seeds was evaluated by lipoxygenase/4-Nitroso-N, N-dimethylaniline, ORAC and the Trolox assays (Laus et al. 2012). The antioxidant compounds were reported high, even more than that of durum and emmer wheats.

It was reported that glycemic index (GI) for quinoa was slightly lower than that of gluten-free pasta and bread (Berti et al. 2004). It was found that quinoa rich in quercetin derivatives exerts high antioxidant activity (86%) and holds promise in management of type 2 diabetes (Ranilla et al. 2009).

9.2.3 Anti-Obesity Effect

Obesity an epidemic has overwhelmed all age groups setting the stage for onset of other health complications. Low-calorie, low-glycemic index foods are highly sought-after to deal with the metabolic dysfunctionalities. The effect of quinoa in high-fructose-fed male Wistar rats was determined (Pasko et al. 2010b). The blood analysis indicated that these seeds effectively reduce serum total cholesterol, LDL and triglycerides. Also, quinoa significantly demoted the level of glucose and plasma total protein. Further, it reversed the fall in HDL level. The effects of quinoa in the form of a cereal bar given daily to 22 volunteers for 30 days were investigated (Farinazzi-Machado et al. 2012). The analysis of blood samples indicated that quinoa could reduce the levels of total cholesterol, triglycerides and LDL. An extract was derived from quinoa seeds following enzymatic hydrolysis. The hydrolyzed quinoa extract was found to be rich in essential amino acids, particularly those with branched chains (leucine, isoleucine and valine). No hepatic or renal toxicity was documented after 30 days supplementation of hydrolyzed quinoa (2000 mg/kg). Moreover, decrease in food intake, body weight, fat deposition and blood triacylglycerol level was observed in the above two groups. These results suggest a potential use of hydrolyzed quinoa extract in human nutrition to combat obesity (Meneguetti et al. 2011). Ecdysteroids are steroid hormones with crucial pharmacologic and metabolic roles in mammals. An ecdysteroid, 20-hydroxyecdysone (20E) is detected in quinoa. The ability of quinoa extract enriched in 20E was investigated in prevention of the onset of diet-induced obesity and regulation of the expression of adipocyte-specific genes in high-fat mice (Foucault et al. 2012). The supplementation of diet with the extract for 3 weeks reduced the adipose tissue development. The reduced adipocyte size and a decrease in the expression of several genes involved in lipid storage (lipoprotein lipase and phosphoenolpyruvate carboxykinase) were credited for the adipose tissue inhibition. The extract treatment led to marked attenuation of mRNA levels of several inflammation markers (monocyte chemotactic protein-1, CD68) and insulin resistance (osteopontin, plasminogen activator inhibitor-1). Also, it altered the high-fat diet-induced downregulation of the uncoupling protein levels in muscle. The antiobesity effect of quinoa owing to the presence of the ecdysteroid was revealed.

9.2.4 Management of Celiac Disease

Celiac disease is an immune-mediated enteropathic disease, triggered by gluten and related prolamins in cereals *viz*. wheat, rye, barley (Saturni et al. 2010; Bakshi et al. 2012). Gluten intolerance is a major health issue in the Western world. It causes chronic inflammation of the intestinal mucosa and atrophy of intestinal villi. Nutritional intervention is considered the only effective treatment for it (Arendt et al.

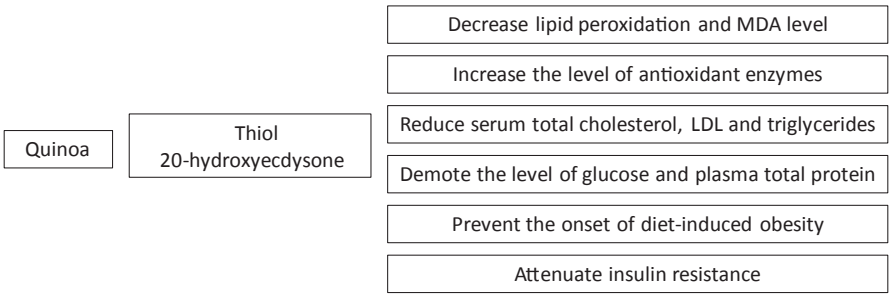

Fig. 9.2 Major biochemical modulations caused by quinoa seed consumption

2011). Lifelong exclusion of gluten-rich food is required by the patients. On the other hand, gluten protein network is vital for the texture and the overall quality of the food products, especially bakery. So, the gluten-free products show low baking performances and are poor in nutritional quality. In this regard, the pseudocereal quinoa seeds hold great promise, as they are gluten-free and have high levels of protein, fat, fibre and minerals. Several products were formulated from quinoa, rice and corn flours and starches for celiac patients. Among the foods prepared, scones and pancakes were accepted for their quality proteins, good textural characteristics and nutritional content (Del Castillo et al. 2009a). Cookies were prepared from defatted hazel nut and quinoa flour for enhancing nutritional food supply to celiac population. The ingredient and process parameters were optimized by Taguchi methodology. The optimum conditions turn out to be hazel nut flour 24.3%; quinoa flour 7.1%; ammonium bicarbonate 0.6% and 22 min baking time. The shelf life study expressed as conjugated dienes was 3.6% after 45 days of storage at 30 °C, proving its stability to rancidity. The products were approved by all, the acceptance level ranging from liking very much to liking (Del Castillo et al. 2009b). Quinoa-based diet was recommended for celiac patients considering its high nutrition density and absence of gluten (Hager et al. 2012).

The major biochemical changes elicited by quinoa seed consumption have been presented in Fig. 9.2.

9.3 Storage and Processing

The properties of starch in raw and heat-treated samples of quinoa were investigated (Ruales and Nair 1994). The starch gelatinised at 67 °C and showed degradation on heat treatment. The cooked samples manifested the highest degree of gelatinisation (97%), followed by the drum-dried (96%) and autoclaved (27%) samples. The total dietary fibre content in the cooked sample (11.0%) was significantly lower than that in the autoclaved (13.2%), drum-dried (13.3%) or raw samples (13.3%), while the insoluble dietary fibre fraction in the samples did not change with heat treatment. The amino acid composition and the physicochemical and functional properties of

quinoa flour proteins were evaluated using elrectrophoresis, differential scanning calorimetry, UV and fluorescence spectroscopy (Abugoch et al. 2009). During storage, loss of protein solubility and water absorption occurs. To avoid these undesirable changes, quinoa flour can be stored at ambient temperature (between 20 and 30 °C) and packed in double kraft paper bags.

9.4 Antinutrition and Detrimental Effects

Saponins, coating the quinoa seeds are bitter-tasting, antinutritional factors. They irritate immune system and perforate microvilli of gut mucosa. Various triterpene saponins were reviewed, emphasizing on the biological action of mono- and bidesmosidic triterpene saponins and aglycones (Kuljanabhagavad and Wink 2009). The adjuvant activity of quinoa on the humoral and cellular immune responses of mice, subcutaneously immunized with ovalbumin was evaluated (Verza et al. 2012). The saponin fraction enhanced the amount of anti-ovalbumin-specific antibodies in its serum. Also, a saponin fraction significantly enhanced the Concavalin A-induced splenocyte proliferation. Zevallos et al. (2012) reported the presence of celiac-toxic epitopes in some cultivars of quinoa (Ayacuchana and Pasankalla). Though, the concentrations were below the permissible limit for a gluten-free food, they could activate the adaptive and innate immune responses in some patients with celiac disease. Further epidemiologic investigation must be carried out to dispel this disparity of result.

