IntechOpen

Phytochemicals

Isolation, Characterisation and Role in Human Health

Edited by A. Venket Rao and Leticia G. Rao

PHYTOCHEMICALS - ISOLATION, CHARACTERISATION AND ROLE IN HUMAN HEALTH

Edited by **A. Venket Rao** and **Leticia G. Rao**

Phytochemicals - Isolation, Characterisation and Role in Human Health
http://dx.doi.org/10.5772/58648
Edited by A. Venket Rao and Leticia G. Rao

Contributors

Wagner Luiz Ramos Barbosa, Marcos Soto-Hernández, Nagi Kumar, Rohanie Maharaj, Aldina Kesić, Nadira Ibrišimović-Mehmedinović, Almir Šestan, João Xavier de Araújo-Júnior, Pedro Aquino, Antônio Euzébio Goulart Sant'Ana, Thiago Mendonça De Aquino, Barbara Santos, Magna Suzana Alexandre-Moreira, Dario Donno, Gabriele Loris Beccaro, Alessandro Cerutti, Giancarlo Bounous, Maria Gabriella Mellano, Gaspar Diaz, Leseilane Johannes Mampuru, Victor Patrick Bagla, Matlou Mokgotho, Everaldo G. Attard, Henrietta Attard, Antoine Tanti, Jurgen Azzopardi, Mario Sciberras, Victor Pace, Neville Buttigieg, Andrew Mangion Randon, Bernardette Rossi, Marie Josette Parnis, Karin Vella, Michelle Zammit, Anthony Serracino Inglott, Patricia Isabel Manzano Santana, Mariana Albulescu, Estela Guardado Yordi, Venketeshwer Rao, Leticia Rao

© The Editor(s) and the Author(s) 2015
The moral rights of the and the author(s) have been asserted.
All rights to the book as a whole are reserved by INTECH. The book as a whole (compilation) cannot be reproduced, distributed or used for commercial or non-commercial purposes without INTECH's written permission.
Enquiries concerning the use of the book should be directed to INTECH rights and permissions department (permissions@intechopen.com).
Violations are liable to prosecution under the governing Copyright Law.

Individual chapters of this publication are distributed under the terms of the Creative Commons Attribution 3.0 Unported License which permits commercial use, distribution and reproduction of the individual chapters, provided the original author(s) and source publication are appropriately acknowledged. If so indicated, certain images may not be included under the Creative Commons license. In such cases users will need to obtain permission from the license holder to reproduce the material. More details and guidelines concerning content reuse and adaptation can be foundat http://www.intechopen.com/copyright-policy.html.

Notice

Statements and opinions expressed in the chapters are these of the individual contributors and not necessarily those of the editors or publisher. No responsibility is accepted for the accuracy of information contained in the published chapters. The publisher assumes no responsibility for any damage or injury to persons or property arising out of the use of any materials, instructions, methods or ideas contained in the book.

First published in Croatia, 2015 by INTECH d.o.o.
eBook (PDF) Published by IN TECH d.o.o.
Place and year of publication of eBook (PDF): Rijeka, 2019.
IntechOpen is the global imprint of IN TECH d.o.o.
Printed in Croatia

Legal deposit, Croatia: National and University Library in Zagreb

Additional hard and PDF copies can be obtained from orders@intechopen.com

Phytochemicals - Isolation, Characterisation and Role in Human Health
Edited by A. Venket Rao and Leticia G. Rao

p. cm.

ISBN 978-953-51-2170-1

eBook (PDF) ISBN 978-953-51-5406-8

We are IntechOpen, the world's leading publisher of Open Access books

Built by scientists, for scientists

3,800+
Open access books available

116,000+
International authors and editors

120M+
Downloads

151
Countries delivered to

Our authors are among the
Top 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Meet the editors

Dr. Rao completed his M. Sc. and Ph. D. degrees in Food Science from Oregon State University, USA. After being a Full Professor in the Department of Nutritional Sciences, Faculty of Medicine, he currently holds the position of Professor Emeritus at the University of Toronto. He also served as the Director of the Program in Food Safety, Nutrition and Regulatory Affairs, Director of the Collaborative Program in Toxicology, Member of the Institute of Environmental Studies, and Undergraduate Coordinator for the Department of Nutritional Sciences. He is a member of several national and international scientific and professional organizations including the International Bifidus Foundation (Japan). He has established a major focus in the area of diet and health with particular emphasis on the role of dietary phytochemicals and intestinal microflora. His research has focused on the role of probiotics and prebiotics in human health and oxidative stress and antioxidants in the causation and prevention of chronic diseases, with particular emphasis on the role of carotenoids and polyphenols. He is one of the pioneering researchers to study the bioavailability, metabolism, mechanisms of action, and biological role of lycopene, a carotenoid antioxidant present in tomatoes and other fruits and vegetables. Dr. Rao served as a member of the Provincial and National Expert Committees in Canada in the areas of nutrition, health, food safety and agriculture. He has a distinguished academic career spanning over 45 years as an educator and a researcher. He serves as a Senior Scientific Consultant to provincial and federal government agencies and food and pharmaceutical industries globally. He is popularly sought by the international media to express his opinions on the subjects of nutrition and health.

Dr. Leticia Rao is an Associate (Adjunct) Professor of Medicine and a full member of the Graduate Faculty, Institute of Medical Sciences, at the University of Toronto. She is also the Director of the Calcium Research Laboratory, Department of Medicine and a Staff Scientist at St Michael's Hospital, Toronto, Ontario. She obtained a Master of Science Degree in Food Science from Oregon State University and a Ph.D. degree in Biochemistry from the University of Toronto. Her current expertise is in the area of bone cell biology and calcium metabolism, and her long term research goal is to uncover the causes and prevention of osteoporosis by studying bone cells in the laboratory. She has extensive basic research experience on drugs that have been approved for osteoporosis prevention and treatment such as raloxifene and estrogen. She has conducted clinical studies to find out how the fat-soluble carotenoid antioxidant lycopene present predominantly in tomatoes and tomato products and the water-soluble antioxidant polyphenols in colourful fruits

and vegetables and natural food supplement can help in the prevention of postmenopausal osteoporosis. She has presented her research data to a number of symposia and has been invited to give talks on her clinical research on osteoporosis. Her publications in peer reviewed scientific journals are extensive. She co-authored a book entitled “Bone Building Solution” and presently co-editing the book “Polyphenols.”

Contents

Preface XIII

Section 1 Phytochemical Composition 1

Chapter 1 **The Phytochemical Constitution of Maltese Medicinal Plants – Propagation, Isolation and Pharmacological Testing 3**
Everaldo Attard, Henrietta Attard, Antoine Tanti, Jurgen Azzopardi, Mario Sciberras, Victor Pace, Neville Buttigieg, Andrew Mangion Randon, Bernardette Rossi, Marie Josette Parnis, Karin Vella, Michelle Zammit and Anthony Serracino Inglott

Chapter 2 **Phytochemicals in Antitumor Herbs and Herbal Formulas 45**
Mariana Albulescu

Chapter 3 **Advances in Studies of Vernonanthura patens (Kunth) H. Rob. Growing in Ecuador 81**
P.I. Manzano, M. Miranda, M.F. Quijano and L. Monzote

Chapter 4 **Phytochemical Profile of Honey 99**
Aldina Kesić, Nadira Ibrišimović-Mehmedinović and Almir Šestan

Section 2 Specific Bioactive Phytochemicals 111

Chapter 5 **Coumarins — An Important Class of Phytochemicals 113**
Maria João Matos, Lourdes Santana, Eugenio Uriarte, Orlando A. Abreu, Enrique Molina and Estela Guardado Yordi

Chapter 6 **Quinolines, Isoquinolines, Angustureine, and Congeneric Alkaloids — Occurrence, Chemistry, and Biological Activity 141**
Gaspar Diaz, Izabel Luzia Miranda and Marisa Alves Nogueira Diaz

Chapter 7 **Aspidosperma Terpenoid Alkaloids — Biosynthetic Origin, Chemical Synthesis and Importance 163**
Pedro Gregório Vieira Aquino, Thiago Mendonça de Aquino, Magna Suzana Alexandre-Moreira, Bárbara Viviana de Oliveira Santos, Antônio Euzébio Goulart Santana and João Xavier de Araújo-Júnior

Section 3 Extraction and Identification of Phytochemicals 185

Chapter 8 **Bud Extracts as New Phytochemical Source for Herbal Preparations — Quality Control and Standardization by Analytical Fingerprint 187**
D. Donno, G.L. Beccaro, A.K. Cerutti, M.G. Mellano and G. Bounous

Section 4 Effect of Stress on Phytochemical Activation 219

Chapter 9 **Effects of Abiotic Stress (UV-C) Induced Activation of Phytochemicals on the Postharvest Quality of Horticultural Crops 221**
Rohanie Maharaj

Section 5 Biological and Health Significance of Phytochemicals 245

Chapter 10 **Oxidative Stress and Antioxidants in the Risk of Osteoporosis — Role of Phytochemical Antioxidants Lycopene and Polyphenol-containing Nutritional Supplements 247**
L.G. Rao and A.V. Rao

Chapter 11 **Phytochemicals and Cancer – Possible Molecular Targets of Phytochemicals in Cancer Prevention and Therapy 261**
Victor P. Bagla, Matlou P. Mokgotho and Leseilane J. Mampuru

Chapter 12 **Lead Compounds from Cucurbitaceae for the Treatment of Cancer 289**
Marcos Soto-Hernández, Jorge Cadena Iñiguez, Lourdes C. Arévalo-Galarza, Edelmiro Santiago-Osorio, Itzen Aguiñiga -Sánchez and Lucero del Mar Ruíz-Posadas

Chapter 13 **Green Tea Catechins for Prostate Cancer Chemoprevention 305**
Ganna Chornokur and Nagi B. Kumar

Chapter 14 **Eleutherine Plicata – Quinones and Antioxidant Activity 323**
Luiz Claudio da Silva Malheiros, João Carlos Palazzo de Mello and
Wagner Luiz Ramos Barbosa

Preface

Global dietary recommendations emphasize the consumption of plant-based foods for the prevention and management of chronic diseases. Plants contain many biologically active compounds referred to as 'phytochemicals' or 'functional ingredients'. These compounds play an important role in human health. Prior to establishing the safety and health benefits of these compounds, they must first be isolated, purified, and their physico-chemical properties established. Once identified, their mechanisms of actions are studied. They can them be evaluated for their biological activity using *in vitro* and *in vivo* techniques including the use of cell cultures, animal models and clinical studies. Recognizing the importance of the phytochemicals in human health, the contents of this book include all the above aspects. The chapters are arranged in the order from isolation, purification and identification to in vivo and clinical studies, there by covering not only the analytical procedures used but also their nutraceutical and therapeutic properties. This book will be beneficial to researchers, health professionals, government regulatory agencies and industrial personnel. We are confident that the readers of this book which follows two previously published books on the same topic by In Tech Publication will find it highly useful as a standard reference book.

Dr. L. G. Rao
Associate (Adjunct) Professor and
Director Calcium Research Laboratory
St. Michael's Hospital
University of Toronto

Dr. A. V. Rao
Professor Emeritus
Department of Nutritional Science
Faculty of Medicine
University of Toronto, Canada

Section 1

Phytochemical Composition

Chapter 1

The Phytochemical Constitution of Maltese Medicinal Plants – Propagation, Isolation and Pharmacological Testing

Everaldo Attard, Henrietta Attard, Antoine Tanti, Jurgen Azzopardi, Mario Sciberras, Victor Pace, Neville Buttigieg, Andrew Mangion Randon, Bernardette Rossi, Marie Josette Parnis, Karin Vella, Michelle Zammit and Anthony Serracino Inglott

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/60094

1. Introduction

In spite of its small size (31,500 hectares), the Maltese Archipelago hosts a large number of medicinal and aromatic plants that have been utilised medicinally for several centuries. The Maltese Archipelago lies in the middle of the Mediterranean Sea, 35°50′ north of the Equator and 14°35′ east of Greenwich. The climate is characterized by hot dry summers, mild wet winters (an average rainfall of 500 mm and temperatures ranging between 13°C in winter and 35°C in summer) and a high relative humidity all the year round. Most of the wild plants thrive in very shallow soil pockets that, in some cases, contribute to the production of phytochemicals as a means of protection against other plants or other organisms. In general, Maltese soils contain a high amount of calcium carbonate (>53%), which is the parent rock material, a high pH (>8) and a high clay content with a good physical structure but lacking organic matter (<4.5 %).

The Maltese flora comprises around 1284 vascular plants 66% originating from the Mediterranean region while the other 34% originating from the cold European and warm subtropical regions [1]. Out of these, there are about 458 medicinal taxa with approximately 300 originating from the Mediterranean region. The main plant families of medicinal importance are Asteraceae (15%), Lamiaceae (7%), Fabaceae (6%), Umbelliferae (4%) and Rosaceae (4%) amongst others. The biodiversity in medicinal flora is high probably due to several reasons that include:

© 2015 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

- favourable Mediterranean climate
- availability of fertile calcareous soils
- considerable area of uncultivated land (wastelands)
- former conquerors of the Maltese Islands
- Maltese interest in herbal medicine

The number of medicinal species is on the decline to the extent that some have already become extinct. This is not mainly attributed to overuse problems but due to various human activities. There were isolated cases where a medicinal plant was under treat due to over-harvesting. One typical example was the seaside squill (*Drimia maritima*) which was over-harvested due to export.

2. Medicinal flora of the Maltese islands

The pharmacological assessment of the Maltese medicinal flora, contributed to a portion of the research conducted on these species. Intensive research has been conducted in other fields, particularly in the ethnobotanical, agronomic, *in vitro* propagation and phytochemical fields. Phytochemistry plays a very important role in medicinal plant research (figure 1). The quality and safety of these plants depends mainly on their phytochemical constitution. These metabolites determine the categorization of plants; whether a medicine, food supplement or cosmetic. The quality and quantity of these metabolites depends mainly on the growing conditions. This instigated researchers to study different aspects of medicinal plants with phytochemistry as the common aspect.