9.5 Cultivars

Several varieties of quinoa are commercialized. Each cultivar has their merits and demerits in terms of cultivation period, hardiness, seed taste etc. Dave, Temuco, Brightest Brilliant, Faro, Isluga, Cahuil are some popular varieties. The cultivars vary in terms of distribution and composition of saponins. Novel triterpene aglycones were detected and characterized according to their fragmentation reactions in ESI-MS/MS and electron ionization mass spectrometry (EI-MS) (Mad et al. 2006). The nutritional content of six Chilean quinoa ecotypes was assessed to determine the effect of climatic and edaphic conditions (Miranda et al. 2012). Quinoa of the Villarrica ecotype showed the highest protein, vitamins E and C content; Regalona ecotype showed the highest content of vitamins B1 and B3; Ancovinto ecotype showed highest content of vitamins B2, while Cancosa ecotype contained the highest potassium. The USDA National Institute of Food and Agriculture is providing grant to research on quinoa cultivation. Focus is being put on ascertaining the seeding rate, planting date, row spacing, nutritional value, heat and salinity tolerance etc. North American farmers are attempting to grow quinoa along the Rocky Mountain Range from Colorado to Saskatchewan. Quinoa was cultivated in South

Eastern Europe as a field trial (Stikic et al. 2012). Even, rainy climatic and fertilizer-free conditions resulted in seed yield of good quantity and quality. The seeds contained protein ranging from 15.16 to 17.41 %. These results raise possibility of wider cultivation of this valuable crop. RNA interference (RNAi) technology is being implicated for nutritional enhancement and antinutrients reduction of several crops. Quinoa cultivation might be benefitted from this gene suppression strategy (Katoch and Thakur 2013).

9.6 Future Scopes

Quinoa is being incorporated in nutritional supplements designed for pregnant and nursing women. Also, it is integrated in school breakfasts in Peru. Even NASA is considering quinoa chow for long-duration planetary space flights. Looking at the potential of quinoa in eradication of hunger, malnutrition and poverty, 2013 has been launched as the 'Internation year of quinoa' by Food and Agricultural Organization (FAO). Already a paradigm shift has been recorded in cultivation trends in Peru, Bolivia, Ecuador, Chile, Colombia and Argentina. It is being speculated that given due impetus, it can improve cash flow to the country growing quinoa crop. Also, it is being harvested in the United States, Canada, France, United Kingdom, Sweden, Denmark, Italy, Kenya and India. Drought resistance and great adaptation qualities have rendered it easy to grow. Even birds don't harm the crop due to the saponin-coated seeds. The high protein and amino acid content of quinoa indicates that selenium is contained as selenoamino acid derivatives. Selenized quinoa was cultivated in selenium-fortified soil (Kitaguchi et al. 2008). On extraction and subsequent HPLC analysis, total selenium concentration was measured to be about 102 μg/g wet weight, that is high enough to exert health benefits. Several research bodies are investigating the functional food potential of quinoa. Advanced technologies are required for extracting quinoa oil, starch and saponin.

9.7 Conclusion

The findings above build prospect that quinoa can be a worthy alternative to conventional staple grains amidst the current food crisis. Its wholesome nutrient profile and flexible adaptability pitches it as a suitable crop for global cultivation. If granted substantial attention, it might bring a massive shift in staple food trend and become a mainstay in global food platter. This chapter is expected to play significant role in augmenting the public awareness about the emergent supergrain quinoa.

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Chapter 10
A Promising CAM Therapeutic For Multiple Cancers: Milk Thistle (Silybum)

Abstract Milk thistle or *Silybum marianum*, a member of Asteraceae family has shown great promise as an emerging anticancer agent. Pronounced inhibition towards a broad range of cancers *viz*. skin, larynx, lungs, breast, liver, pancreas, ovary, prostate, colon, kidney, cervix and blood have been documented. The compounds silymarin and silibinin (a flavonolignan) in the seed extract have been credited with most of the therapeutic potency. This chapter presents a latest account of the preventive role of milk thistle, with emphasis on anti-neoplastic, anti-angiogenesis, anti-metastasis, synergy with chemotherapeutics and side-effect amelioration. Thrust has been put on the biochemical and genetic modulations. Hurdles like short half-life and poor bioavailability and scopes of efficacy augmentation have been discussed. This chapter is of importance for progress in onco-treatment and the development of a robust adjunct therapy.

Keywords Milk thistle · Anticancer · Antimetastasis · Apoptosis · Silymarin · Silibinin

10.1 Introduction

Milk thistle (genus Silybum), a member of Asteraceae family is a medicinally-potent plant. It is a native of the Mediterranean region and is one of the most widely used herbs in the United States with a long history of health remedial use. The plant has erect and branched stems, broad lobed leaves with spines, purple flowers and black achene fruits (Fig. 10.1a and b). Silymarin and silibinin ($C_{25}H_{22}O_{10}$) are polyphenolic flavonolignans, extracted from the fruits and seeds. These bioactives are known to confer antioxidant, antilipemic, anticancer, antidiabetic, hepato- and renoprotective effects. The efficacy against a range of cancers has grabbed much interest from drug discovery fraternity. Vaid and Katiyar (2010) reviewed the UV-induced skin cancer preventive potential of silymarin and the antioxidant, anti-inflammatory and immunomodulatory properties were accounted for the protective role. Cheung et al. (2010) reviewed the pharmacokinetics, mechanisms, effectiveness and adverse effects of silibinin-based anticancer therapy. The influence of histologic subtype, hormonal status, stromal interactions and drug-metabolising gene polymorphisms

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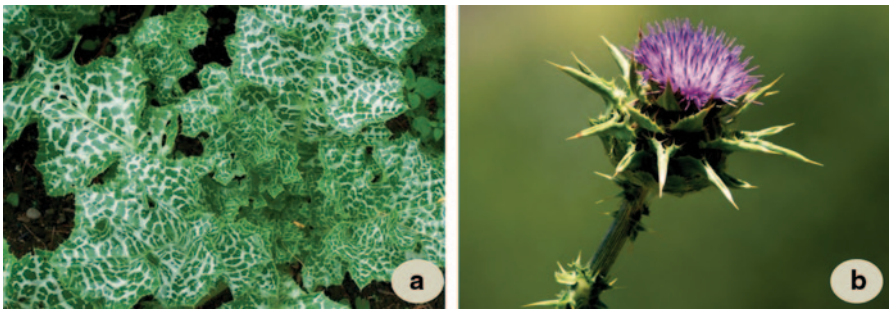

Fig. 10.1 Milk thistle. **a** Whole plant. **b** Flower

were emphasized. Li et al. (2010) reviewed the regulatory roles of silibinin in antiproliferative signaling pathways. Ozten-Kandas and Bosland (2011) also reviewed on natural anticancer drugs, including milk thistle and its derivative silibinin. Weidmann (2012) reviewed the therapeutic roles and mode of action of dihydroquercetin (taxifolin), a potent flavonoid found in many plant sources, including milk thistle. It's mechanisms of action, the activation of the antioxidant response element and detoxifying phase II enzymes, inhibition of cytochrome P450 and fatty acid synthase in carcinogenesis were proposed. TNF-α and NF-κB dependent transcription in hepatitis C infections, the scavenging effect, the effects on cholesterol biosynthesis as well as the effects on apob/apoA-I, HMG-CoA reductase and apoptosis were reviewed. Deep and Agarwal (2013) reviewed the effect of silibinin on cellular and non-cellular components of the tumour microenvironment. It influenced the microenvironment constituents by disrupting tumour growth, angiogenesis, inflammation and metastasis. Targeting the signaling molecules for epithelial-to-mesenchymal transition, proteases activation, adhesion, motility, invasiveness *etc*. resulted in the above changes.

10.2 Actions of Milk Thistle Bioactive Components on Various Cancers

The inhibitory actions of milk thistle extracts on various types of cancers are increasingly being validated. The crucial outcomes have been discussed in the following sections.

10.2.1 Skin

Yu et al. (2012b) investigated the contribution of silibinin to the induction of apoptosis and autophagy via generation of ROS and NO in human epidermoid carci-

noma A431 cells. It inhibited the cell growth in a dose-and time-dependent manner. At a high dose (400 μM) silibinin induced apoptosis through both the intrinsic and extrinsic apoptotic pathways. Silibinin treatment (50 mg/kg) daily for 4 days reversed dermal and epidermal autophagy levels from UVB irradiation-induced improper autophagy intervention, repaired the balance between cell survival and death, protected skin against damage through mediation of p53 activation in dermal and epidermal cells (Wang et al. 2013). Lee et al. (2013) investigated the effect of silybin on melanoma cell growth and elucidated its molecular targets. Treatment of melanoma cells with silybin attenuated the phosphorylation of ERK-1/2 and ribosomal S6 kinase (RSK)-2, which are regulated by the upstream kinases MAPK1/2. The blockade of MAPK1/2-ERK1/2-RSK2 signaling by silybin resulted in a reduced activation of NF-κB, activator protein-1, and STAT3, which are transcriptional regulators of a variety of proliferative genes in melanomas. It attenuated the growth of melanoma xenograft tumors in nude mice. Silybin inhibited the kinase activity of MAPK-1/2 and RSK-2 in melanoma cells. Cell cycle arrest was induced at the G1 phase and inhibited melanoma cell growth *in vitro* and *in vivo*. It was inferred that silybin suppresses melanoma growth by directly targeting MAPK- and RSK-mediated signaling pathways.