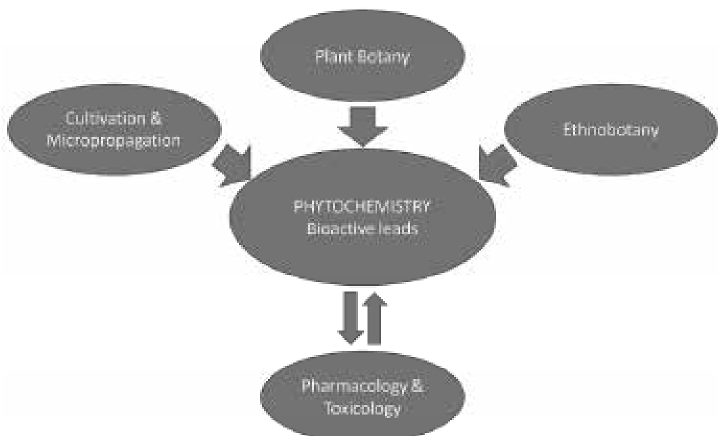


Figure 1. The importance of phytochemistry in medicinal plant research

Medicinal plants have been classified either on their phytochemical constitution or else on their pharmacological activities. These plant contain a myriad of metabolite classes and single

metabolites. In most cases, more has to be discovered as the information is either unavailable or else still uninvestigated yet. Locally, medicinal plants have been classified on their pharmacological activity. Some would include the following effects: cardiotonic (e.g. squill, oleander), anticancer (e.g. squirting cucumber, borage), immunomodulatory (e.g. squirting cucumber, olive tree), antiflammatory and skin disorders (e.g. marigold, aloe, erica), antihypertensive (e.g. hawthorn), antimicrobial and antifungal (poison ivy, sage, garden basil, sticky fleabane, couch grass, garlic, fig tree, caper plant, pellitory of the Wall), antidiabetic (karela), insect repellents and insecticides (pennyroyal, tree tobacco), antihelmintic (pumpkin), spasmodic and antispasmodic (vervain, henbane), sedative (blue passion flower, orange-flower water, chamomile), kidney stone problems (micromeria), volatile oil (lavander, garden rue, lemon balm, rosemary, laurel, spearmint) and fixed oils (olive tree, castor oil plant). Some of these plants are listed in table 1.

Local ethnobotanical research has contributed towards the discovery of new leads. In such studies, the traditional claims are challenged using scientific methods. Possible conservation strategies were also considered, particularly for endangered species. However, there are limitations since there are no national incentives to conserve these plant species unless cultivated or sold as pot plants. However, there are few plants that are legally bound. A typical example is the carob tree. The grower cannot uproot a carob tree to pursue cultivation needs.

Latin name	Family	Common name	Maltese name
Drimia maritima (L.) Stearn	Asparagaceae	Seaside squill	Basla tal-għansar
Ecballium elaterium (L.) A.Rich.	Cucurbitaceae	Squirting cucumber	Faqqus il-ħmir
Mentha pulegium L.	Lamiaceae	Pennyroyal	Plejju
Salvia officinalis L.	Lamiaceae	Garden sage	Salvja
Verbena officinalis L.	Verbenaceae	Vervain	Buqexrem
Hedera helix L.	Araliaceae	Common ivy	Liedna
Crataegus monogyna Jacq.	Rosaceae	Common hawthorn	Anżalor salvaġġ
Calendula officinalis L.	Asteraceae	Pot marigold	Suffejra
Melissa officinalis L.	Lamiaceae	Lemon balm	Burieħa
Olea europea L.	Oleaceae	Olive tree	Żebbuġa
Urtica dubia Forsk.	Urticaceae	Stinging nettle	Ħurrieqa
Capparis spinosa L.	Capparaceae	Caper plant	Kappara
Ephedra fragilis Desf.	Ephedraceae	Mormon tea	Efedra
Nicotiana glauca RC Graham	Solanaceae	Tree tobacco	Tabakk tas-swar

Table 1. The Maltese medicinal plants in this study.

2.1. *Drimia maritima* (L.) Stearn

Drimia maritima or *Urginea maritima* is one of the local medicinal plants which was harvested and exported. It is a member of the Asparagaceae family, with cardiac glycosides that reside

in the bulb of this plant. It is renowned for its emetic, diuretic, cardiotonic [2], expectorant, rodenticide [3] and anticancer activities. The seaside squill has been extensively studied for its propagation potential. Locally, cultivation studies have been associated with the cardiac glycosidic content while micropropagation has been linked to biomass production.

The main constituents of the seaside squill are the cardiac glycosides and phenolic compounds [4]. It also contains mucilage and calcium oxalate crystals. The squill cardiac glycosides are bufadienolides. In principle, these are similar to triterpenoids having a sugar group and a lactone ring at C17. Scillaren A accounts for about 70% of the total glycosidal content of squill. It contains one unit of rhamnose and one unit of glucose. When scillaren A is hydrolyzed by enzymes, it breaks down to proscillaridin A and D-glucose (Figure 2).

Figure 2. The structure of cardiac glycosides of *Drimia maritima* (L.) Stearn.: (A) Scillaren A and (B) Proscillaridin A

These glycosides act by binding to the Na^+/K^+ ATPase pumps. This occurs due to the presence of the lactone group [5, 6]. These bufadienolides are therefore important cardiotonic, blood pressure stimulating and antitumour agents. The main glycosides with digoxin-like effects are scillaren A and proscillaridin A [7].

Several cultivation parameters were studied for *Drimia maritima* in relation to dry matter yield and the total glycosidal content [8]. These include methods of propagation, planting at different depths, effects of nitrogen (N), phosphorus (P) and potassium (K) fertilizers, cultivation in different soil types, age of harvesting and seasonal timing of harvesting. Propagation by bulb division only takes 10 weeks to produce a seedling as opposed to seed propagation that requires 56 weeks. The type of soil does not contribute to the variation of glycosides in the squill bulb. In fact, Maltese squill grown on four soil types, namely terra soil, xerorendzina soil, carbonate raw and sandy soil exhibited average glycosidal contents of 0.575 % (w/w). Fertiliser studies revealed that the use of different ratios of N, P and K affect the rate of growth but no change in glycosidal content (average of 0.59 % w/w). For the best annual production of dry weight and glycosidal content, it is advisable to harvest squill in the third year after transplanting (table 2) immediately after flowering. The highest glycosidal content is obtained from the roots (Figure 3)

http://dx.doi.org/10.5772/60094

Year of harvest	Treatment	Mean glycosidal content(%)
First	Control	0.25
	Fertiliser-treated	0.26
Second	Control	0.66
	Fertiliser-treated	0.68
Third	Control	0.57
	Fertiliser-treated	0.58
Fourth	Control	0.58
	Fertiliser-treated	0.58
Fifth	Control	0.38
	Fertiliser-treated	0.40
Sixth	Control	0.31
	Fertiliser-treated	0.34

Table 2. The mean percentage glycosidal content in the squill bulb with year of harvest [8].

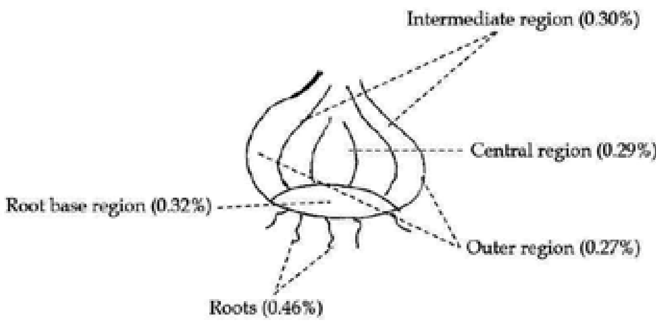


Figure 3. The mean percentage glycosidal content in different parts of the squill bulb [8].

Micropropagation of squill was carried out by direct and indirect organogenesis. Regeneration was successfully achieved using bulb explants by direct organogenesis. Although the process of regeneration was slow, callus cultures maintained in high auxin concentrations (4 mg/l 2,4-D + 2 mg/l NAA) induced root formation, when the plant growth regulators (PGRs) were removed [9].

2.2. Ecballium elaterium (L.) A.Rich.

Ecballium elaterium (squirting cucumber), a member of the Cucurbitaceae family, is a Mediterranean medicinal plant in a monotypic genus. In the past, the squirting cucumber was used as

a purgative, emetic, for the treatment of jaundice and oedema. It was also used for the treatment of otitis, hydrophobia and malarial fever. Locally, it was prepared in various dosage forms such as powders, solutions, semisolid blocks and dried cubes for exportation. It also used to be prepared in the form of lozenges with gum Arabic. The fresh fruit juice was renowned for several pharmacological effects mainly as antibilirubinaemic, antihepatotoxic and lacrimation stimulant. The dried juice, also known as the elaterium, was effective as a laxative, anti-inflammatory, antitumour and as an aflatoxin suppressor [10, 11]. Most of these pharmacological effects have been proven through various scientific investigations.

The main constituents of this plant are the cucurbitacins (Cu), the major ones being CuE and CuB (Figure 4), particularly present in the fruit juice. Other cucurbitacins include cucurbitacins D, G, H, I, R, L, hexanorcucurbitacin I, 16-deoxy-Δ^{16}-hexanorcucurbitacin O, anhydro-22-deoxo-3-epi-isocucurbitacin D, and their glycosides [12-14]. The squirting cucumber also contains sterols, fatty acids, elaterases, tannins [15], complex phenolic compounds and flavonoids [16], amino acids and their derivatives as well as the *Ecballium elaterium* protease inhibitors (EEPIs). These EEPIs are obtained from seed extracts and are effective against at least four different serine proteinases [17]. In fact, these are termed as trypsin inhibitors I, II, and III, also known as the trypsin isoinhibitors (EETIso), chymotrypsin inhibitor (8 kDa), subtilisin inhibitor (9 kDa), and elastase inhibitor, and Astacus protease inhibitor [18].

Figure 4. The structures of (A) cucurbitacin E and (B) Cucurbitacin B found in *Ecballium elaterium* (L.) A.Rich.

Although this plant is abundant in wastelands throughout the Maltese Archipelago, micro-propagation was attempted for two main reasons. These were as a means to study the responses of explants from the squirting cucumber to different plant growth regulators, and to determine the potential propagation of high-yielding mother plants. In this attempt, seeds were germinated in Murashige-Skoog (MS) medium. Different concentrations and types of PGRs, mainly auxins and cytokinins, were added. Subculturing with the different PGRs was performed every 4 weeks and explants were maintained at about 25 ± 1 °C and 3250 ± 250 lx. Once developed, the plantlets were transferred to Jiffy® pots until rooting and then repotted (compost:peat:perlite, 2:2:1) until flowering [19]. The main four responses of explants were bud multiplication, shoot elongation, callus production and rooting, as illustrated in Figure 5.

A regeneration protocol was devised as follows. Briefly, the seeds were germinated on MS medium (8 - 9 weeks). Bud multiplication of node explants was performed on naphthalene-acetic acid/6-benzylaminopurine (NAA/BAP) medium (for 2 - 3 subcultures every 4 weeks).

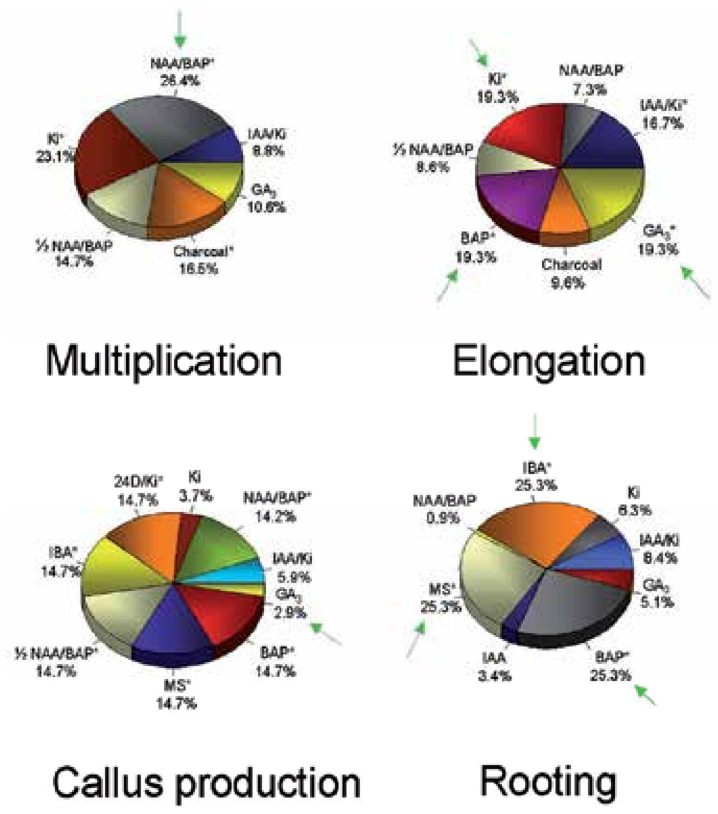


Figure 5. The effects of plant growth regulators on *Ecballium elaterium* explants in tissue culture [20].

shoot elongation was obtained on Gibberellic Acid (GA_3) medium (4 weeks), followed by an auxin shock on Indole-3-acetic acid (IAA) medium (1 week) then, treated with rooting hormone powder and finally transfer to Jiffy® pots (3 - 4 weeks). The plants were then repotting and acclimatised for 4 - 5 weeks [19]. The whole process takes between 24 and 35 weeks.

The *Ecballium elaterium* explants produced a high amount of callus and this led to further studies to determine the production of cucurbitacins in these undifferentiated cells. Callus masses were treated with different PGRs at different concentrations. The best PGR combination for biomass accumulation was 2,4-Dichlorophenoxyacetic acid/kinetin (2,4D/Ki) while for metabolite production, the NAA/BAP combinations showed optimum yields [20]. A growth-linked accumulation of metabolites was observed (figure 6).

The production of cucurbitacins from cultivated sources, is significantly higher in fruit compared to stems and leaves (figure 7). A drop in ambient temperature results in lower production of cucurbitacins [21].

Pharmacological testing has been extensively carried out on this plant. Extracts exhibited a marked effect on prostate cancer cells (IC_{50}= 9.35 nM) and moderate effects on melanoma and breast cancer cells (IC_{50} = 0.87 and 1.95 μM, respectively) *in vitro*. Negligible cytotoxic effects

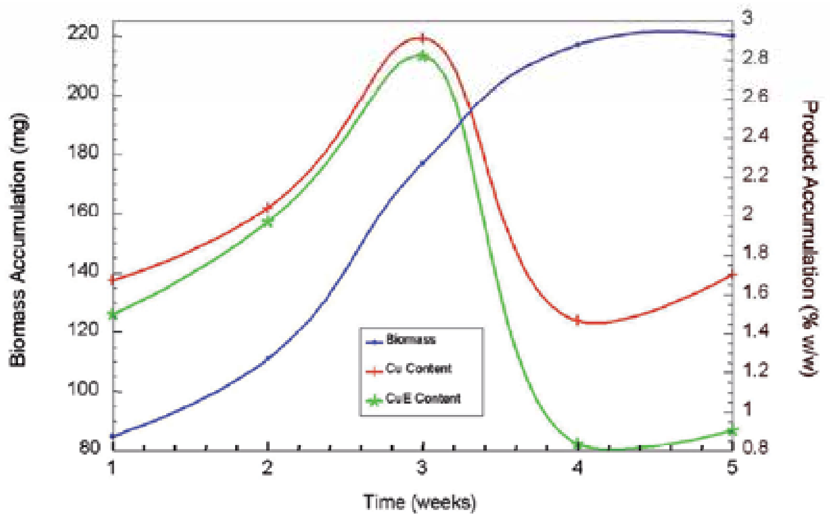


Figure 6. Growth-linked accumulation of metabolites in *Ecballium elaterium* cultures.

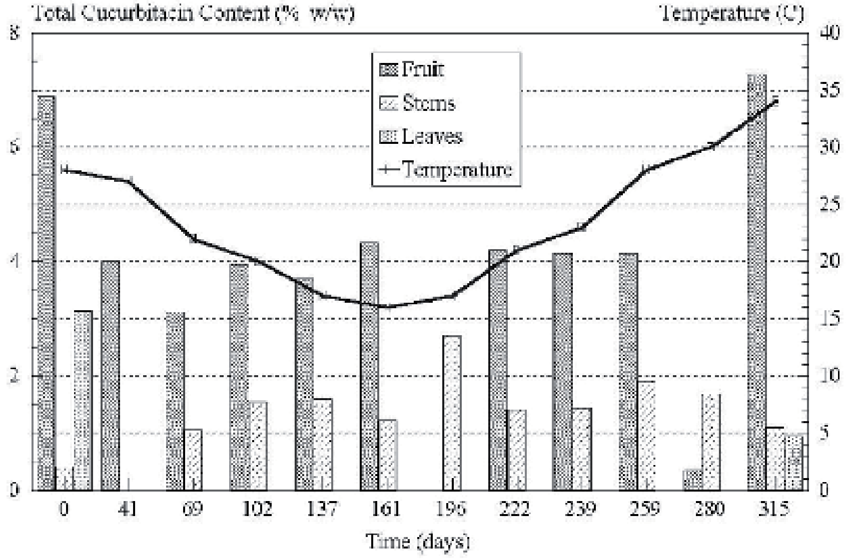


Figure 7. The total cucurbitacin content in elaterium produced from *Ecballium elaterium* fruit, stems and leaves with time and temperature [21].

were observed on normal fibroblasts (IC_{50} = 93.8 μM) [22]. It was demonstrated that CuE provoked apoptosis in cancer cell lines. This was exhibited by the condensation of chromatin and also DNA fragmentation using gel electrophoresis. CuE was also effective as an immune modulator. Human peripheral T-lymphocytes were freshly isolated and challenged with phytohaemagglutinin (PHA) and *Ecballium elaterium* extracts [23]. Cucurbitacins in the juice

extract of *Ecballium elaterium*, also exhibited potential anti-inflammatory, analgesic and antipyretic activities in rodents [10, 24].

2.3. Mentha pulegium L.

Mentha pulegium L. is a perennial plant, belonging to the Lamiaceae family. During Roman times, the plant was used for several ailments particularly for headaches, flatulence and even as an abortifacient. The name 'pulegium' derived from the Latin word 'pulex' for flea, indicates that in Roman times the plant was used as a flea repellent [25]. Locally, it was well-reputed as a treatment for common cold, as a carminative, emmenagogue but also as an insect repellent [26]. *Mentha pulegium* used to be hung in wardrobes to ward off fleas and placed on windowsills to repel mosquitoes especially during the summer months. The most important extract from this plant is the essential oil, known as the pennyroyal oil.

In a study by [27], pennyroyal oil contained 38.0% piperitone, 33.0% piperitenone, 4.7% α-terpineol and 2.3% pulegone as the major components (Figure 8). The authors concluded that Iranian pennyroyal oil is rich in piperitone/piperitenone. In another study, the pulegone content of Iranian pennyroyal oil ranged between 1.3 – 52.0%, when extracted by supercritical fluid extraction, while hydrodistillation yielded around 37.8% of pulegone. Piperitenone consituted only 6.8% to the extracted essential oil [28]. Similarly, in another study [29], the content of pulegone in Greek pennyroyal oil was in the range of 42.9% and 90.7% attributed to two populations. In other wild populations, the pulegone content did not exceed 35.6%. Such populations were rich in either menthone/isomenthone or in piperitone/piperitenone. In Tunisian pennyroyal oil, 41.8 % of the oil was pulegone [30] while Portuguese pennyroyal oil contained 23.2 % of pulegone [31]. The pennyroyal oil was extracted from wild Maltese populations using hydrodistillation with a yield of 0.73 % [32]. The pulegone content in the oil was 85.8 %, followed by other constituents; (-) limonene (0.984 %), myrcene (0.109 %) and β-pinene (0.191 %). This was determined by GC-FID.

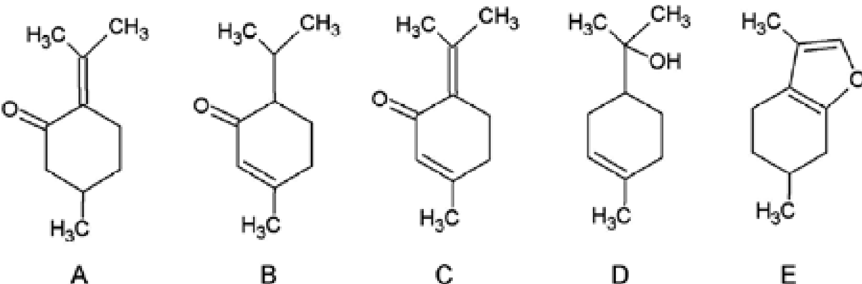


Figure 8. The most abundant monoterpenoids of *Mentha pulegium* L. essential oil: (A) pulegone, (B) piperitone, (C) piperitenone, (D) α-terpineol and (E) menthofuran.

Apart from its abortifacient activity, pennyroyal oil is also hepatotoxic and causes pulmonary necrosis. Hepatotoxicity is mainly attributed to the conversion of pulegone into its epoxide or menthofuran derivatives [33-35].

Insect repellent activity of pennyroyal was determined by using two setups (Figure 9) with citronella oil and distilled water used as positive and negative controls, respectively [32]. Setup 1 consisted of a trough with a diameter of 30 cm and a height of 12 cm. Four zones were designated within the trough (Figure 9A). The mosquitoes were introduced inside the container, and the oil sample was then injected by a syringe. Sixteen mosquitoes were observed every two minutes for a period of 20 minutes and their position within the trough was recorded. After the second minute, 75 % of the mosquitoes were found in the compartment furthest from the injection site. A gradient was achieved at this time interval and the mosquitoes moved away from the source. After the tenth minute, this compartmental difference was no longer observed, most probably due to the fact that the oil must have saturated the trough and hence there was no trend in mosquito distribution. Setup 2 consisted of a glass tube with an internal diameter of 2.5 cm and a length of 150 cm. Seven zones were designated within the tube (Figure 9B). Twenty mosquitoes were observed every two minutes for half an hour and their position recorded, following injection of the pennyroyal oil. As with setup 1, there was a statistical difference between zone 1 and zone 7 of the tube, but this difference became negligible with time. Similar results were observed with citronella. In spite of this similarity, GC-FID determination of the citronella oil revealed the presence of geraniol (60.0 %), citronellal (15.0 %) and camphene (> 15.0 %), but no significant pulegone content. With water a more random distribution of mosquitoes was observed [32].

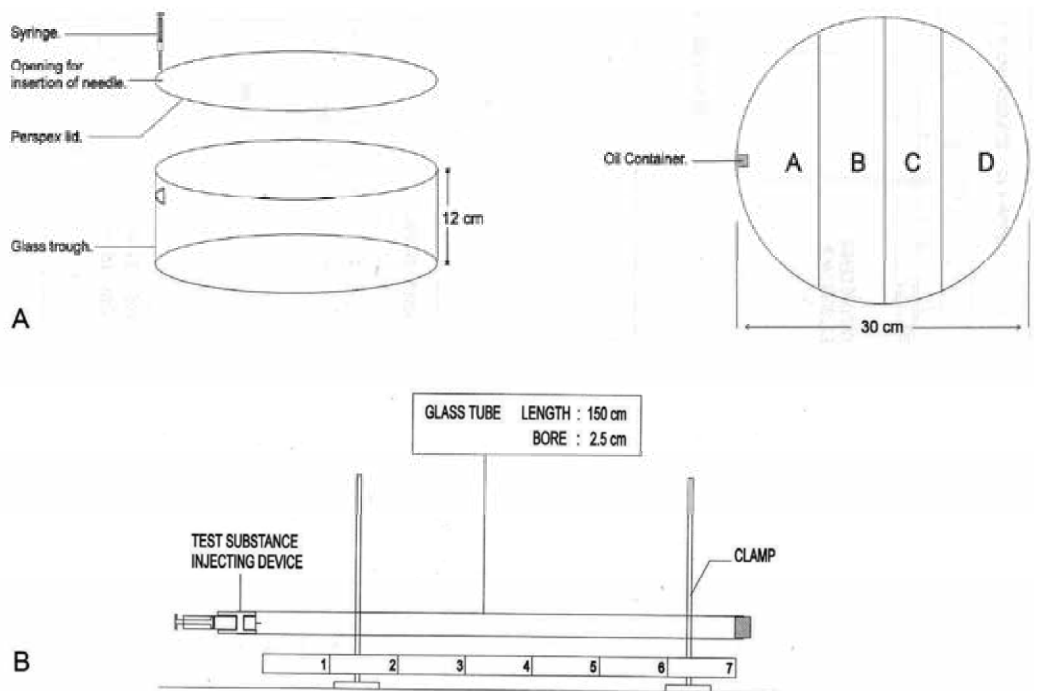


Figure 9. The experimental setups used to determine the insect repellent properties of pennyroyal oil [32].

Pennyroyal oil exhibited repellent and insecticidal effects. After 90 minutes exposure, none of the mosquitoes were airborne and those that were in contact with the oil were dead. The insect repellent activity was attributed to the high pulegone content [36].

2.4. Salvia officinalis L.

Salvia officinalis, more commonly known as garden sage, is a member of the Lamiaceae family. Sage has been renowned for its healing properties since the Ancient Greeks. The Romans inherited the medicinal knowledge on sage and used it to enhance diuresis, menstruation and to stop bleeding of wounds. It was also used to treat pain associated with colds and rheumatism [37]. Scientifically, sage has several medicinal properties, such as, antioxidant [38, 39], antibacterial [40], anti-inflammatory [41] and antiviral effects [42] and is also used to control Alzheimer's disease [43]

Sage contains several metabolites primarily monoterpenoids and sesquiterpenoids, diterpenoids [43], triterpenoids, such as ursolic and oleanolic acid [41, 44], and also flavonoids and phenolic glycosides [45]. The essential oil of Portuguese sage according to [46] contains α-thujone (17.4 %), α-humulene (13.3 %), 1,8-cineole (12.7 %), *E*-caryophyllene (8.5%) and borneol (8.3%) as major constituents. In another study [47], the sage essential oil contained mainly α-thujone (29.1 %), camphor (26.3 %), 1,8-cineole (9.3 %), α-humulene (4.4 %) and terpinen-4-ol (4.0%). Similar results were obtained in a local study [48], where the Maltese sage oil was found to contain mainly α-thujone (29.28 %), camphor (26.61 %) and 1,8-cineole (15.53 %) as the major constituents (Figure 10).

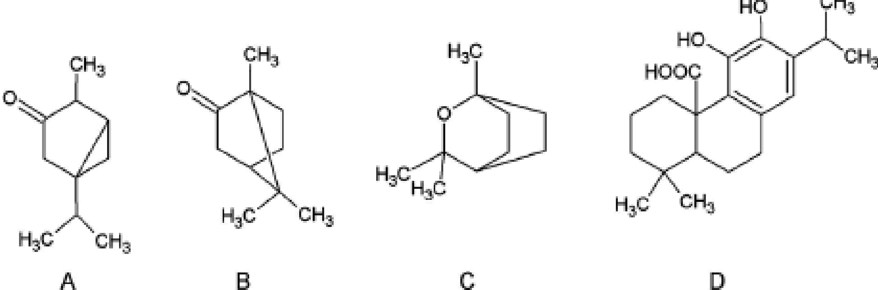


Figure 10. The common constituents of *Salvia officinalis* L. essential oil: (A) thujone, (B) camphor, (C) 1,8-cineole and (D) carnosolic acid.

Another significantly important metabolite in sage is carnosolic acid, a bitter abietane diterpenoid derivative with a carboxylic acid structure. This compound possesses antimicrobial, antioxidant, antiviral and anticancer activities [49]. Carnosolic acid was extracted using Soxhlet extraction and petroleum ether as extractant. The extract was dried and dissolved in pyridine/acetic anhydride. The neutral fraction was then chromatographed using silica gel as support [48].

Cultivation studies revealed that sage is best cultivated under shade conditions with irrigation. Propagation is best performed by cuttings every three weeks during spring after the plants

have ceased to flower. The recommended planting distance is 30 cm in a row with a cultivation density of 10 plants per m^2. Plants should be irrigated immediately after planting of cuttings and twice weekly in summer. The monthly harvesting of leaves produced a variable content of essential oil on fresh weight basis with the peak reached during the month of August (2.24 % v/v) and the least during December (0.52 % v/v) (Figure 11).