10.2.2 Larynx and Lungs

Bang et al. (2008) demonstrated that silibinin induces apoptosis of laryngeal squamous carcinoma SNU-46 cells by a mechanism involving decreased survivin (the protein inhibitor of apoptosis) expression in a dose- and time-dependent manner. Silibinin (200 μmol/L) treatment raised the population of apoptotic cells from 7 to 40%. Mateen et al. (2013) investigated the efficacy of silibinin either alone or in combination with epigenetic therapies to modulate E-cadherin expression in a panel of non-small cell lung cancer (NSCLC) cell lines. Silibinin combined with HDAC inhibitor (Trichostatin A) or DNMT inhibitor (5'-Aza-deoxycytidine) significantly restored E-cadherin levels in NSCLC cells. Treatment of NSCLC cells with basal E-cadherin levels, by silibinin further increased their expression and inhibited migratory and invasive potential. Also, silibinin alone or in combination with the inhibitors down-regulated the expression of Zeb1, known to be a major transcriptional repressor of E-cadherin.

10.2.3 Breast

Kim et al. (2009) determined the effect of silibinin on 12-O-tetradecanoyl phorbol-13-acetate (TPA)-induced MMP-9 and COX-2 expression in the human breast cancer MCF-7 and MDA-MB231 cells. The flavonoid downregulated MMP-9 and COX-2 expression, most likely by blocking NF-κB, PI3K/Akt, and ERK1/2 signaling. Wang et al. (2010) observed the sustained O^{2-} production capability of silibinin in MCF-7 cells. It was correlated to the apoptosis enhancement ability of

exogenous SOD. Tamaki et al. (2010) evaluated the inhibitory effects of various herbal extracts and isoflavonoids, including milk thistle, on intestinal breast cancer resistance protein (BCRP). It inhibited BCRP-mediated transport of the xenobiotic methotrexate, though faintly. Kim et al. (2010) reported the enhancement of TPA-induced G2/M phase arrest in breast cancer cells and p21 expression by silibinin. It was derived that silibinin has an additive effect on TPA-induced growth arrest through the PI-3-kinase/Akt-dependent pathway. Kim et al. (2011) investigated the effect of silibinin on the epidermal growth factor (EGF) ligand-induced CD44 expression in human breast cancer SKBR3 and BT474 cells. CD44 and MMP-9 expression was reduced by silibinin treatment in a dose-dependent manner. Also, silibinin suppressed the phosphorylation of EGF receptor and the downstream signaling molecule ERK1/2. Noh et al. (2011) investigated the effect of silibinin in MCF-7 cells and determined whether it enhances UVB-induced apoptosis. Analysis by MMT assay,Western blot and flow cytometry furnished promising results. A dose- and time-dependent reduction in viability was observed which was correlated to increased p53 expression and induction of apoptosis. The combination of silibinin and UVB resulted in an additive effect on apoptosis in MCF-7 cells. Dunnick et al. (2011) studied the effect of milk thistle extract in rodents and reported a decrease in spontaneous background tumours including mammary gland tumours. The radical-scavenging and antioxidant properties of silibinin and allied flavonolignans were accredited to confer the protection. Dastpeyman et al. (2011) investigated the effect of silibinin on proliferation, migration and adhesion capacity of MDA-MB-231 cells by MTT assays. Dose-dependent inhibition of Cdc42 and D4-GDI mRNAs expression was recorded by RT-PCR. Further, silibinin modulated β1-integrin signaling pathway and RAF1 function, indirectly but effectively. Oh et al. (2013) reported that TPA-induced MCF7 cell migration and MMP-9 expression was significantly decreased and phosphorylation of MEK and ERK was inhibited by silibinin.

10.2.4 Liver and Pancreatic

Ramakrishnan et al. (2009) investigated whether the ingestion of silymarin has any role in mast cell density (MCD) and in the expressions of MMP-2 and MMP-9 in N-nitrosodiethylamine induced-liver cancer in rats. Silymarin treatment inhibited the abnormal increase in MCD (associated with neoplasm) and down-regulated the expressions of MMP-2 and MMP-9 (involved in invasion and angiogenesis). Brandon-Warner et al. (2010) demonstrated that silibinin inhibits *in vitro* cytochrome p4502E1 induction, ethanol metabolism and ROS generation in hepatocellular carcinoma HCC cells *in vitro*. Bousserouel et al. (2012) reported the silibinin-mediated activation of TRAIL death receptor apoptotic signaling pathway both *in vitro* and in hepatocarcinoma grafts in mice. Silibinin activated the extrinsic apoptotic pathway in Hep55.1C cells, manifested in the up-regulation of TRAIL and death receptor 5 transcripts as well as by the activation of caspase-3 and -8. Grafting of the cancer cells into mouse liver, followed by oral administration of silibinin (700 mg/kg) for four weeks, caused shrinkage

of tumour growth, associated with the down-regulation of MMP-7, MMP-9, IL1β and the up-regulation of TRAIL, DR5, and caspase-3 activation. Nambiar et al. (2012) investigated *in vitro* and *in vivo* effects of silibinin against both primary and advanced stages of human pancreatic carcinoma cells. Silibinin (25–100 μM) treatment for 24–72h caused a dose- and time-dependent cell growth inhibition in BxPC-3 cells (27–77%) and in PANC-1 cells (22–45%). Feeding of silibinin to nude mice (0.5%, w/w in AIN-93M diet for 7 weeks) inhibited BxPC-3 and PANC-1 tumor xenograft growth. Tumour volume and weight were significantly reduced, though the efficacy differed in the cells.

10.2.5 Ovary

Cho et al. (2013) examined the effect of silibinin *in vitro* and *in vivo* on tumour growth in human ovarian cancer cells. Silibinin increased ROS generation inside the cancer cells and compromised their viability. Oral administration of silibinin to animals with subcutaneous A2780 cells reduced tumour volume. Tumour tissue analysis showed that silibinin treatment induced a decrease in Ki-67-positive cells, an increase in transferase-mediated dUTP nick end labeling (TUNEL)-positive cells, activation of caspase-3, and inhibition of p-ERK and p-Akt.

10.2.6 Prostate

Silibinin or silipide (silibinin formulated with phospholipids) when given in diet delayed tumour development in transgenic adenocarcinoma of the mouse prostate (TRAMP) model. Silipide decreased plasma levels of insulin-like growth factor (IGF)-1 (involved in cell transformation and proliferation) by 36% and reduced tumour size by 31% (Verschoyle et al. 2008). Mokhtari et al. (2008) reported that PC-3 cells when incubated with silibinin, dose- and time-dependent inhibition of viability, motility and adhesion of highly metastatic cells occurred. Raina et al. (2008) assessed the dietary efficacy of silibinin in TRAMP model. The mice fed with 1% silibinin-enriched diet for 8–15 weeks, showed less severe lesions. The tumour suppressive effect was mediated by antiproliferation as well as inhibition of angiogenesis. Decreased expressions of platelet endothelial cell adhesion molecule-1/CD-31, VEGF and associated receptors, HIF-1α, and iNO synthase were correlated to the lowered tumorigenicity. Metastasis to distant organs was decreased in silibinin-fed mice, which was associated with a decreased expression of MMPs, mesenchymal markers snail-1, and fibronectin in the prostatic tissue and retention of epithelial characteristics. Jung et al. (2009) investigated the ability of silibinin to inhibit HIF-1α expression in LNCaP and PC-3 prostate cancer cells and found that it was associated with the suppression of global protein translation. Vidlar et al. (2010) determined whether a daily administration of a silymarin and selenium combination for 6 months would alter basic clinical chemistry and oxidative stress markers, and improve the quality