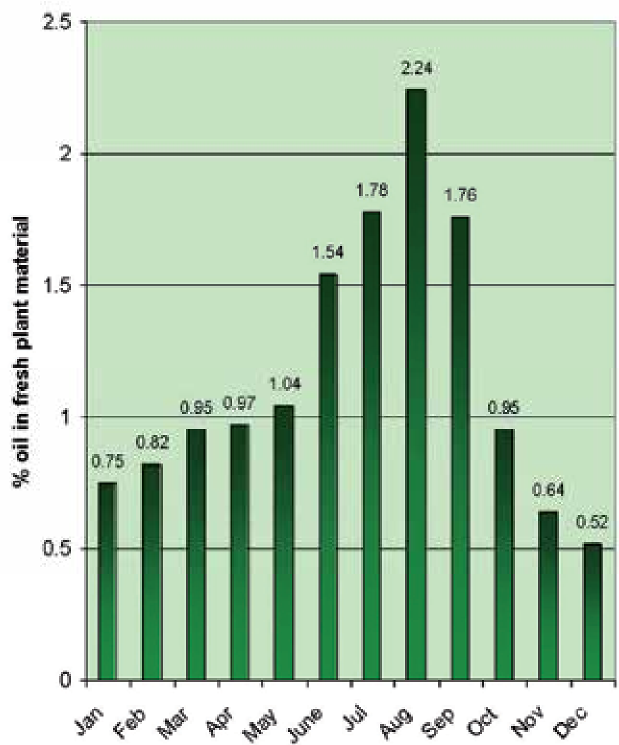


Figure 11. The yield of Maltese sage essential oil throughout the year [48].

2.5. Verbena officinalis L.

Verbena officinalis, a member of the Verbenaceae family, is also known as vervain. This plant is indigenous to Europe, North Africa and Asia but has been introduced to North America and Australia. Some of the common traditional uses of vervain, worldwide, were in the treatment of respiratory problems such as cough, wheezing and shortness of breath [37], as a purgative, in the treatment of haemorrhoids, eye problems [50], wounds, fever and stomach upsets [51]. In Malta, vervain was used in the treatment of many ailments particularly, carbuncles, boils, wounds, eczema, high blood pressure, diarrhoea, dysentery, cough and arthritis [52].

The main constituents of *Verbena officinalis* are iridoid glycosides, namely verbenalin [53], hastatoside [54] and aucubin [55]. It yields an essential oil, with citral, geraniol, limonene and verbenone as main constituents [56]. Other constituents include the flavone derivative artemetin, phenylpropane glycosides verbascoside and eukovoside and the triterpenes ursolic

acid, β-sitosterol and lupeol [57]. Some of these constituents are highlighted in figure 12. The volume of oil obtained from Maltese sources was negligible [58].

Figure 12. Typical constituents of *Verbena officinalis* L.: (A) verbenalin, (B) artemetin and (C) verbascoside

A hydromethanolic extract of the dried aerial parts of Maltese vervain was obtained by Soxhlet extraction [58]. The constitution of verbenalin was determined by HPLC using Supelcosil LC-18 column, acetonitrile/water-phosphoric acid (pH 2) gradient mobile phase with a flow rate of 1.5 ml/min. The content of verbenalin expressed as dry weight of plant material was 2.09 % (w/w). Previous reports [59] declared that contents of verbenalin were less than 0.1 % when extracted with ether but the content in methanolic extracts varied between 0.12 and 0.50 % [60].

Several pharmacological activities are attributed to vervain, namely, anti-inflammatory [54, 61], neuroprotective [62], antioxidant, antifungal [63], antileukaemic [64] and hepatoprotective [65]. Verbenalin, from Maltese vervain sources, was tested on mammalian intestinal smooth muscle *in vitro* and compared to acetylcholine [58]. Final molar concentrations of acetylcholine (40nM to 10 μM) and verbenalin (21.3 μM to 2.6 mM) were prepared. The smooth muscle was placed in an organ bath with a 30 ml-muscle chamber in freshly prepared Tyrode's solution maintained at 37°C. The muscle was challenged for a period of 30 seconds with the two substances at the stated concentrations (Figure 13). Between additions, the muscle was allowed to achieve baseline activity. The median effective concentration for acetylcholine and verbenalin were 1.54 μM and 0.32mM, respectively, with acetylcholine being approximately 200 times more potent than verbenalin. In spite of its mild effects, the presence of verbenalin in vervain is not recommended in pregnancy [66].

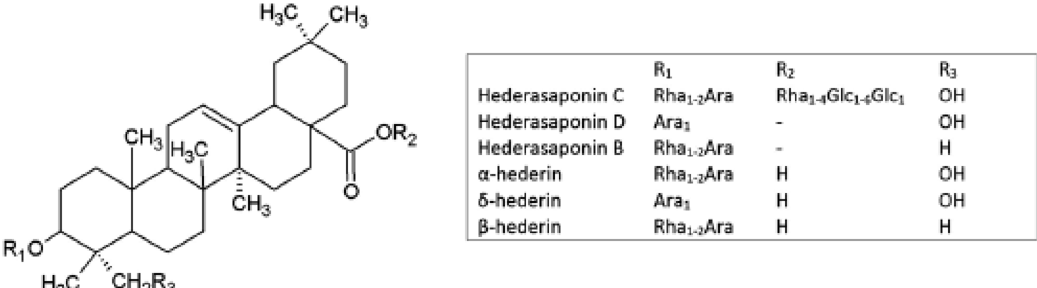
2.6. Hedera helix L.

Hedera helix L. or common ivy, a member of the Araliaceae family, is indigenous to Europe but its presence has been reported in Asia (as far as Japan), Africa and North America. Records of the use of ivy as a medicinal plant, dates back to the times of Hippocrates. The flowers were used to treat dysentery, earache and headache, while the leaves were usedas an emmenagogue [67). Others claimed it to be effective in the treatment of sunburn, ulcers, tuberculosis, bronchitis, whooping-cough, constipation, wounds and various skin diseases [68-70].

The main constituents of *Hedera helix* are the saponins, more commonly known as hederasaponins. This is a group of structurally related triterpenoid glycosides with an oleanane

Figure 13. The spasmodic response of the smooth intestinal muscle with (a) acetylcholine and (b) verbenalin [58].

backbone (Figure 14). These are divided into mono- and bidesmosides. Monodesmosides include α-hederin and hederagenin 3-O-β-glucoside, while bidesmosides include hederasaponins C, A, B, D, E, F, G, H and I [71, 72].



	R_1	R_2	R_3
Hederasaponin C	$Rha_{1\text{-}2}Ara$	$Rha_{1\text{-}4}Glc_{1\text{-}6}Glc_1$	OH
Hederasaponin D	Ara_1	-	OH
Hederasaponin B	$Rha_{1\text{-}2}Ara$	-	H
α-hederin	$Rha_{1\text{-}2}Ara$	H	OH
δ-hederin	Ara_1	H	OH
β-hederin	$Rha_{1\text{-}2}Ara$	H	H

Figure 14. The main pentacyclic triterpenoids of *Hedera helix* L.

Another important group is that represented by phenolics (flavonoids, anthocyanins, coumarins and phenolic acids) [71, 73]. The essential oil from ivy stems and leaves contains germacrene D, β-caryophyllene, sabinene, β-pinene, limonene, and α-pinene [74]. Hederasaponins, from ivy grown in Malta, were extracted with 70 % ethanol by Soxhlet extraction [75]. Spring, summer, autumn and winter leaves yielded 12.75 %, 11.82 %, 10.74 % and 10.97 % (w/w) of dried extract. The hederosaponin content was determined by HPLC using Supelcosil LC-18 column, acetonitrile/water-phosphoric acid (0.01 N) gradient mobile phase with a flow rate of 1 ml/min. Hederasaponin C and α-hederin were used as standards. The 70 % ethanolic extract contained 46.7 % hederasaponin C and 6.1 % α-hederin totaling 52.8 %. Purification of the ethanolic extract through an alumina column with methanol as solvent resulted in 62.2 % hederasaponin C and 9.2 % α-hederin. This goes in accordance with other authors [76, 77] who confirmed that hederasaponin C is the main saponin in common ivy.

Hedera helix was investigated for its pharmacological potential, by many scientists. Typical reported activities include anti-inflammatory [78, 79], antiviral [80], antifungal [81], antibacterial, mucolytic, antispasmodic agent and *in vitro* bronchodilatory [82, 83].

The ivy leaf extracts, obtained from Maltese sources, and the standards were tested for their antimicrobial activity [75]. The tested organisms were *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella* sp., *Serrata* sp. and *Candida albicans*. Pure α-hederin was inactive against all organisms presumably due to its poor solubility in water as was reported by [84]. On the other hand, pure hederasaponin C was active against all the tested organisms. It was more active than both ivy extracts against *Staphylococcus aureus*, *Enterobacter aerogenes*, *Klebsiella* sp. and *Serrata* sp. It was just as effective as the purified ivy extract against *Escherichia coli* and *Candida albicans*. The only difference between hederasaponin C and α-hederin is that the former has an extra sugar group. Being a bidesmoside, hederasaponin C is more water soluble. There are no other structural differences that may have contributed to a better antimicrobial activity. In conclusion, the purified ivy extract (62.2 % hederasaponin C) and pure hedersaponin C were more active against *Staph. aureus* and least active against *Candida albicans* (table 3).

Microorganism	Minimum Inhibitory Concentrations (mg/l)			
	hederasaponin C	α-hederin	Ethanolic extract	Purified ethanolic extract
Staph. aureus	0.312	-	1.25 – 2.50	0.625 – 1.25
Escherichia coli	5	-	10	5 - 10
Enterobacter aerogenes	2.5	-	5 – 10	5 – 10
Klebsiella sp.	1.25	-	5 – 10	2.5 – 5
Serrata sp	2.5	-	5 – 10	-
Candida albicans	10	-	-	10

Table 3. Minimum Inhibitory Concentrations (mg/l) for *Hedera* extracts [75].

2.7. Crataegus monogyna Jacq.

Crataegus monogyna (may, quick or common hawthorn) belongs to the Rosaceae family. Records show that it has been used since the Ancient Roman times. Dioscorides and later Paracelsus reported the effects of the shrub in heart conditions [85]. Mediterranean folk medicine utilized the shrub as an astringent, febrifuge, sedative, in the treatment of diarrhoea, whitlow's, heart disease, high blood pressure and to improve circulation [86].

Hawthorn contains several constituents, most of which are either pharmacologically active or have a nutritional value. Triterpenoids, flavonoids, coumarins and amines are the main groups of compounds that possess a significant activity in the treatment of cardiovascular diseases [87].

The two triterpenoids, abundantly found in hawthorns, are ursolic and oleanolic acids (figure 15). These account for 90 % of the total pentacyclic triterpenoids present in the shrub [88]. The triterpenoids oleanolic, ursolic and crataegolic acids were extracted as a crude mixture with 96 % alcohol [89, 90], as an acid-ether extract [91] and as a tincture of *Crataegus monogyna* [92].

Figure 15. Structure of oleanolic acid and derivatives. For oleanolic acid, R_1 and R_2 are hydrogen atoms. For the triterpenoid glycosides, R_1 and R_2 represent different sugar groups.

Crataegus species are renowned for their flavonoid content [93]. Flavonoids include vitexin, hyperoside [94], rutin, quercetin, luteolin-7-glucoside [95] and apigenin [96]. The most abundant in flowers was the hyperoside [94]. Other flavonoids included catechin, luteolin, epicatechin, quercetrin, quercetrin-3-rhamnogalactoside and luteolin-3',7-diglucoside [87, 97]. Hawthorn contains a large variety of cardiotonic amines in different plants parts especially the leaves and flowers. These include di- and trimethylamine, ethanolamine, ethylamine [87], isoamyl and isobutylamines [92]. Choline and acetylcholine are also present. It contains other minor constituents [98].

Hawthorn extracts have been tested for several pharmacological activities such as antimicrobial, antioxidant [99, 100], peroxysmal tachycardia [101], prevention of cardiac necrosis [102-104], hyperglycaemia [105], atherosclerosis [106] and hypertension [107].

The hydroethanolic extract of *Crataegus monogyna* was studied for its angiotensin-converting enzyme (ACE) inhibitory activity [108]. The direct interaction of extracts and pure compounds with ACE was performed using a microtiter plate method modified for the ACE detection kit (Sigma, MO) at 430 nm (Figure 16). The crude extract contained triterpenic acids, flavonoids and coumarins. The ACE inhibitory activity of the crude extract and pure oleanolic acid (a triterpenoid) were compared to captropril, the latter used as a control drug. The hydroethanolic extract and oleanolic acid showed higher IC_{50} values (335.00 μg/ml and 3.61 μM, respectively) in comparison to captopril (46.9 nM). However, these results suggest that the anti-ACE activity of the hydroethanolic extract from hawthorn is due to oleanolic acid and other triterpenic acids present. This was the first study to suggest that triterpenic acids contribute to the antihypertensive activity of hawthorn. In previous studies, the ACE inhibitory activity of *C. monogyna* extracts was always attributed to flavonoids and proanthocyanidins.

http://dx.doi.org/10.5772/60094

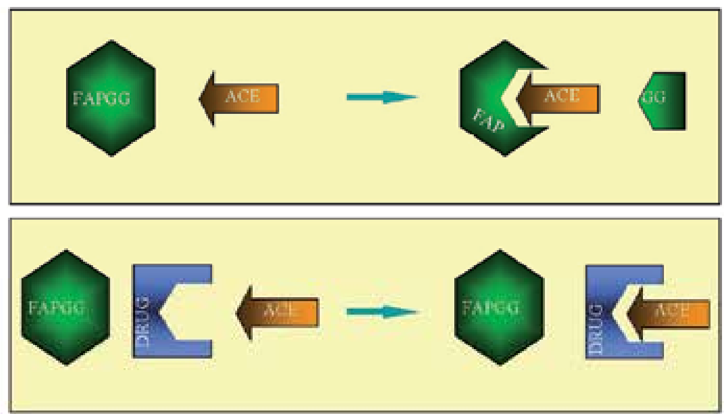


Figure 16. The interaction of angiotensin converting enzyme and compounds (such as captopril and oleanolic acid) with the chromophore N-[3-(2-furyl)acryloyl]-L-phenylalanylglycylglycine (FAPGG).

2.8. Calendula officinalis L.

Calendula officinalis, more commonly known as the pot marigold, belongs to the Asteraceae family. The use of pot marigold for therapeutic purposes has been recognised since the time of St. Hildeguard (1098-1197), who described in her work Causae et Curae and Physica the curative properties of ringula [109]. *Calendula officinalis* was being used internally during the twelfth century for digestive disturbances and also as an antidote against man and animal intoxication. It was also used externally for the treatment of impetigous eczema. Hundred years later (1193-1280), Albert the Great utilized the *Calendula* which he called Sponsa Solis, against animal bites and also to alleviate hepatic pain and pain of the spleen. This plant can also be seen in the herbals of the Renaissance. Leonard Fuchs stated that if the plant was to be boiled and held in the mouth for some time it relieved dental pain. Later during the sixteenth Century, Mattioli (1500-1577) attributed the therapeutic properties of the pot marigold in the constriction of the heart and palpitations as a consequence of menstrual fluid retention. According to this author, the water of the marigold has sudatory properties. He was also the first physician to recommend the herb for its therapeutic use against cancer and accordingly called it Herba Cancri. Mattioli's recommendations of *Calendula officinalis* as a remedy against cancer were fully approved by Osiander and Hufeland [109]. The pharmacist, J. W. Weinmann (1683 - 1741), in his work "Phytantoza iconographica" recommended the aqueous marigold extract for the alleviation of red and inflamed eyes and also used the plant in the treatment of goitre.

Pharmacologically-active classes of compounds, in the marigold, include the terpenoids including the carotenoids, flavonoids, coumarins and polysaccharides [110-112]. The saponosides are particularly abundant in the plant. There are also numerous triterpenoid alcohols which are derived from tarassene, lupene, oleanene and ursene. These are present as free or esterified as monols, diols and triols. The content of monoesters of the triterpenoid diols is between 2 and 45 %, of which 1.85 % is made up of faradiol esters. The most common triter-

penoid is oleanolic acid (Figure 15). The colour of the flowers is determined by the amount of carotenoids which can vary from 1.5 to 3 %. The orange flowers are made up mainly of carotenes particularly lycopene whereas the yellow flowers contain mainly xanthophylls [113]. The heterosides of quercetin and isorhamnetin (flavonoids) are present in the dry *Calendula* drug [114]. Their content varies between 0.25 and 0.88 %. The *Calendula* drug contains 14.75 % of polysaccharides (PS), which are soluble in water. The three main ones are PS I (molecular weight of 15,000), PS II (molecular weight of 25,000) and PS III (molecular weight of 35,000). These are made up of galactose, rhamnose and arabinose subunits. Other constituents include the essential oil, triterpene alcohols, phenolic acids, tannins, sterols, tocopherols, N-paraffins, pyrethrins, sesquiterpenes and coumarins. Monoterpenes and sesquiterpenes make up the essential oil. However, the latter does not contain sesquiterpene lactones. Moreover, 50 - 60 % of the oil present in the seeds is made up of calendulic acid, an unsaturated fatty acid having an unusual chemical structure.

Flowerheads of *Calendula officinalis* were extracted with methanol and following concentration, the extract was hydrolysed with 0.5 M hydrochloric acid. The mixture was centrifuged and the residue was dissolved in chloroform. This was then dried and subjected to column chromatography (silica gel; mobile phase - petroleum ether:dichloroethylene:acetic acid 50:50:0.7). The collected fractions were analysed by melting point determination, Infrared and Ultraviolet spectroscopy. The content of oleanolic acid extracted from the dried flowerheads was 0.13% (w/w) [115].

The marigold has been investigated for its anti-microbial, anti-inflammatory [116, 117], anti-tumour [110] activities, effects on the cardiovascular and nervous systems [118, 119] as well as oestrogenic [120], hypolipidaemic [121], anti-ulcer [122] and spermicidal properties [123].

The antimicrobial activity was conducted for oleanolic acid against a number of organisms [115]. Due to the insoluble nature of oleanolic acid in water, it was incorporated in the nutrient agar for bacterial strains and in Sabouraud's dextrose agar for fungi. In fact, [124] stated that the anti-bacterial agent was soluble in alcohol but not in water. According to the results obtained after 24 hours in the study performed, oleanolic acid was active against Gram-positive organisms (*Strep. faecalis, Strep. viridans* and *Staph. aureus*) except *Staph. albus*. However, for *Staph. albus*, there was slight inhibition which resulted in hazy growth. On the other hand, it was inactive against Gram-negative strains. Although for *Morganella* species and *Pseudomonas aeruginosa*, there was some degree of inhibition, the plate which contained the ethanol instead of oleanolic acid showed the same degree of inhibition. Hence, the anti-bacterial activity in these two cases might be attributable to the presence of ethanol. Oleanolic acid did not show any activity against *Candida albicans*.

The topical in vivo effects of oleanolic acid (2.5 %) on inflamed bites induced by mosquitoes (*Culex pipiens*) was studied [115]. The positive and negative controls included indomethacin (2.5 %), hydrocortisone (1 %) and petroleum jelly. The topical anti-inflammatory activity of oleanolic acid, over a 24-hour period, was comparable to that of hydrocortisone, after being applied at 8 hourly periods. However, when compared to indomethacin, oleanolic acid was found to be less effective ($P<0.01$). In accordance with the study conducted by [125], oleanolic acid was found to have similar effects to those of hydrocortisone. However, other studies relate oleanolic acid to non-steroidal anti-inflammatory agents, like indomethacin [126].

http://dx.doi.org/10.5772/60094

2.9. Melissa officinalis L.

Melissa officinalis L. is a member of the Lamiaceae family. It is also known as lemon balm or simply as balm. The Latin name ''Melissa'' (balm) refers to the Greek word 'melitos', that is honey. It is believed that the plant attracts honey bees. The plant is found mainly in the Mediterranean region and eastwards to Asia and Siberia. Balm is renowned for its effects on the nervous system and is used to treat nervous agitation, insomnia, hysteria, melancholia, migraine, headache, toothache, earache and nerve pains. It is also useful for gastrointestinal problems such as gastric complaints and lower abdominal pain [127, 128].

Lemon balm contains a volatile oil [129], flavonoids (cynaroside, rhamnocitrin, isoquercitrin, cosmosin), phenolic acids (carnosic acid and rosmarinic acid), and triterpene acids (particularly ursolic and oleanolic acid) [130]. The study by [131] focused mainly on the cultivation parameters that affect the quantity and quality of the lemon balm oil. The oil yield was 0.1 % (v/w) with cis-citral and trans-citral as the major constituents (figure 17).

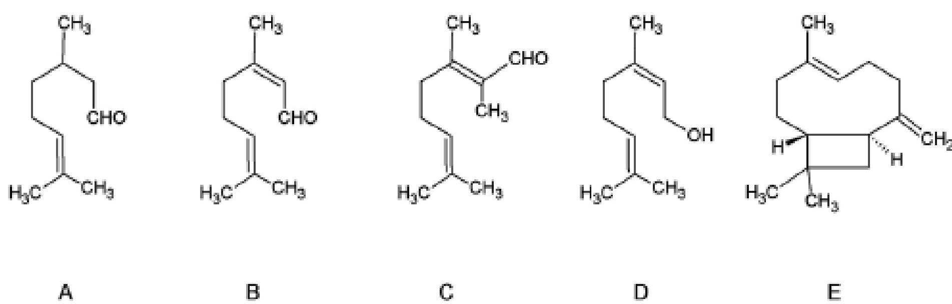


Figure 17. The main constituents of *Melissa officinalis* essential oil: (A) citronellal, (B) geranial (trans-citral), (C) neral (cis-citral), (D) trans-caryophyllene

Seeds were procured from four sources: Maltese (Argotti Gardens), Swiss (Basel Botanic Gardens), German A (Botanischer Garten der Martin-Luther-Universität) and German B (Botanischer Garten der RWTH). The planting distance was of 20 cm in a row with a distance of 50-60 cm between rows. The cultivation density was of 10 – 12 plants per m^2. The plants were irrigated immediately after transplanting and then once every fortnight in winter but twice weekly in summer. Plots were divided into two: half treated with fertiliser (NPK Mg (12+12+17+2) + Trace elements) while the other half left untreated, as a control. The leaves were harvested in May and subjected to steam distillation extraction and GC-MS analysis. Table 4 illustrates the results obtained in this study.

In most cases, the use of fertilizer improved content of the two main terpenoids, geranial and neral. This goes in accordance with [132], stating that nitrogen fertilisers increased the yield of these constituents. In some cases citronellal also showed significant increases with fertilizer application. In another study, the oil yield was found to vary between 0.16 and 0.25% [133]. With farmyard manure, the content of neral (28.23%) and geranial (39.86%) was higher than with other treatments. Oil yield was also significantly affected by planting spacing and nutrient amendments.

Sample		Citronellal	Nerol	Geranial	Neral	Caryophyllene
Maltese	w/o Fertiliser	0.00	0.00	37.11	47.39	1.02
	Fertiliser	0.52	0.00	36.82	47.74	1.26
Swiss	w/o Fertiliser	0.55	0.00	36.08	48.92	2.11
	Fertiliser	1.31	0.55	30.73	45.13	2.67
German A	w/o Fertiliser	1.24	0.57	30.96	45.79	2.62
	Fertiliser	1.25	0.71	32.11	47.23	1.84
German B	w/o Fertiliser	1.65	0.56	31.39	47.63	2.13
	Fertiliser	1.31	0.74	33.42	49.19	1.94

Table 4. The composition of essential oils obtained from lemon balm of different seed origins [131].

2.10. Olea europea L.

Olea europea L. is a typical Mediterranean plant within the Oleaceae family with culinary and medicinal virtues. The typical extract from this plant is the fixed oil obtained from the fruit. In the ancient world, by 2000 BC the olive tree was already in cultivation. Olive and olive oil was used and traded by the Egyptians, Phoenicians, Greeks and Romans. Today, a large number of olive varieties are recognised internationally as table olives and olives for oil production. Extracts from the olive tree were used in the treatment of hypertension, hyperglycaemia, hyperacidity [134], constipation, for treatment of wounds, sunburn and muscle aches [135, 52] amongst others.

The bioactive phenolic compounds present in the olive fruit include phenolic acids, phenolic alcohols, flavonoids and secoiridoids. The main phenolic acids are cinnamic, syringic, p-coumaric, vanillic, caffeic, 3,4-dihydroxyphenylacetic and protocatechuic acid [136]. Phenolic alcohols include 3,4-dihydroxyphenylethanol (hydroxytyrosol) and p-hydroxyphenylethanol (tyrosol) [137-139]. Flavonoids include taxifolin, apigenin, luteolin and lignans represented by pinoresinol and its metabolites [140]. However, an important class of metabolites found in the leaves and fruit of *Olea*, is that of the secoiridoid glycosides. These include oleuropein (figure 18), demethyloleuropein, oleuropein aglycone and elenolic acid [141-144].

Oleuropein was extracted from Maltese olives as follows [145].The leaves were defatted with petroleum ether and then extracted with 50% ethanol for 6-8 hours. The dried extract was then treated with water and sodium chloride was added until saturation was achieved. Chloroform was added and the aqueous extract was collected. Ethylacetate was added to the aqueous extract and following partitioning, the ethylacetate extract was collected. The extract was then subjected to dryness in order to obtain a yellow crystalline substance. Oleuropein in the olive leaf ethanolic extract amounts to 20.6 %, as mentioned by [146], with a content varying from 20 to 25% (w/w) total dry weight.

Olea europea was tested for its antimicrobial [147, 148], antiviral [149], antioxidant [146, 150], antihypertensive, antiatherosclerotic [151, 152] and antidiabetic [153] activities amongst

http://dx.doi.org/10.5772/60094

Figure 18. Structures of polyphenolic compounds from olive oil: (A) hydroxytyrosol and (B) oleuropein glucoside.

others. Maltese olive leaf extract was studied for its immunomodulatory activity [145]. Human peripheral blood lymphocytes were isolated and cultured on RPMI medium. Oleuropein (540 – 0.054 μg/ml) was tested alongside phytohaemagglutinin (m-form, Gibco BRL, UK - 1 – 0.0001 % as positive control and *Olea* extracts to final concentrations ranging from 540 – 0.054 μg/ml (oleuropein content). The cells were studied for their survival, death and morphological characteristics using WST-1 assay, LDH (Boehringer-Mannheim, Germany) and the Papanicolau staining procedure, respectively. Oleuropein possesses three α,β -moieties; two α, β - unsaturated keto systems at the 3,4-dihydroxyphenyl part and one α, β -unsaturated aldehyde system on the secoiridoid part, which are important for the non-toxic but stimulatory activity on lymphocytes. From the results obtained, oleuropein was more effective when it formed part of the extract (SC_{50}, < 0.054 μg/ml) than when used in its pure form (SC_{50}, 0.146 μg/ml).

2.11. Urtica dubia Forsk.

Urtica dubia Forsk., stinging nettle, is a member of the Urticaceae family. The *Urtica* species are common weeds found growing wild throughout the temperate zones of both hemispheres worldwide. These species are renowned for their stinging sensation when touched. Since Ancient Greek times, stinging nettle was used as a medical treatment for septic wounds, nosebleeds and as an emmenagogue [154]. They were later used as diuretics and laxatives, in the treatment of asthma, pleurisy, dog bites, tinea and mouth ulcers [155]. In Malta, *Urtica dubia* was used in the treatment of pneumonia, chilblains, as a metabolic stimulant, to improve blood circulation and as a diuretic [156].

Stinging nettle contains bioactive amines such as 5-hydroxytryptamine; flavonoids such as quercetin, kaempferol and their glycosides; coumarins such as scopoletin; organic acids such as caffeic acid and chlorogenic acid; fatty acids such as erucic acid, α-linolenic acid and linoleic acid; an essential oil; carotenoids such as lutein, β-carotene, neoxanthin, violaxanthin and lycopene; agglutinins such as *Urtica dioica* agglutinin (Figure 19); and phytosterols such as β-amyrin, stigmasterol, oleanolic acid and β-sitosterol [157-163]. The isolation of *Urtica dubia* agglutinin (UDuA) was based on a procedure described by [164] with some modification [165]. Briefly, the fresh plant materials (rhizomes, leaves and stems) were homogenised with 0.1N

HCl (200 g/l) and allowed for 24 h shaking. The filtrate was passed through series of extractions with 2N NaOH and $(NH_4)_2SO_4$ solutions. The final agglutinin purified extract was washed with phosphate buffer saline (PBS) which was used as the medium for the bioassays. Phytohaemagglutinin (PHA, Invitrogen) was prepared likewise in PBS. The content of UDuA in the rhizomes, leaves and stems was 0.49 %, 0.65 % and 0.16 %, respectively.