of life score in men after radical prostatectomy. Thirty seven participants, 2–3 months after the surgery, were randomly assigned to receive 570 mg of silymarin and 240 μg of selenium as selenomethionine or placebo daily for 6 month. The six month administration of silymarin and selenium improved the quality of life score, decreased LDL and total cholesterol while increasing the serum selenium levels. Also, the combinatory treatment reduced the markers of lipid metabolism linked with prostate cancer progression. Iczkowski (2010) conducted luciferase promoter construct to test for CD44 promoter activity of silibinin. Oncogenic microRNAs, miR-373 and miR-520c bind to the 3' untranslated region of the CD44 RNA and suppress CD44 in prostate cancer. It was observed that stable re-expression of CD44s reduces metastasis of cancer cells. Wu et al. (2010) found that silibinin treatment results in the up-regulation of cytokeratin-18 and down-regulation of vimentin and MMP2 in bone metastatic prostate ARCaPM cell line. Also, it inhibited NF-kB p50 translocation via the up-regulation of IkB-α protein. Further, it down-regulated the expression of two major epithelial-mesenchymal transition regulators ZEB1 and SLUG, thus checking cancer progression. Kavitha et al. (2012) examined the effect of silibinin on bone metastatic prostate cancer PCA cells-induced osteoclastogenesis employing human PC3MM2, PC3, and C4-2B and murine macrophage RAW264.7 cells. At 30–90 μM dose, the flavonoid inhibited PCA cells-induced osteoclast activity and differentiation in RAW264.7 cells by modulating the expression of several cytokines (IGF-1, TGF-β, TNF-α, I-TAC, M-CSF, G-CSF, GM-CSF) that are important in osteoclastogenesis. In RAW264.7 cells, silibinin decreased the RANKL-induced expression and nuclear localization of NFATc1, which is considered the master regulator of osteoclastogenesis. Also, the DNA binding activity of NFATc1 and its regulators NF-κB and AP1, and the protein expression of osteoclast specific markers (TRAP, OSCAR, and cathepsin K) were decreased. Silibinin also decreased the expression of osteomimicry biomarkers (RANKL, Runx2, osteocalcin, and PTHrP) in cell culture (PC3 and C4-2B cells) and/or in PC3 tumors. Lu et al. (2012) found that silibinin inhibits LRP6 promoter activity and decreases LRP6 mRNA levels in in prostate cancer PC-3 and DU-145 cells. Deep et al. (2012) considered different isomers of silybin (silybin A, silybin B, isosilybin A and isosilybin B) for combating advanced prostate cancer. Ingestion of these flavonolignans (50 and 100 mg/kg body weight) effectively inhibited the growth of advanced human prostate cancer PCA DU145 xenografts. Immunohistochemical assays revealed that these isomers selectively inhibit tumour angiogenesis biomarkers (CD31 and nestin) and signalling molecules regulating angiogenesis (VEGF, VEGFR1, VEGFR2, phospho-Akt and HIF-1α). Ting et al. (2013) reported that silibinin is capable of modulating cell signalling, proliferation, apoptosis, epithelial-mesenchymal transition, invasion, metastasis and angiogenesis. Thus, it might be developed as a prostate cancer chemopreventive agent.

10.2.7 Colon

Colorectal cancer is infamous for claiming countless lives and there is always a search for effective drugs to combat it. Toyoda-Hokaiwado et al. (2011) investigated the suppressive effects of silymarin against carcinogenicity and genotoxicity induced by 1,2-dimethylhydrazine (DMH) injection followed by oral DSS in the colon of F344 gpt delta transgenic rats. The test animals were fed diets containing silymarin for 4 weeks, starting 1 week before DMH injection and samples were collected at 4, 20 and 32 weeks after the DMH treatment. Silymarin (100–500 ppm) suppressed the tumour formation in a dose-dependent manner. Also, it substantially reduced (20%) the mutant frequency in the colon. Genotoxicity of DMH was decreased in a dose-dependent manner in bacterial mutation assay with *Salmonella typhimurium* YG7108. Colombo et al. (2011) studied whether silymarin pretreatment amplifies the effect of chemotherapy. Silymarin-doxorubicin and silymarin-paclitaxel treatments were evaluated on two colon carcinoma LoVo and the multidrug-resistant isogenic LoVo/DX cell lines. Pre-treatment with low dose silymarin worked in synergy with both doxorubicin and paclitaxel in LoVo cells, whereas high dose of silymarin showed additive effect with both the chemotherapeutics. However, silymarin favourably interfered with uptake and cell cycle effects of the chemotherapeutics in LoVo cells only. Kauntz et al. (2011) investigated the mechanisms of silibinin-induced cell death using primary tumor SW480 and their derived metastatic SW620 cells. Silibinin triggered apoptosis in both types of cells manifested by DNA fragmentation and activation of caspase-3. It enhanced the expression TRAIL and death receptors at the cell surface in SW480 as well as in SW620 cells. Extrinsic nature of the apoptotic pathway was confirmed from the involvement of Caspase-8 and -10. The protein Bid was cleaved in SW480 cells indicating a cross-talk between extrinsic and intrinsic apoptotic pathway. Silibinin activated also the intrinsic apoptotic pathway in both cell lines, including the perturbation of the mitochondrial membrane potential, the release of cytochrome c into the cytosol and the activation of caspase-9. Yan et al. (2012) assessed the effect of a blend of polyphenon E (green tea) and siliphos (main component silibinin) on the growth of subcutaneous colon cancer CT-26 in several murine models. When administered daily for 7–9 days preprocedure and for 7–21 days postoperatively, the blend significantly inhibited tumor mass, number and size of hepatic metastaes, and proliferation and apoptosis rates. Kauntz et al. (2012a) used azoxymethane -induced colon carcinogenesis model of rats to study the effect of silibinin. One week after the genotoxic agent injection, the rats received daily intragastric dose of 300 mg silibinin/kg body weight daily until being killed after 7 weeks of treatment. Silibinin-treated rats exhibited a 2-fold reduction in the number of -induced hyperproliferative crypts and aberrant crypt foci in the colon. Silibinin-induced apoptosis occurred via down-regulation of the anti-apoptotic protein Bcl-2 and up-regulation of the pro-apoptotic protein Bax. Also, this treatment significantly decreased the genetic expression of biomarkers of the inflammation (IL1β, TNFα) and their downstream target MMP7. Silibinin effec-

tively checked colon carcinogenesis by anti-inflammation and eventual apoptois. Kauntz et al. (2012b) investigated the effect of silibinin and TRAIL in SW480 and SW620 cells. Reverse transcriptase PCR (RT-PCR) and flow cytometry showed synergistic induction of cell death in both the cell lines, manifested in up-regulation of death receptor 4 and 5. Synergistic activation of caspase-3, -8, and -9 by silibinin and TRAIL was shown by colorimetric assays. Silibinin and TRAIL potentiated activation of the mitochondrial apoptotic pathway and down-regulated the anti-apoptotic proteins Mcl-1 and XIAP. Karim et al. (2013) evaluated the effect of silibinin on the Cdk4 pathway in Apc-/+ mice (Cdk4 pathway alterations have been linked to many forms of tumour development, including colon cancer). The flavonoid effectively targeted this pathway causing hypophosphorylation of the retinoblastoma protein (a tumour suppression protein), inhibited cell growth and induced apoptosis. Silibinin blocked the development of intestinal adenomas in the mice by 52 %. Raina et al. (2013) reported that silibinin induces oxidative stress in SW480 cells due to ROS generation accompanied with disruption of mitochondrial transmembrane potential and cytochrome c release. These changes activate procaspase 3, ultimately resulting in apoptosis. With increased exposure to silibinin, decrease in apoptotic response and increase in autophagic events occurs. Kauntz et al. (2013) investigated the epigenetic effects of silibinin in primary adenocarcinoma cells SW480 and their derived metastatic cells SW620. Silibinin could significantly inhibit DNA methyltransferase (DNMT) activity in both SW480 and SW620 cells. The clinically used HDAC inhibitor (suberoylanilide hydroxamic acid and trichostatin A) combined with silibinin synergistically induced cell death. Selective targeting of the inhibitors and non-toxicity of silibinin held promise for cancer inhibition.

10.2.8 Kidney

Kaur et al. (2010) investigated the protective effect of silymarin against iron nitrilotriacetate-mediated renal oxidative stress, inflammation and tumour promotion response. Intraperitoneal administration of the nephrotoxicant to mice induced marked oxidative stress in kidney, evident from the high renal metallothionein expression, depletion of glutathione content and enhanced production of aldehyde products. Feeding of 0.5 and 1 % silymarin diet lowered oxidative stress and inflammation by promoting metallothionein expression, reducing NF-kB activation and decreasing the expression of proinflammatory mediators. Silymarin also suppressed the induced hyperproliferation in kidney, ameliorating renal ornithine decarboxylase activity and DNA synthesis.

10.2.9 Cervix

Garcia-Maceira and Mateo (2009) derived that silibinin inhibits the accumulation and transcriptional activity of hypoxia-induced HIF-1α protein in HeLa cells. The attenuation was correlated with strong dephosphorylation of mammalian target of rapamycin (mTOR) and its effectors ribosomal protein S6 kinase (p70S6K) and eukaryotic initiation factor 4E-binding protein-1 (4E-BP1), a pathway known to regulate HIF-1α expression at the translational level. Also, silibinin reduced hypoxia-induced vascular VEGF release by HeLa cells, and this effect was potentiated by the PI3K/Akt inhibitor LY294002. Yu et al. (2012a) evaluated the potential action of silymarin against cervical cancer C-33A cells and investigated its mechanism of action. Silymarin induced apoptosis of the cells through the modulation of Bcl-2 family proteins and activation of caspase 3. Further, it inhibited the phosphorylation of Akt with an increase in expression of phosphatase and tensin homolog (PTEN). Also, silymarin significantly inhibited the expression of MMP-9 in C-33A cells.

10.2.10 Leukemia

Ladas et al. (2010) conducted a double-blind, randomized study in children with acute lymphoblastic leukemia to investigate the therapeutic feasibility of milk thistle. The subjects were adminstered with it for 28 days. Biochemical analyis revealed significantly lower aspartate amino transferase enzyme in the milk thistle group. Chemotherapy doses were reduced in 61 % of the milk thistle group compared with 72 % of the placebo group. *In vitro* study revealed a modest synergistic effect with vincristine (anticancer alkaloid extracted from *Vinca rosea*). Further, it did not hamper the effects of chemotherapy agents used for the treatment of leukemia.

10.3 Antimutagenic Effect

Kaleeswaran et al. (2009) assessed the antimutagenic activity of silymarin using an *in vitro* Ames bacterial reverse mutation assay. It showed stronger antimutagenic effect against 2-aminofluorene and 4-nitroquinoline N-oxide induced mutation in *Salmonella typhimurium* tester strains TA97a and TA98.

10.4 Side Effect Amelioration/Supportive Care Regimen

Ninsontia et al. (2011) determined whether silymarin is capable of protecting renal cells from cisplatin-induced cell death. The pretreatment with 25–200 μM of silymarin significantly offered protection in a dose-dependent manner. At a dose

of 25–100 μM, it amplified the drug-induced apoptosis in melanoma G361 cells. These findings reveal the selectivity of silymarin in shielding renal cells against chemotherapeutic abuse, which could be vital for development of renoprotective agent. McBride et al. (2012) reported the efficacy of milk thistle as a supportive care agent against the chemotherapy side effect transaminitis. The patient had an immediate decrease of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) level in blood.

10.5 Efficacy Bolstering Strategy

The medicinal potency of milk thistle has garnered enough credence, but the clinical trials do not always yield positive result, unlike the *in vitro* tests. Issues are often encountered owing to the hydrophobic nature, low water solubility, feeble absorption and poor bioavailability. Flaig et al. (2010) studied the effect of silibinin in subjects with localized prostate cancer scheduled to undergo prostatectomy. The patients received 13 g of silybin-phytosome daily for 14–31 days prior to surgery. High-dose intake of the complex helped attain high blood concentrations. Yet, silibinin level in prostate tissue was measured low. Its meagre tissue penetration was correlated to the small half-life, the brief duration of therapy in this study or any active process purging silibinin from the prostate organ. Hydrophobicity of silibinin is deemed another responsible factor for its restricted bioavailability. Cho et al. (2012) attempted to enhance the efficacy of silibinin by formulating a biodegradable poly (organophosphazene) hydrogel-based injectable drug carrier. The aqueous solution of the hydrogel enhanced the solubility of silibinin up to 2000 fold compared to that of phosphate buffered saline. A faster *in vitro* degradation and drug release rate from the gel was recorded at pH 6.8 at temperature 37 °C. Propelled by diffusion, silibinin was released from the hydrogel in a sustained manner. In the HT-29 xenografted mice model, the intra-tumorally injected silibinin containing-hydrogel exhibited better antitumor effect compared to the control groups, the superiority credited to the sustained release of the phytochemical. The milk thistle active compounds often succumb to short half-life due to conjugation, particularly glucuronidation. Sy-Cordero et al. (2013) attempted to generate analogues with lowered metabolic lability and longer half-life. Five methylated analogues of silybin B were synthesized and their potencies were determined in a 72 h growth-inhibition assay against a panel of three prostate cancer (DU-145, PC-3, and LNCaP) and a human hepatoma (Huh7.5.1) cell lines. The compounds were evaluated for inhibition of both cytochrome P450 2C9 (CYP2C9) activity in human liver microsomes and hepatitis C virus infection in Huh7.5.1 cells. Among the derivatives, the monomethyl and dimethyl analogues possessed enhanced activity in terms of cytotoxicity, CYP2C9 inhibitory potency (CYP2C9 is a crucial cytochrome P450 enzyme with key role in oxidation of drugs), compared to the parent compound. Agarwal et al. (2013) attempted to intensify the potency of silybin by structural modification. The derivatives 2, 3-dehydrosilybin, 7-O-methylsilybin and

7-O-galloylsilybin were synthesized and employed against human bladder HTB9, colon cancer HCT116 and prostate carcinoma PC3 cells. In all the 3 cell lines, the modified products demonstrated better inhibitory effects than the native compound. Also, the optical isomers of the derivatives fared better than the silybin isomers. Ebrahimnezhad et al. (2013) devised a method to improve silibinin-basesd drug delivery method. The flavonoid was loaded in PLGA-PEG-Fe_3O_4 and the cytotoxic effect of this nano-drug on breast cancer T47D cell line was determined by MTT assay. Real Time PCR analysis showed that the level of telomerase gene expression more efficiently decreased with this drug composite than with free silibinin alone.

10.6 Conclusion

The above discussed findings furnish enough testimony for the undisputed role of milk thistle and novelty of silibinin in onco-therapy regimen. Its efficacy against different cancers at various stages of malignancies unfolds many prospects. Mechanistic exploration of the chemo-preventive efficacy of milk thistle components must be intensified. The mechanisms of actions need further elucidation in order to be well-versed with the molecular interactions. Better profiling of bioactive principles, functional food formulations, CAM drug inventions remains areas to be addressed. Given due investigative input, it might offer attractive supportive care in the endeavor to ease cancer burden.

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Chapter 11
Chaga (*Inonotus Obliquus*) Mushroom: Nutraceutical Assesement Based on Latest Findings

Abstract Chaga mushroom (*Inonotus obliquus*) is a white-rot fungus growing on birch trees. It looks like burnt coal, has edibility and is regarded a traditional medicinal mushroom in China, Russia, Poland, other Baltic countries. It is hailed as a folk remedy against diabetes, digestive disorders, intestinal worms, liver ailments, cardiovascular diseases, tuberculosis and cancer *etc*. Recently, it has grabbed attention from mainstream medicine as a therapeutic mushroom. The discovery of a diverse range of secondary metabolites including, phenolic compounds, polysaccharides, melanins, lanosterol, inotodiol, ergosterol and trametenolic acid have promoted it as a potential drug candidate. Biological roles as antioxidant, antiinflammation, anticancer, immunomodulation, antiallergy, antilipemic, hypoglycaemic, antiviral, neurostimulation have been validated. This chapter delves into the biological roles and mechanisms governing the effects.