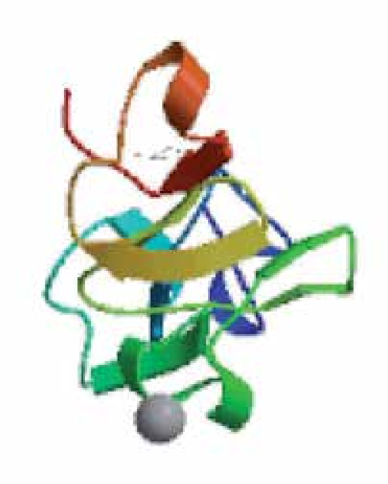


Figure 19. The crystal structure of *Urtica dioica* agglutinin isolectin I [166].

The stinging nettle possesses several pharmacological activities, namely antioxidant, antimicrobial, antiulcer and analgesic activities [167], anti-inflammatory effects [168] and cardiovascular effects [169]. The UDuA extracts from the Maltese *Urtica dubia* were tested for haemagglutination activity on human red blood cells (RBCs) [165]. Briefly, a 1% suspension of RBCs was prepared and 100 μl aliquots were tested with different concentrations of UDuA and PHA. The agglutination was quantified by lysing the precipitated agglutinated cells and read spectrophotometrically at a wavelength of 405 nm at 20, 40, 60 and 80 minutes. Over the 80-min period, the best results were obtained after 60 min, as was observed by [170] for the snowdrop lectin. Extracts from all three plant parts exhibited superior haemagglutination activity (AgA) to the standard PHA lectin (AgA - 3.996 ± 0.259). The highest activity was exhibited by the stems, followed by roots and leaves (AgA - 4.824 ± 0.301, 4.693 ± 0.368 and 4.594 ± 0.417, respectively at 1% concentrations).

2.12. Capparis spinosa L.

Capparis spinosa L. is a member of the Capparaceae family, also known as the caper plant. Today it is renowned for its culinary uses, particularly in the Mediterranean region. When stored in brine, the intensive and slightly pungent taste of the capers is preserved. Capers were used since prehistoric times, although it is believed that other *Capparis* species were actually utilised rather than *Capparis spinosa* [171]. In the past, the root bark and leaves were used as aperient,

tonic, diuretic and expectorant while the flowers were used as anthelmintic, emmenagogue, analgesic, antimicrobial, antifertility, anti-inflammatory, hepatoprotective, antihyperglycemic, immuno-stimulant and in the treatment of anaemia, diabetes, heart problems, amongst other uses [172]. In Malta, caper extracts were used as diuretics, in the treatment of skin rashes and pain associated with gout [135].

According to [173], the capers contain 79% moisture, 1.6% ash, 5.8% protein, 1.6% fat and 5.4% raw fibre. It contains several minerals such as, Ca (871 ppm), Mg (636 ppm), K (542 mg/100mL), Na (226 ppm), Fe (13 ppm) and P (21 mg/100g). Other valuable constituents include the flavonoids such as rutin, kaempferol and its glycosides; alkaloids (Figure 20) such as cadabicine [174], capparisine A, capparisine B, capparisine C; 2-(5-hydroxymethyl-2-formylpyrrol-1-yl) propionic acid lactone and N-(3′-maleimidy1)-5-hydroxymethyl-2-pyrrole formaldehyde [175]. Other constituents include aldehydes, esters, sesquiterpenes, monoterpenes and sulphur compounds with methyl-isothiocyanate as the main constituent [176], carotenoids with lutein as the main constituent [177], sterols such as β-sitosterol, campesterol, stigmasterol, 5-avenasterol, cholesterol and campestanol [178], and a lectin (*Capparis spinosa* lectin) [179].

A

B

Figure 20. Typical *Capparis spinosa* L. alkaloids: (A) capparisine and (B) cadabicine.

Metabolites from the Maltese caper plant were obtained by extracting the plant material with four different solvents [180]. The xanthoproteic test for proteins [181], Fehling's test for carbohydrates, Sudan IV test for fats and lipids [182], Dragendorff's test for alkaloids [183], triphenyltetrazolium test for terpenoids and the acidified vanillin test for flavonoids [184] were carried out on the extracts. The petroleum ether extract (0.020 % w/w plant material) contained fats and lipids, the aqueous/methanol extract (2.401 % w/w plant material) contained proteins and terpenoids, the methanol extract (1.398 % w/w plant material) contained alkaloids, while the aqueous extract (3.015 % w/w plant material) contained carbohydrates and terpenoids.

The caper plant was tested for several pharmacological activities such as antiviral [185], anti-arthritic [186], anti-oxidant [187], hypolipidaemic [188], antihyperglycaemic [189], chondrocyte protective [190], antiallergic, antihistaminic [191], antifungal [192], anti-Leishmania [193] and antimicrobial [194]. The Brine shrimp test was conducted for the extracts derived from the Maltese caper plants [180]. Briefly, Artemia salina eggs were hatched and challenged with

various concentrations of the extracts ranging between 0.0001 and 1 % as 1 in 10 dilutions. After 24 hour the number of dead larvae (nauplii) was determined. The aqueous extract exhibited the lowest LC_{50} (0.014%) compared to the methanol (0.0475%) and the aqueous/ methanol (0.08%) extracts. The chloroform extract did not reach a 50% lethal effect and therefore the LC_{50} could not be determined. According to [195] the methanol, aqueous and aqueous/methanol extracts were all active as their LC_{50} was below the 0.1% threshold.

2.13. Ephedra fragilis Desf.

Ephedra fragilis Desf., a member of the Ephedraceae family, is also known as Mormon tea. *Ephedra* has been listed amongst the most important herbs used by Ancient Chinese civilisations. It was known as Ma Huang and was used to treat coughs, colds, headache and fever. It was later used by the Chinese to treat asthma [196] and acute nephritis [197]. This plant contains alkaloids [198], amino acids, proteins [199], tannins and fatty acids [200]. The volatile oil of *Ephedra fragilis* contains (E)-phytol (10.1%), pentacosane (5.2%), 6,10,14-trimethyl-2-pentadecanone (5.3%), cis-thujopsene (3.5%), and α-terpineol (3.0%) as the major components [201]. Flavonoids, minerals, and vitamins are also present. The principle alkaloid present in this plant is ephedrine [198] (Figure 21).

Figure 21. Ephedrine, pseudoephedrine and methylephedrine

Aerial parts of local cultivated *Ephedra fragilis* specimen were dried in an oven at 30ºC for 48 h, pulverized and dispersed in distilled water for 25 min. After thorough mixing for 30 min at 30ºC (twice), the filtrate was treated with sodium carbonate (15 g). An equal volume of benzene was added and then acidifed and treated with acidifed water. After neutralisation to a pH of 7, the precipitate was oven dried. The alkaloidal content in the different plant parts was determined; 1.8675 % (w/w) in flowers, 0.6234 % in seeds, 0.5198 % in pods and seeds, 0.1389 % in dried pods and 0.0547 % in branches [202].

Clinically, ephedra has been tested for its anti-hypertensive [203], bronchodilator [204], decongestant [205], diuretic [206] and immune booster [207]. The immunomodulatory response of ephedrine and the *Ephedra* extract were studied on human peripheral lymphocytes [202]. Cell viability, cytotoxicity and morphological characteristics were recorded for the test substances and phytohaemagglutinin (PHA), a mitogen known to stimulate cell division of T-lymphocytes. Over the 96-hour treatment, ephedrine and *Ephedra* extracts exhibited high cell viability (> 97% viability) and blastogenesis when compared to the untreated control. The control cells measured 6-10 μm, while treated cells measured 20-40 μm in diameter. The ephedrine present in *Ephedra* extracts exhibited a direct effect on lymphocytes *in vitro*.

2.14. Nicotiana glauca RC Graham

Nicotiana glauca RC Graham belongs to the *Solanaceae* family and is known as tree tobacco. This was native to South America but is now naturalized in North America, the Mediterranean, and Africa. Since, this plant was considered as poisonous [208], it has been rarely used in tradition. The more toxic counterpart, *Nicotiana tabacum* was used for several conditions particularly to expel leeches [209], against snakebite [210] and scabies [211].

Tree tobacco contains pyridine alkaloids [212], as for other *Nicotiana* species. The major pyridine alkaloids are nicotine and anabasine (figure 22). Nicotine predominated in *Nicotiana tabacum* [213] and *Nicotiana* rustica [214] whereas anabasine predominates in *Nicotiana glauca* [214, 215].

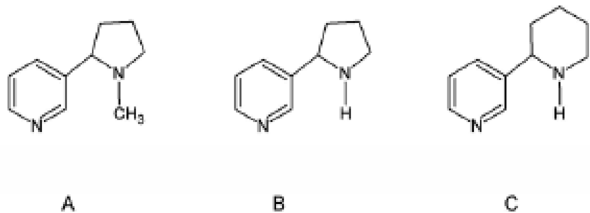


Figure 22. (A) Nicotine, (B) Nornicotine and (C) Anabasine, the main pyridine alkaloids of *Nicotiana* species.

The leaves of Maltese *Nicotiana glauca* were dried and extracted with 200ml of 0.5% sodium hydroxide [216]. After volume reduction, chloroform was added to extract the alkaloids in this organic phase. This phase was then treated with acidified water (0.05M hydrochloric acid) and then neutralised with ammonia solution to a pH of 7. The presence of alkaloids was tested at each step using the Dragendorff's reagent [217] and the anabasine content was determined by HPLC. A Shimadzu LC-10A HPLC (Shimadzu, Kyoto, Japan) using a C18 MicroBondapak column, 250 x 4.6mm, 10mm was used. The mobile phase consisted of 40 % methanol containing 0.2 % phosphoric acid buffered to pH 7.25 with triethylamine [218]. The anabasine standard was used for calibration and for the determination of anabasine in Nicotiana extracts. In the Maltese study, the anabasine content (0.258 ± 0.0042 %) concords very closely with the results obtained in a study in Arizona (0.233 ± 0.0061 % anabasine) [219]. In another HPLC determination, the anabasine content of *Nicotiana glauca* plants in California, was 0.143 % [220].

The nicotine and anabasine have been widely used as pesticides. Nicotine is a powerful insecticide towards aphids [221] and larvae of lepidopterous pests [222]. Anabasine and nicotine exert their insecticidal effect by interacting with nicotinic acetylcholine receptors [222, 223]. Anabasine and *Nicotiana glauca* extracts were tested for their effects against *Pieris rapae* larvae [216]. The paralysis of the larvae was an indicator of activity. Standard anabasine produced an effect on *Pieris rapae* larvae (EC_{50} - 0.572 mg/larva or 0.286 %) which was higher to that provoked by the extract (EC_{50} - 1.202 mg/larva or 0.601 %). It is possible that alongside anabasine there may be other metabolites that interfered with anabasine hence reducing the response of the caterpillars to anabasine.

3. Conclusion and further directions

The studies on the fourteen Maltese medicinal plants, presented herein, demonstrate a wide array of experimental work that is all associated with phytochemical research. This is a very small fraction of the Maltese medicinal flora, but in terms of research, this represents a diversity of research protocols that may be adopted for medicinal plant research. In some cases, phytochemical analysis is the end-point of the research whereas in others, phytochemical analysis leads on to further studies, including pharmacological testing.

Author details

Everaldo Attard[1*], Henrietta Attard[1], Antoine Tanti[2], Jurgen Azzopardi[2], Mario Sciberras[2], Victor Pace[2], Neville Buttigieg[2], Andrew Mangion Randon[2], Bernardette Rossi[1], Marie Josette Parnis[1], Karin Vella[2], Michelle Zammit[2] and Anthony Serracino Inglott[2]

*Address all correspondence to: everaldo.attard@um.edu.mt

1 University of Malta, Institute of Earth Systems, Division of Rural Sciences and Food Systems, Msida, Malta

2 University of Malta, Faculty of Medicine and Surgery, Department of Pharmacy, Msida, Malta

References

[1] Attard E. Status of Medicinal and Aromatic Plants in Malta. In: Baricevic, D., Bernath, J., Maggioni, L. and Lipman E. (eds.) *Report of a Working Group on Medicinal and Aromatic Plants*, First Meeting, 12-14 September 2002, Gozd Martuljek, Slovenia. Rome: International Plant Genetic Resources Institute; 2004.

[2] Kropp B, Krenn L, Draxler M, Hoyer A, Terkola R, Vallaster P, Robien W. Bufadienolides from *Urginea maritima* from Egypt. Phytochemistry 1996;42:513-522.

[3] Dewick PM. Medicinal natural products. A biosynthetic approach. New York: John Wiley & Sons Inc; 1997.

[4] Knittel DN, Stintzing FC, Kammerer DR. Simultaneous determination of bufadienolides and phenolic compounds in sea squill (*Drimia maritima* (L.) Stearn) by HPLC-DAD-MSn as a means to differentiate individual plant parts and developmental stages. Analytical and bioanalytical chemistry 2014: 1-16.

[5] Liu J, Tian J, Haas M, Shapiro JI, Askari A, Xie Z. Ouabain interaction with cardiac Na^+/K^+-ATPase initiates signal cascades independent of changes in intracellular Na^+ and Ca^{2+} concentrations. Journal of Biological Chemistry 2000;275(36) 27838-27844.

[6] Keenan SM, DeLisle RK, Welsh WJ, Paula S, Ball Jr WJ. Elucidation of the Na^+/K^+-ATPase digitalis binding site. Journal of Molecular Graphics and Modelling 2005;23(6) 465-475.

[7] Bayazıt, V, Konar V. Analgesic effects of scilliroside, proscillaridin-A and taxifolin from squill bulb (*Uriginea maritima*) on pains. Digest Journal of Nanomaterials & Biostructures 2010;5(2).

[8] Scicluna-Spiteri A. *Drimia maritima (L) stearn in Malta: the growth, quality and commercial potential of Maltese squill, Drimia maritima*. PhD thesis. University of Bradford; 1989.

[9] Cassar E. Studies on the micropropagation of the local squill: *Drimia maritima* (L.) Stearn. M.Sc. thesis. University of Malta; 1998.

[10] Yesilada E, Tanaka S, Sezik E, Tabata M. Isolation of an anti-inflammatory principle from the juice of *Ecballium elaterium*. Journal of Natural Products 1988; 51(3) 504 – 508.