Keywords Chaga · *Inonotus obliquus* · Polysaccharide · Antioxidant anticancer · Antiviral

11.1 Introduction

Chaga (*Inonotus obliquus*) is a member of Hymenochaetaceae family. It grows mostly on birch trees. The dark sclerotia are harvested for food and medicinal purposes. The crushed powder is generally consumed as tea, latte, soups, stews, smoothies *etc*. Polysaccharide, phenolic compounds, hispidin analogues, lanostane triterpenoids (inotodiol and spiroinonotsuoxodiol) and trametenolic acid have been discovered to be the active components in this mushroom (Du et al. 2011). Different solvents have resulted in extraction of variable bioactive compounds (Zheng et al. 2011). Owing to these ingredients, chaga is recognized as an adaptogen and synergistic medicine. It is validated to possess antioxidant, anti-inflammation, immunomodulatory, anticancer, antilipemic, hypoglycaemic and cognitive properties. Also, the culinary possibilities of chaga are being explored as entrepreneurs are experimenting with chaga-based food manufacturing. Several reviews have been published in recent times that reflect various biological aspects of chaga mushroom. Zheng et al. (2010) examined the current progress in the discovery of its chemical

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diversity, biological activities and the strategies to enhance bioactive production. Lee and Yun (2011) also interpreted the structural diversity, healthy effects and biogenesis of styrylpyrone-class polyphenols. Sysoeva et al. (2012) reviewed on antioxidant-rich additives and drugs development from chaga meal extracted by ethanol, chloroform and *tert*-butyl methyl ether. Song et al. (2013) summarized the recent findings on the mechanisms underlying the anticancer influence of *I. obliquus*. This chapter discusses the augmented phenolic compound production by submerged fermentation, co-culture and response surface methodology. Also, the *status quo* of functional food and CAM development is emphasized. Overall, this chapter presents a holistic appraisal of the latest nutraceutically and pharmaceutically-relevant findings and prospects ahead.

11.2 Beneficial Effects on Health

Recent times have witnessed an upsurge in medicinal vigor of chaga. The validated therapeutic effects are discussed below (Fig. 11.1).

11.2.1 Antioxidant Activity

Intracellular generation of ROS leads to oxidative stress and subsequent damage of cellular and nuclear components. Ample evidences validating the significant antioxidant effect of *I. obliquus* exist. Liang et al. (2009) investigated the antioxidant activities of the ethanolic crude extracts and three subfractions (ethyl acetate, n-butanol and aqueous) of *I. obliquus* by sequential partitioning. The total antioxidant

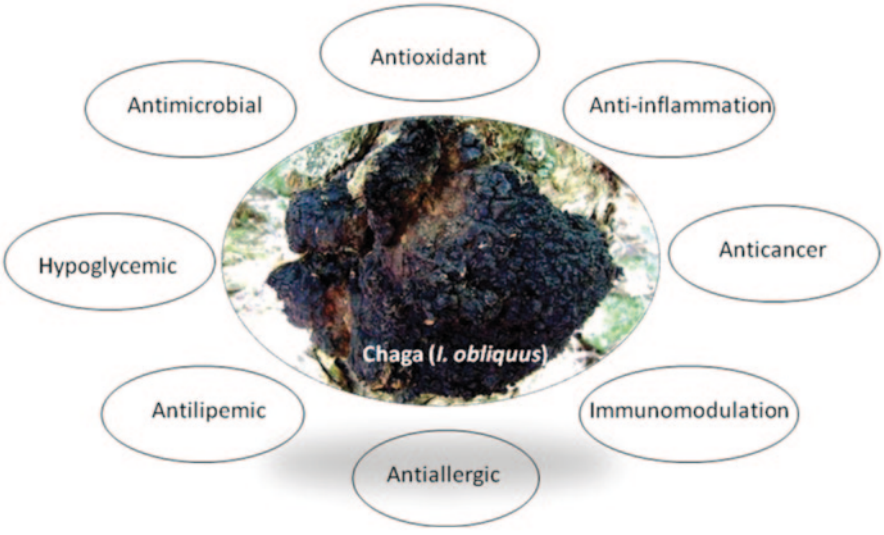

Fig. 11.1 Chaga mushroom with its therapeutic spectrum

capacity was measured by FRAP and DPPH assays. The total phenolics and total flavonoids were also determined by spectrophotometry. The ethyl acetate fraction showed maximum antioxidant activity followed by n-butanol, crude extract and aqueous fraction. A strong correlation between the antioxidant activities and the phenolics and flavonoids contents were established. Nakajima et al. (2009) investigated the protective effects of chaga extracts against H_2O_2-induced oxidative stress in PC12 cells. The extracts and phenolic ingredients, 3,4-dihydroxybenzalacetone and caffeic acid effectively suppressed intracellular ROS level in the cells. Also, 3,4-dihydroxybenzalacetone selectively inhibited the phosphorylation of p38-MAPK. Zheng et al. (2011b) investigated the effects of solvents (ethyl acetate, acetone, ethanol and water) on metabolic profiles and antioxidant activities of chaga extracts. Polyphenols were most effective in quenching free radicals while polysaccharides and some lanostane-type triterpenoids strongly scavenged DPPH and hydroxyl radicals. Yun et al. (2011) investigated the cytoprotective effects of chaga against oxidative stress-induced apoptosis and premature senescence. Pretreatment with the mushroom scavenged intracellular ROS and prevented lipid peroxidation in H_2O_2-treated human fibroblasts. Also, it inhibited MMP-1 and MMP-9 activities in the fibroblasts, leading to increase in collagen synthesis. Further, it could avert the deleterious effects of UV, *i*.e. skin thickening and wrinkle formation in hairless mice. Mu et al. (2012) isolated polysaccharides from chaga and assayed antioxidant properties of the carbohydrate-rich fractions, which dose-dependently protected PC12 cells aginst H_2O_2 abuse. Huang et al. (2012) purified five polysaccharides with antioxidant effects, the activity exerted by their uronic acid and proteinous substance content and molecular weights. Lin et al. (2012) inoculated chaga in cooked embryo rice and monitored the mycelial growth. The chaga-grown rice had similar composition to embryo rice yet it contained a substantial amount of ergothioneine (101 mg/kg), higher amounts of soluble sugars and polyols, and umami taste compounds, including monosodium glutamate (MSG)-like components and flavor 5'-nucleotides. Zhao et al. (2012) investigated the effects of lanosterol, inotodiol and trametenolic acid in CCl_4-induced oxidative damage in mice. When administered at a dose of once daily for 3 days prior to stress exposure, significant alteration in antioxidative profile was noticed. Increase in the activities of SOD, CAT and GSH-Px and decrease in the MDA content of serum and liver homogenate was pronounced. Also, significant inhibition of the ALT and AST activities as well as decrease in the IL-6 concentration in the serum was monitored.

11.2.2 Antiinflammatory Effect

Park et al. (2005) investigated the anti-inflammatory and anti-nociceptive effects of its methanol extract both *in vivo* and *in vitro*. The extract reduced carrageenin-induced acute paw edema in rats. Also, it demonstrated analgesic activity in mice as tested by acetic acid-induced abdominal constriction test and a hot plate test. The extract significantly inhibited the productions of NO, PGE2 and TNF-α in LPS-stimulated RAW 264.7 macrophages. Also, it suppressed the protein and mRNA

expressions of iNOS and COX-2 via the down-regulation of NF-kB binding activity. Van et al. (2009) assessed its anti-inflammatory effects in LPS-induced murine macrophage RAW 164.7 cells. Water-soluble polysaccharide, polyphenolic as well as ethyl acetate faction of the ethanolic extract significantly inhibited the levels of inflammatory cytokines IL-1β, IL-6 and TNF-α. Each fraction showed a dose-dependant anti-inflammatory effect and significant reduction in NO production was reported. Choi et al. (2010) investigated anti-inflammation of chaga extract in DSS-induced colitis mice models. The incorporation of mushroom in diet decreased the expression of TNF-α and STAT1. The expressions of IL-4 and STAT6 as well as the serum IgE level decreased moderately. Also, the extract exerted toxicity towards RAW264.7 cells, to a low degree. Yan et al. (2011) explored the protective effects of its ethanol extract on ovalbumin-injected asthmatic mice. The concentrations of IL-4, IL-5, IL-13 and IFN-γ in BALF as well as the phosphor-p38 MAPK in lung tissues were measured. In the pathologic mice, all these immunological parameters had increased than normal level, whereas IFN-γ level had fallen. The extract treatment ameliorated the level of inflammatory cells and histopathological damage. It was inferred from the study that chaga extract effectively treats asthma by inhibiting the expression of phosphor-p38 MAPK, correcting the unbalance of IFN-γ/IL-4 and decreasing the number of inflammatory cells. Mishra et al. (2012) determined the effects of chaga water extract on DSS-induced colitis mice model. Histological examinations revealed the suppressive effects of the extract on edema, mucosal damage and the loss of crypts. The extract lowers iNOS levels and myeloperoxidase accumulation in colon tissues, demonstrating its suppressive effect on infiltration of immune cells. Significant inhibition of mRNA expression of pro-inflammatory cytokines TNF-α, IL-1β, IL-6, and IFN-γ was measured. Debnath et al. (2012) evaluated the *in vivo* antiinflammatory activity of ethanol extracts from chaga grown on germinated brown rice. Administartion of chaga to DSS-induced colitis mice model decreased the expression of TNF-α, COX-2, IL-4, IFN-γ, STAT1 and STAT6. Also, it led to the decrease in IgE and IgA in the spleen and mesenteric lymph node.

11.2.3 Anticancer Effect

A bulk of findings underlines the anticancer potential of chaga. Nomura et al. (2008) investigated its antitumor effect both *in vitro* and *in vivo*. As determined by MTT assay, the extract inhibited the proliferation of leukemia P388 cells. Inotodiol was found responsible for the inhibition of proliferation, DNA fragmentation, caspase-3/7 activation and subsequent apoptosis. The intraperitoneal administration of 10 mg/kg inotodiol prolonged the number of survival days of the test mice. Ham et al. (2009) evaluated the antimutagenic potential of the subfractions of its ethyl acetate extract. At a dose of 50 μg/plate, the subfractions strongly inhibited the mutagenesis induced in *Salmonella typhimurium* strain TA100. The components 3β-hydroxy-lanosta-8, 24-dien-21-al and inotodiol were credited for the antimutagenesis. Youn et al. (2009) examined its anti-proliferative effects on melanoma B16-F10 cells and mice. The water extract inhibited the growth of the cancer cells

by arresting cell cycle at G0/G1 phase and apoptosis, also by inducing cell differentiation. The down-regulation of pRb, p53 and p27 as well as reduction of cyclin E/D1 and Cdk 2/4 expression levels were deduced responsible for the effects. Intraperitoneal administration of this extract at dose of 20 mg/kg daily for 10 days significantly inhibited the growth of tumor mass in the implanted mice. Lee et al. (2009) demonstrated that the hot water extract of chaga exerts inhibitory activity against the proliferation of human colon cancer HT-29 cells. A dose-dependent inhibition was observed with maximum effect recorded at mg/mL incubated for 48 h. The apoptotic cell percentage was closely associated with down-regulation of Bcl-2 and up-regulation of Bax and caspase-3. Nakjima et al. (2009) examined the phenolic ingredients of chaga against normal a panel of cancer cell lines. The 80% methanol extract of fruiting body showed significant selective cytotoxicity towards tumor cell lines. Fluorescence-activated cell sorting analysis further revealed that 3,4-dihydroxybenzalacetone have high potentiality for apoptosis induction in human ovarian teratocarcinoma PA-1 cells. Chung et al. (2010) tested the *in vitro* and *in vivo* growth inhibition of chaga subfractions on human carcinoma cell lines and S-180 implanted mice. Lung carcinoma A-549, stomach adenocarcinoma AGS, breast adenocarcinoma MCF-7 and cervical adenocarcinoma HeLa cells succumbed to the subfractions, though at various extents. The cancerous mice when fed a normal chow supplemented with different subfractions (0.1–0.2 mg/daily) caused significant shrinkage of tumor volume (23.96–33.71%). Mazurkiewicz et al. (2010) investigated the components from acid hydrolysate of chaga against A549 human lung carcinoma cells. Formation of of benzaldehyde *in situ* in the cells culture was assumed to mediate the antiproliferative effect of the extract. Kim et al. (2011) conducted *in vitro* cytotoxic assessment of methanol extract of chaga sclerotia against a panel of cancer cell lines. 3, 4-dihydroxybenzalacetone exhibited the strongest cytotoxic activities against A549 and HL-60 cells. Zhong et al. (2011) observed the effects of inotodiol extracts on human lung carcinoma A549 cells. The compound inhibited the proliferation and induced apoptosis of A549 cells. The molecular mechanism was mediated by the upregulation of p53 and bax proteins and downregulation of Bcl-2 protein. Lemieszek et al. (2011) evaluated the *in vitro* anticancer activity of chaga fraction. It could decrease the tumor cell proliferation, motility and morphological changes induction in human lung carcinoma (A549), colon adenocarcinoma (HT-29) and rat glioma (C6) cells. Fan et al. (2012) purified a water-soluble polysaccharide from chaga which exerted *in vivo* antitumor activity, also promoted immunity. Ma et al. (2013) observed the cytotoxicity of petroleum ether and ethyl acetate fractions of chaga against human prostatic carcinoma PC3 and breast carcinoma cell MDA-MB-231 cells. Among the constituents identified, ergosterol peroxide and trametenolic acid offered the cytotoxicity. Kuriyama et al. (2013) examined the inhibitory activities of seven low molecular weight polyphenolics on DNA polymerase and DNA topoisomerase. Among the components, caffeic acid and 3, 4-dihydroxybenzalacetone proved most to be the promising. These compounds with catechol propanoid moiety suppressed the proliferation of human colon HCT116 carcinoma cells by arresting the the cell cycle in the G2/M phase, which was correlated to their ability to inhibit DNA topoisomerase II.

11.2.4 Immunomodulation and Antiallergic Effect

Won et al. (2011) investigated the immunostimulating activity of polysaccharides isolated from fruiting body of chaga. In the RAW264.7 macrophage cells, the polysaccharide showed capability of promoting NO/ROS production, TNF-α secretion and phagocytic uptake in macrophages. It was able to induce the phosphorylation of three MAPKs as well as the nuclear translocation of NF-κB, leading to the activation of RAW264.7 cells. Ko et al. (2011) investigated the immunomodulating effects of chaga ingestion by immunizing mice with ovalbumin. The intake of hot water extract significantly suppressed the increase in serum IgE and IgG. It was determined that the extract inhibits the secretion of NO from LPS-stimulated peritoneal macrophages *ex vivo*. Concavalin A stimulation in spleen cells decreased the IL-4 production, while enhancing the IFN-γ production. Also, the lectin reduced IL-4, IFN-γ, and IL-2 production from CD4+ T cells. It was inferred that chaga modulates immune responses through secretion of Th1/Th2 cytokines in immune cells and regulates antigen-specific antibody production. Yoon et al. (2013) investigated the possible anti-allergic activity of chaga extract in mice. This activity was assessed through the levels of the IgE antibody produced in response to ovalbumin. Oral administration of the extract prophylactically inhibited the systemic anaphylactic shock induced by the histamine-releasing polymer 48/80. Significant reduction in the total IgE levels was observed. Also, the spleen cell cultures harvested from the extract-treated mice showed a significant increase in Th1-derived responses.

11.2.5 Hypoglycaemic Effect

Diabetes mellitus has responded well to chaga extract. Xu et al. (2010) investigated the antihyperglycaemic effects of the ethanol extract of the dry matter of a culture broth of chaga in alloxan-induced diabetic mice. The treatment for three weeks showed a significant decrease in blood glucose level. Further, the ethanol extract decreased serum contents of free fatty acids, total cholesterol, triglycerides and LDL-cholesterol, whereas it effectively increased HDL-cholesterol, insulin levels and hepatic glycogen contents. The extract restored the damage of pancreatic tissues in the tested mice. Lu et al. (2010) investigated the effects of chaga ethyl acetate fraction on hyperglycaemia. The treatment led to a significant decrease in blood glucose level in alloxan-induced diabetic mice. The extract decreased the levels of triglyceride and MDA while increasing the HDL cholesterol level in serum and the hepatic glycogen level in liver. Inhibitory effect of inotodiol and trametenolic acid on α-amylase activity was witnessed. Zhang et al. (2011) compared the effect of cultured and wild chaga on hyperglycemic mice. The vanadium content in the cultured mushroom was 3 mg/g while in the wild variety is 1/100th of that amount. At that concentration

of vanadium, the cultured chaga possessed significantly better anti-diabetic effect than the latter, its bioavailability and low toxicity rendering it suitable for consumption. Geng et al. (2013) demonstrated that chaga mycelium powder possesses significant antihyperglycemic effects. The chloroform extract of mycelium was potential inhibitory against DPP-4, an enzyme with crucial role in glucose metabolism.