[11] Dymock W. Pharmacographia Indica, A history of the principal drugs of vegetable origin. Pakistan: The institute of Health and Tibbi Research; 1972.

[12] Hegnauer R. Chemotaxonomie der Pflazen. Germany: Birkhauser Verlag Basel und Stutgart; 1964.

[13] Rao MM, Lavie D, Meshulam H. The Constituents of *Ecballium elaterium L.* Part XXIII. Cucurbitacins and Hexanorcucurbitacins. Journal of the Chemical Society, Perkin translation 1974; 2552 - 2556.

[14] Seifert K, and Elgamal MH. New cucurbitacin glucosides from *Ecballium elaterium* L. Pharmazie 1977;32(10) 605 – 606.

[15] Jodral MM, Martin JJ, Agil AM, Navarro Moll MC, Cabo Cires MP. *Ecballium elaterium* (L.) A. Richard II - Morphological and phytochemical studies. Boletin da Sociedade Bioteriana 1990;63 213-224.

[16] El-Haci IA, Atik-Bekkara F. Antioxidant activity of stems and leaves organic fractions of *Ecballium elaterium* L. Annals of Biological Research 2011;2 327-332.

[17] Favel A, Mattras H, Coletti-Previero MA, Zwilling R, Robinson EA, Castro B. Protease inhibitors from *Ecballium elaterium* seeds. International Journal of Peptide and Protein Research 1989;33 202-208.

[18] Attard E, Attard H. Antitrypsin activity of extracts from *Ecballium elaterium* seeds. Fitoterapia 2008;79(3) 226-228.

[19] Attard E, Attard H. A Micropropagation Protocol for *Ecballium elaterium* (L.) A. Rich. Cucurbits Genetics Cooperative Reports 2002;25 67-70.

[20] Attard E. *Ecballium elaterium* (L.) A. Rich.: *In Vitro* Regeneration, and the Production of Cucurbitacins in culture. In: Govil JN., Singh VK., Arunachalam C. (eds.) Recent Progress in Medicinal Plants: Search for New Drugs. Volume 13. USA: Studium Press LCC; 2006. p49 – 72.

[21] Attard E, Scicluna-Spiteri A. The Cultivation and Cucurbitacin Content of *Ecballium elaterium* (L.) A. Rich. Cucurbits Genetics Cooperative Reports 2003;26 66-69.

[22] Attard E, Cuschieri A. Cytotoxicity of Cucurbitacin E extracted from *Ecballium elaterium* and anticancer agents *in vitro*. Journal of Natural Remedies 2004;4(2) 137-144.

[23] Attard E, Brincat MP, Cuschieri A. Immunomodulatory activity of cucurbitacin E isolated from *Ecballium elaterium*. Fitoterapia 2005;76 439-441.

[24] Agil MA, Risco S, Miró M, Navarro MC, Ocete MA, Jiménez J. Analgesic and antipyretic effects of *Ecballium elaterium* (L.) A. Richard. Extract in rodents. Phytotherapy Research 1995;9 135–138.

[25] Howard M. Traditional folk remedies: a comprehensive herbal. Australia: Century; 1987.

[26] Lanfranco G. Duwa u Semm il-Ħxejjex Maltin. Malta: Edizzjoni Klabb Kotba Maltin; 1975.

[27] Mahboubi M, Haghi G. Antimicrobial activity and chemical composition of *Mentha pulegium* L. essential oil. Journal of Ethnopharmacology 2008;119(2) 325-327.

[28] Aghel N, Yamini Y, Hadjiakhoondi A, Pourmortazavi SM. Supercritical carbon dioxide extraction of *Mentha pulegium* L. essential oil. Talanta 2004;62(2) 407-411.

[29] Kokkini S, Hanlidou E, Karousou R, Lanaras T. Variation of pulegone content in pennyroyal (*Mentha pulegium* L.) plants growing wild in Greece. Journal of Essential Oil Research 2002;14(3) 224-227.

[30] Mkaddem M, Boussaid M, Fadhel NB. Variability of volatiles in Tunisian *Mentha pulegium* L.(Lamiaceae). Journal of Essential Oil Research 2007;19 (3) 211-214.

[31] Teixeira B, Marques A, Ramos C, Batista I, Serrano C, Matos O, Neng NR, Nogueira JMF, Saraiva, JA, Nunes ML. European pennyroyal (*Mentha pulegium*) from Portugal: Chemical composition of essential oil and antioxidant and antimicrobial properties of extracts and essential oil. Industrial Crops and Products 2012;36(1) 81-87.

[32] Tanti A. The Insect Repellent Activity of *Mentha pulegium* L. Essential Oil. BPharm (Hons) thesis, University of Malta; 1994.

[33] Sullivan JB, Rumack BH, Thomas H, Peterson RG, Bryson P. Pennyroyal oil poisoning and hepatotoxicity. Journal of the American Medical Society 1979;242(26) 2873-2874.

[34] Gordon WP, Forte AJ, McMurtry RJ, Gal J, Nelson SD. Hepatotoxicity and pulmonary toxicity of pennyroyal oil and its constituent terpenes in the mouse. Toxicology and Applied Pharmacology 1982;65(3) 413-424.

[35] Sztajnkrycer MD, Otten EJ, Bond GR, Lindsell CJ, Goetz RJ. Mitigation of pennyroyal oil hepatotoxicity in the mouse. Academic Emergency Medicine 2003;10(10) 1024-1028.

[36] Duke J. Focus on American Pennyroyal. The International Journal of Aromatherapy 1991;3(4) 18-19.

[37] Culpeper N. The Complete Herbal (1953). Birmingham: Kynoch Press; 1653.

[38] Lu Y, Yeap Foo L. Antioxidant activities of polyphenols from sage (*Salvia officinalis*). Food chemistry 2001;75(2) 197-202.

[39] Wang M, Li J, Rangarajan M, Shao Y, LaVoie EJ, Huang TC, Ho CT. Antioxidative phenolic compounds from sage (*Salvia officinalis*).Journal of Agricultural and Food Chemistry 1998;46(12) 4869-4873.

[40] Longaray Delamare AP, Moschen-Pistorello IT, Artico L, Atti-Serafini L, Echeverrigaray S. Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil. Food chemistry 2007;100(2) 603-608.

[41] Baricevic D, Sosa S, Della Loggia R, Tubaro A, Simonovska B, Krasna A, Zupancic A. Topical anti-inflammatory activity of *Salvia officinalis* L. leaves: The relevance of ursolic acid. Journal of Ethnopharmacology 2001;75(2) 125-132.

[42] Tada M, Okuno K, Chiba K, Ohnishi E, Yoshii T. Antiviral diterpenes from *Salvia officinalis*. Phytochemistry 1994;35(2) 539-541.

[43] Akhondzadeh S, Noroozian M, Mohammadi M, Ohadinia S, Jamshidi AH, Khani M. *Salvia officinalis* extract in the treatment of patients with mild to moderate Alzheimer's disease: a double blind, randomized and placebo-controlled trial. Journal of clinical pharmacy and therapeutics 2003;28(1) 53-59.

[44] Horiuchi K, Shiota S, Hatano T, Yoshida T, Kuroda T, Tsuchiya T. Antimicrobial activity of oleanolic acid from *Salvia officinalis* and related compounds on vancomycin-resistant enterococci (VRE). Biological and Pharmaceutical Bulletin 2007;30(6) 1147-1149.

[45] Lu Y, Yeap Foo L. Flavonoid and phenolic glycosides from *Salvia officinalis*. Phytochemistry 2000;55(3) 263-267.

[46] Lima CF, Carvalho F, Fernandes E, Bastos ML, Santos-Gomes PC, Fernandes-Ferreira M, Pereira-Wilson C Evaluation of toxic/protective effects of the essential oil of *Salvia officinalis* on freshly isolated rat hepatocytes. Toxicology in Vitro 2004;18-465.

[47] Duke JA. CRC Handbook of Medicinal Plants. Florida: CRC Press Inc; 1989.

[48] Azzopardi, J. An Evaluation of local *Salvia officinalis* L. BPharm (Hons) thesis. University of Malta; 1994.

[49] Aeschbach R., Philippossian G. Procédé d'obtention d'acide carnosique et son utilisation pur ses propriétes anticarcinogénes et antivirals. European Patent No. 0480077A1. 1992.

[50] Brimble LJ. Flowers in Britain: Wild, Ornamental and Economic. London: Macmillan and Co. Ltd; 1944.

[51] Sfikas G. Medicinal Plants of Greece. Athens: P.Efstathiadis & Sons: 1981.

[52] Lanfranco G. Some Recent Communications on the Folk Medicine of Malta, L-Imnara 1980;3 87.

[53] Buchi G, Manning RE. Constitution of Verbenalin. Tetrahedron 1962;18 1049-1059.

[54] Deepak M, Handa SS. Quantitative determination of the major constituents of *Verbena officinalis* using high performance thin layer chromatography and high pressure liquid chromatography. Phytochemical Analysis 2000;11(6) 351-355.

[55] Makboul AM. Chemical constituents of *Verbena officinalis*. Fitoterapia 1986;57, 50-51.

[56] Shamsardakani MR, Mosaddegh M, Shafaati A. Volatile constituents from the aerial part of *Verbena officinalis* L. (Vervain). Iranian Journal of Pharmaceutical Research 2010; 39-42.

[57] Khaled AA, Adnan AE, Salwa MN. Some pharmacochemical investigations on Verbena Tenuisecta. Research Journal of Agriculture and Biological Sciences 2009;5(5) 649-659.

[58] Sciberras M. Verbenalin: A glucoside found in *Verbena officinalis* L. BPharm (Hons) thesis. University of Malta; 1994.

[59] Horodysky AG, Waller GR., Eisenbraun EJ. Biosynthesis of Methylcyclopentane Monoterpenoids IV. Verbenalin. Journal of Biological Chemistry 1969;244(12) 3110-3116.

[60] Liu Z, Xu Z, Zhou H, Cao G, Cong XD, Zhang Y, Cai BC. Simultaneous determination of four bioactive compounds in *Verbena officinalis* L. by using high-performance liquid chromatography. Pharmacognosy Magazine 2012;8(30) 162.

[61] Calvo MI. Anti-inflammatory and analgesic activity of the topical preparation of *Verbena officinalis* L. Journal of Ethnopharmacology 2006;107(3) 380-382.

[62] Lai SW, Yu MS, Yuen WH, Chang RCC. Novel neuroprotective effects of the aqueous extracts from *Verbena officinalis* Linn. Neuropharmacology 2006;50(6) 641-650.

[63] Casanova E, García-Mina JM, Calvo MI. Antioxidant and antifungal activity of *Verbena officinalis* L. leaves. Plant foods for Human Nutrition 2008;63(3) 93-97.

[64] De Martino L, D'Arena G, Minervini MM, Deaglio S, Fusco BM, Cascavilla N, De Feo V. *Verbena officinalis* essential oil and its component citral as apoptotic-inducing agent in chronic lymphocytic leukemia. International Journal of Immunopathology and Pharmacology 2008;22(4) 1097-1104.

[65] Singh B, Saxena A, Chandan BK, Anand KK, Suri OP, Suri KA., Satti NK. Hepatoprotective activity of verbenalin on experimental liver damage in rodents. Fitoterapia 1998;9(2) 135-140.

[66] Abascal K, Yarnell E. Nervine herbs for treating anxiety. Alternative & Complementary Therapies 2004;10(6) 309-315.

[67] Dioscorides P, Goodyer J. The Greek Herbal of Dioscorides. In: Gunther RWT. (ed.). New York: Hafner Publishing Co; 1959. p210.

[68] Kingsbury JM. Poisonous Plants of the United States and Canada. New Jersey: Prentice-Hall. 1964. p341.

[69] Da Legnano LP. Le Piante Medicinali Nella cura delle Malattie Umane. Roma: Edizioni Mediterranee. 1973. p780.

[70] Grieve M. A Modern Herbal: The Medicinal, Culinary, Cosmetic and Economic Properties, Cultivation and Folklore of Herbs, Grasses, Fungi, Shrubs and Trees with All Their Modern Scientific Uses. London: Penguin Books Limited. 1976. p440-442.

[71] Hänsel R, Keller K, Rimpler H, Schneider G. Drogen E-O. Berlin: Springer-Verlag. 1993. p399-404.

[72] Crespin F, Elias R, Morice C, Ollivier E, Balansard G, Faure R. Identification of 3-O-β-D-glucopyranosylhederagenin from the leaves of *Hedera helix*. Fitoterapia LXVI 1995; (5) 477.

[73] Trute A, Nahrstedt A. Identification and quantitative analysis of phenolic compounds from the dry extract of *Hedera helix*. Planta Med 1997;63(2) 177-9.

[74] Tucker AO, Maciarello MJ. Essential oil of English ivy, *Hedera helix* L. 'Hibernica'. Journal of Essential Oil Research 1994;6(2) 187-188.

[75] Pace, V. The Saponins of *Hedera Helix* L. with Antibacterial Activity. BPharm (Hons) thesis. University of Malta; 1994.

[76] Cioaca C, Margineanu C, Cucu V. The saponins of *Hedera helix* with antibacterial activity. Die Pharmazie 1978;33(9) 609.

[77] Elias R, Diaz Lanza AM, Vidal-Ollivier E, Maillard C, Crespin F, Balansard G, Boudon G. Influence du proced de sechage et du degree alcoolique sur l'extraction

del'Hederasaponine C et de l'Alpha-Hederine A partir des feuilles de *Hedera helix* L. Journal de pharmacie de Belgique 1991;46(3) 177-181.

[78] Suleyman H, Mshvildadze V, Gepdiremen A, Elias R. Acute and chronic antiinflammatory profile of the ivy plant, *Hedera helix*, in rats. Phytomedicine. 2003;10 370–374.

[79] Gepdiremen A, Mshvildadze V, Suleyman H, Elias R. Acute anti-inflammatory activity of four saponins isolated from ivy: alpha-hederin, hederasaponin-C, hederacolchiside-E and hederacolchiside-F in carrageenan-induced rat paw edema. Phytomedicine 2005;12 440–444.

[80] Song JH, Yeo SG, Hong EH, Lee BR, Kim JW, Kim JH, Jeong HG, Kwon JS, Kim HP, Lee SW, Park JH, Ko HJ. Antiviral Activity of Hederasaponin B from *Hedera helix* against Enterovirus 71 Subgenotypes C3 and C4a. Biomolecules and Therapeutics 2014;22(1) 41.