11.2.6 Antiviral Effect

Shibnev et al. (2011) observed that chaga water extract exhibits a cidal effect towards hepatitis C virus cultured on porcine embryo kidney cells, reducing the infectivity to 100-fold within 10 min. Pan et al. (2013) reported that chaga water extract exhibits marked decline in HSV-1 infection, the mechanism being the inhibition of viral-induced membrane fusion leading to the prevention of viral entry.

11.2.7 Neuroprotection, Learning and Memory Enhancement

Giridharan et al. (2011) investigated the cognitive stimulation and antioxidant activities of chaga against scopolamine-induced experimental amnesia (memory loss). Chaga methanolic extract at 50–100 mg/kg doses were administered orally for 7 days to test mice. Learning and memory was assessed by passive avoidance task and Morris water maze test. The extract significantly reduced the oxidative-nitritive stress, as evidenced by a decrease in MDA and nitrite levels and restored the GSH-Px and SOD levels. The extract treatment led to decrease in acetylcholinesterase activity in brain homogenates and restored the levels of acetylcholine. Gunjima et al. (2013) investigated the neuroprotective activity of 3,4-dihydroxybenzalacetone against the Parkinson's disease-related neurotoxin 6-hydroxydopamine. It promoted the translocation of Nrf2 to the nucleus, activated the transcription of Nrf2-dependent antioxidative genes, and increased glutathione synthesis in the cells. Activation of the Nrf2/glutathione pathway through PI3 K/Akt led to significant improvement in the survival of human neuroblastoma SH-SY5Y cells against the toxicity.

11.3 Cultivation Methods

Convincing number of recent findings establish that chaga mycelia can be spawned in synthetic medium and the metabolite production adjusted. Chen et al. (2010a) attempted maximization of ultrasonic/microwave assisted extraction of chaga polysaccharides using RSM. The optimal conditions for the process were 90 W

microwave power and 50 W ultrasonic power together with 40 kHz ultrasonic frequency applied for 19 min, respectively. Under the optimal conditions, the yield and purity of polysaccharides achieved were 3.25 and 73.16%, respectively. Further, Chen et al. (2010b) investigated the effect of RSM-optimized fermentation medium on the radical scavenging activity of the chaga polysaccharides. The resultant medium with corn flour 5.30, peptone 0.32, KH_2PO_4 0.26, $MgSO_4$ 0.02 and $CaCl_2$ 0.01 (% w/v) possessed superior hydroxyl radical scavenging rate than basal media. The main monosaccharides components of the polysaccharides were rhamnose, arabinose, xylose, mannose, glucose and galactose with molar proportion at 1.45, 3.63, 2.17, 15.94, 50.00 and 26.81%. Sun et al. (2011) examined whether cultivated chaga retains antitumor activity like the wild-harvested. MTT assay revealed the tumor inhibitory effect of the extract rich in lanosterol, inotodiol, and ergosterol. Ergosterol was quantified to be much higher (27.32%) in cultivated fruiting body, which held promise to be as effective as the wild chaga. Zheng et al. (2011a) evaluated the bioactive metabolite yield from the coculture of chaga and *Phellinus punctatus*. As a result of co-culture, accumulation of phenolic compounds, melanins, and lanostane-type triterpenoids increased. The metabolite profile of mono- and co-culture differed and the constituents exerting radical scavenging effect varied. Phelligridin C, phelligridin H, methyl inoscavin A, inoscavin C, methyl davallialactone, foscoparianol D and inotodisaccharide were the bioactive components scavenging the radicals in co-culture. The findings recommend cost-effective biosynthesis of active ingredients by co-culture. Chen et al. (2011) explored the possibility of enhanced chaga exopolysaccharide and antioxidant production using corn stover submerged fermentation. Compared to basal medium, the production of the polymer dwindled, yet it possessed superior superoxide radical scavenging activity. The monosaccharide components varied significantly from that of polyposaccharides from the basal medium. Xiang et al. (2012) also reported that the chaga exopolysaccharides from the corn stover-containing medium have significantly stronger radical-scavenging activity than the control. Zhu and Xu (2013) investigated the effect of lignocellulosic materials (wheat straw, sugarcane bagasse and rice straw) addition to the liquid culture. Increase in the production and antioxidant activity of extra- and intra-cellular phenolic compounds was observed. Also, the phenolic compound extracts showed higher radical scavenging activity than those from the control medium, attributed to the lignocellulosic substrate decomposition.

11.4 Isolation, Purification and Quantification of Bioactive Components

Gao et al. (2009) determined the steroids in the fruiting bodies and submerged-cultured mycelia of chaga. The HPLC method could successfully recover betulin, ergosterol, cholesterol, lanosterol, stigmasterol and sitosterol. Fu et al. (2010) employed an ultrasonic technique to extract polysaccharides from chaga. The effects

of ultrasonic conditions on the recovery and radical scavenging activity of the polysaccharide were evaluated. Handa et al. (2010) isolated an unusual lanostane-type triterpenoid (spiroinonotsuoxodiol) and two lanostane-type triterpenoids (inonotsudiol A and inonotsuoxodiol A) from the sclerotia of chaga, as determined by NMR studies. Du et al. (2011) used preparative high-speed counter-current chromatography (HSCCC) to isolate inotodiol and trametenolic acid from the chloroform extract of chaga. The purity of the obtained target compounds was analysed effectively by HPLC with evaporative light scatting detection (ELSD). Gracheva and Golovanchikov (2011) reported that the extraction of bioactive ingredients from chaga in an electric field with a constant current density significantly reduces the time.

11.5 Property Enhancement

Several procedures have been attempted to enhance the biological activities of chaga. Kim et al. (2009) evaluated the effect of ionizing radiation on color and antioxidative properties of chaga extract. 10 mg/mL of the extract was subjected to various doses of gamma-irradiation. With increased dose, the lightness and yellowness were increased and redness was decreased. The irradiation led to increased total phenolic compound level and decreased lipid peroxidation. Zhang et al. (2013) modified the chaga polysaccharides by acid and alkali hydrolysis, thermal and ultrasonic treatment. The thermal and ultrasonic-treated polysaccharide showed the properties of lower molecular weight distribution, lower intrinsic viscosity, a hyperbranched conformation, and higher antioxidant abilities on ferric-reducing power and lipid peroxidation inhibition activity.

11.6 Conclusion

The folkloric medicinal knowledge about chaga has now earned credence as indicated from the ample validations. More clinical trials may shed light on its CAM potential against many persistent forms of cancers pathogenic virus. Development of hypoallergenic functional foods by nutrient-valorising is another promising area of chaga utilization. Its bioactive arsenal and therapeutic spectrum ought to be explored, while ensuring prevention of overharvesting for sustainable usage. Rather than superficial documentation of beneficial effects, molecular level intervention must be investigated. Moderation must be followed while consuming chaga, as instances of oxalate-nephropathy have been reported. Chaga has already attained the sobriquet “King of medicinal mushrooms” and time is ripe to verify the claim and derive optimal benefit from it.

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Glossary

α-linolenic acid
Acetylcholinesterase
Adjunct therapy
Adaptogen
Advanced glycation end products
Alternative and Complementary Medicine
Amaranthaceae
Anthocyanin
Antiallergic
Anticancer
Anticonvulsant
Antiinflammatory
Antileptic
Antinutritional factors
Antioxidant
Antiproliferative
Apocyanaceae
Asteraceae
Betanin
Biodiversity
Bioprospecting
Bran
Cactaceae
Dietary fibre
Dietary supplement
Diuretic
eNOS expression
Epigallocatechin
Ergosterol
Estrogenic
Ethnobotany
Fabaceae
Flavonoid
Food fortification
Food insecurity
Foraging
Functional food
Gastroprotective
Hepatoprotective
Hymenochaetaceae
Hyperlipidemic
Hypoglycaemic
Hypotensive
Immunomodulator
Indicaxanthin
Insulin resistance
Lamiaceae
Lignan
Metastasis
Mushroom polysaccharide
Myrtaceae
Nephroprotective
Neuroprotective
Nutraceutical
Polyphenol
Portulacaceae
Proanthocyanidin
Pseudo-cereal
Saponin
Sesquiterpene
Silibinin

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Silymarin
Submerged fermentation
Sustainability
Underutilized crop
Value-added product
ω-3 fatty acids
Xerophyte

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