[81] Balansard G, Timon-David P, Julien J, Bernard P, Gasquet M. Douvicidal and antifungal activities of alpha-hederin extracted from *Hedera helix* leaves. Planta Medica 1980;39(3) 234.

[82] Trute A, Gross J, Mutschler E, Nahrstedt A. *In vitro* antispasmodic compounds of the dry extract obtained from *Hedera helix* Planta Medica 1997;63:125–129.

[83] Sieben A, Prenner L, Sorkalla T, Wolf A, Jakobs D, Runkel F, Haberlein H. Alpha-hederin, but not hederacoside C and hederagenin from *Hedera helix*, affects the binding behavior, dynamics, and regulation of beta 2-adrenergic receptors. Biochemistry 2009;48 3477–3482.

[84] Tschesche R, Wulff G. Antimicrobial action of saponins. Zeitschrift für Naturforschung 1965;206(6) 543-546.

[85] Weihmayr T, Ernst E. Therapeutic effectiveness of *Crataegus*. Fortschritte der Medizin 1996;114(1-2) 27-29.

[86] Lanfranco, G. Ħxejjex mediċinali u Oħrajn fil-Ġżejjer. Maltin, Malta: Pubblikazzjoni Media Centre. 1993

[87] Farnsworth NR. 1996. Napralert Database Search on *Crataegus monogyna*, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois, Chicago. USA.

[88] Griffiths DW, Robertson GW, Shepherd T, Birch AN, Gordon SC, Woodford JA. A comparison of the composition of epicuticular wax from red raspberry (*Rubus idaeus* L.) and hawthorn (*Crataegus monogyna* Jacq.) flowers. Phytochemistry 2000;55(2) 111-116.

[89] Schimert G, Blömer H. Beitrag zur Pharmakologie der Crataegus-Wirkstoffe. Über die Kreislaufwirkung der Triterpensäuren. Archiv für Experimentelle Pathologie und Pharmakologie 1953;217 337.

[90] Dörner J, Kuschke HJ. Besitzen die aus *Crataegus* gewonnenen Triterpensäuren (Crataegussäuren) ein pharmakodynamische Wirkung? Archiv für Experimentelle Pathologie und Pharmakologie 1955;225 144.

[91] Jacobi H, Oberdorf A, W Rummel. Zur Frage der Coronarwirkung von Crataegus-Extrakten, Arzneimittel-Forschung 1956;6 98.

[92] Netien MG. Characterisation of Pharmaceutical Preparations from Hawthorn base by Paper Chromatography. Bulletin des Travaux de la Societe de Pharmacie 1963;7(4) 145-153.

[93] Nikolov N, Batyuk VS, Kovalev IP, Ivanov V. Glycoflavonoids of *Crataegus monogyna* and *C. pentagyna*. Khimiya Prirodnykh Soedinenii 1973;9(1) 116-117.

[94] Lamaison JL, Carnart A. Content of principle flavonoids in flowers and leaves of *Crataegus monogyna* Jacq. and *Crataegus laevigata* (Poiret) DC. (Rosaceae). Pharmaceutica Acta Helvetica 1990;65(11) 315-320.

[95] Nikolov N. Flavonoid composition of *Crataegus monogyna*. II. Separation of flavonoid mixture from the leaves. The isolation of quercetin, hyperoside and 7-glucoluteolin. Farmatsiya 1971;21(4): 42-47.

[96] Nikolov NT, Vodenicharov RI. Di-C-glycosides from *Crataegus monogyna*. Khimiya Prirodnykh Soedinenii 1975;11(3) 423-424.

[97] Petri G, Kery A, Krawczyk U, Herenyi B, Vadasz A. Flavonoids and procyanidins in *Crataegus* species. Bulletin de Liaison Groupe Polyphenols 1988;14 115-118.

[98] Hoffmann D. Hawthorn: The Heart Helper. Alternative and Complimentary Therapies 1995;4 191-192.

[99] Djeddi S, Boutaleb H. Evaluation of antioxidative and antibacterial potentials of *Crataegus monogyna* Jacq. from Mahouna mountain (Algeria). Journal of Applied Science and Applied Engineering 2014;1(1) 60-63.

[100] Petko D, Kratchanova M, Ciz M, Lojek A, Vasicek O, Blazheva D, Nedelcheva P, Vojtek L, Hyrsl P. Antioxidant, antimicrobial and neutrophil-modulating activities of herb extracts. Acta biochimica Polonica 61, no. 2 (2014): 359-367.

[101] Kamikaura T, Kobayashi A, Yamashita T, Hayashi H, Yamazaki N. Effects of Coenzyme Q10 on exercise tolerance in chronic stable angina pectoris. American Journal of Cardiology 1985;56 247-251.

[102] Ciplea AG, Richter KD. The Protective Effect of *Allium sativum* and *Crataegus* on isoprenaline-induced tissue necrosis in rats. Arzneimittel-Forschung 1988;38(11) 1583 - 92.

[103] Nasa Y, Hashizume H, Ehsanul Hoque AN, Abiyo Y. Protective Effects of *Crataegus* Extract on the Cardiac Mechanical Dysfunction in Isolated Perfused Working Rat Heart. Arzneimittel-Forschung 1993;43(9) 945 - 949.

[104] Al Makdessi S, Sweidan H, Muellner S, Jacob RMyocardial Protection by pretreatment with *Crataegus oxyacantha*: An Assessment by Means of the Release of Lactate Dehydrogenase by the Ischaemic and Reperfused Langendorff Heart. Arzneimittel-Forschung 1996;46,(1) 25 - 7.

[105] Román Ramos R, Alarcón-Aguilar F, Lara-Lemus A, Flores-Saenz JL. Hypoglycemic effect of plants used in Mexico as antidiabetics. Archives of Medical Research 1992;23(1) 59-64.

[106] La Cour B, Molgaard P, Yi Z. Traditional Chinese medicine in Treatment of Hyperlipidaemia. Journal of Ethnopharmacology 1995;46(2) 125-9.

[107] von Eiff M. Hawthorn/passionflower extract and improvement in physical capacity of patients with dyspnoea Class II of the NYHM functional classification. Acta Therapeutica 1983;20 47-66.

[108] Attard E, Attard H The Potential Angiotensin-Converting Enzyme Inhibitory Activity of Oleanolic Acid in the Hydroethanolic Extract of *Crataegus monogyna* Jacq. Natural Product Communications 2006;1(5) 381-386.

[109] Golding G. *Calendula officinalis*. Atoms 1994 11-18.

[110] Ukiya M, Akihisa T, Yasukava K, Tokuda H, Suzuki T, Kimura Y. Antiinflammatory, anti-Tumor-Promoting and Cytotoxic Activities of Constituents of Marigold (*Calendula officinalis*) Flowers. Journal of Natural Products 2006;69 1692-1696.

[111] Bako E, Deli J, Toth G. HPLC study on the carotenoid composition of *Calendula* products. Journal of Biochemical and Biophysical Methods, 2002;53 241-250.

[112] Varlijen J. Structural analysis of rhamnoarabinogalactans and arabinogalactans with immunostimulating activity from *Calendula officinalis*. Phytochemistry 1989;28 2379-2383.

[113] Sausserde R, Kampuss K. Composition of carotenoids in Calendula (*Calendula officinalis* L.) Flowers. FoodBalt 2014;13-18.

[114] Palma Tenango M. Cuantificación de flavonoides y carotenoides en variedades de caléndula (*Calendula officinalis* L.) y descriptores varietales. PhD thesis. Colegio de Postgraduados; 2014

[115] Borg H. *Calendula officinalis* L.: A source of oleanolic acid, an anti-inflammatory and a potential anti-bacterial pentacyclic triterpenoid. BPharm (Hons) thesis. University of Malta; 1996.

[116] Khairnar MS, Pawar B, Marawar PP, Mani A. Evaluation of *Calendula officinalis* as an anti-plaque and anti-gingivitis agent. Journal of Indian Society of Periodontology 2013;17(6) 741.

[117] Ferreira Filho JCC, Gondim BLC, da Cunha DA, de Figueiredo CC, Valença AMG. Physical Properties and Antibacterial Activity of Herbal Tinctures of Calendula (*Cal-*

endula officinalis L.) and Cashew Tree (*Anacardium occidentale* L.). Brazilian Research in Pediatric Dentistry and Integrated Clinic 2014;14(1) 49-53.

[118] Ray D, Mukherjee S, Falchi M, Bertelli A, Carlo Braga P, K Das D. Amelioration of myocardial ischemic reperfusion injury with *Calendula officinalis*. Current Pharmaceutical Biotechnology 2010;11(8) 849-854.

[119] Wojcicki J, Bartolomowicz B, Samochowiec L. Effects of saponosides obtained from *Aralia manschurica* rupr. et maxm. and *Calendula officinalis* L. on central nervous system. Herba Pol 1980; 26 119-22.

[120] Banaszkiewicz W, Mrozikiewicz A. Determination of the estrogenic activity of *Calendula officinalis* flowers in biological units. Poznańskie Towarzystwo Przyjaciół Nauk 1962;2;, 35-40.

[121] Liu L, Guo XM. Extraction of Total Saponins from *Calendula officinalis* L and its Effects of Anti-oxidation & Hypolipidemic. Journal of Mountain Agriculture and Biology 2010;3 011.

[122] Iatsyno AI, Belova LF, Lipkina GS, Sokolov SIA, Trutneva EA. Pharmacology of calenduloside B, a new triterpene glycoside from the roots of *Calendula officinalis*. Farmakologiia i toksikologiia 1978;41 556-560.

[123] Singh A, Kala S, Kapoor DN, Gupta R, Virk A, Singh S, Chaudhary J. on human sperm mitochondrial activity by *Piper betle* and *Calendula officinalis*. Annals of Biological Research Effect 2011;2(5) 622-627.

[124] Chaplinska MG, Golovkin VO. Antimicrobial action of some extracts from flowers of *Calendula officinalis*. Farmatsevticheskii Zhurnal 1963;8(2) 56.

[125] Tubaro A, Della Loggia R, Zilli C, Vertua R, Delaveau P. Societa Italiana di Farmacognosia, 2 Congresso nazionale, Dicembre 15-16, 1983, Milano, Italy.

[126] Singh GB, Singh S, Boni S. Oleanolic Acid: Anti-Inflammatory, Drugs of the Future 1994;19(5) 450 - 451.

[127] Cohen RA, Kucera LS, Herrmann E. Antiviral activity of *Melissa officinalis* (Lemon Balm) extract. Proceedings of the Society for Experimental Biology and Medicine 1964;117 431–434.

[128] Kümel G, Stoll L, Brendel M. Therapie mit rezeptfreien Topika. Deutsche Apotheker Zeitung 1991;131 1609.

[129] Sarer E, Kökdil G. (Constituents of the Essential Oil from *Melissa officinalis*. Planta medica 1991;57(1) 89.

[130] Schultze W, König W A, Hilker A, Richter R. Melissenöle. Deutsche Apotheker Zeitung 1995;135 557–577.

[131] Buttigieg N. A Phytochemical Investigation on the Medicinal Constituents of *Melissa officinalis* L. BPharm (Hons) thesis. University of Malta, 1994.

[132] Aziz E E, El-Ashry SM. Efficiency of slow release urea fertilizer on yield and essential oil production of lemon balm (*Melissa officinalis* L.) plant. American-Eurasian Journal of Agricultural and Environmental Science 2009;5(2) 141-147.

[133] Harshavardhan PG, Vasundhara M, Shetty GR, Nataraja A, Sreeramu BS, Gowda MC, Sreenivasappa KN. Influence of spacing and integrated nutrient management on yield and quality of essential oil in lemon balm (*Melissa officinalis* L). Biomedicine 2007;2(3) 288-292.

[134] Gaspar N, Godinho J, Vasconcelos T, Caldas D, Mendes P, Barros O. Ethnobotany in the Center of Portugal (Santarém). In Natural Products in the New Millennium: Prospects and Industrial Application. Netherlands: Springer; 2002. p271-284.

[135] Penza C. Flora Maltija Mediċinali. Malta: Progress Press Co. Ltd.; 1969.

[136] Buiarelli F, di Berardino S, Coccioli F, Jasionowska R, Russo MV. Determination of phenolic acids in olive oil by capillary electrophoresis. Annali di Chimica 2004;94(9-10) 699-706.

[137] Mazza G, Miniati E. Anthocyanins in fruits, vegetables, and grains. Boca Raton: CRC press. 1993.

[138] Romero C, Brenes M, García P, Garrido A. Hydroxytyrosol 4-β-D-glucoside, an important phenolic compound in olive fruits and derived products. Journal of Agricultural and Food Chemistry 2002;50(13) 3835-3839.

[139] Ryan D, Robards K. Critical Review. Phenolic compounds in olives. Analyst 1998;123(5) 31R-44R.

[140] Patumi M, d'Andria R, Marsilio V, Fontanazza G, Morelli G, Lanza B Olive and olive oil quality after intensive monocone olive growing (*Olea europaea* L., cv. Kalamata) in different irrigation regimes. Food Chemistry 2002;77(1) 27-34.

[141] Amiot MJ, Fleuriet A, Macheix JJ. Accumulation of oleuropein derivatives during olive maturation. Phytochemistry 1989;28(1) 67-69.

[142] Esti M, Cinquanta L, La Notte E. Phenolic compounds in different olive varieties. Journal of Agricultural and Food Chemistry 1998;46(1) 32-35.

[143] Romani A, Mulinacci N, Pinelli P, Vincieri FF, Cimato A. Polyphenolic content in five tuscany cultivars of *Olea europaea* L. Journal of Agricultural and Food Chemistry 1999;47(3) 964-967.

[144] Soler-Rivas C, Espín JC, Wichers HJ. An easy and fast test to compare total free radical scavenger capacity of foodstuffs. Phytochemical Analysis 2000;11(5) 330-338.

[145] Mangion Randon, A. Phytochemistry and Pharmacology of Oleuropein derived from *Olea europaea* L. BPharm (Hons) thesis. University of Malta; 2005.

[146] Benavente-Garcia O, Castillo J, Lorente J, Ortuno A, Del Rio JA. Antioxidant activity of phenolics extracted from *Olea europaea* L. leaves. Food Chemistry 2000;68(4) 457-462.

[147] Pereira AP, Ferreira ICFR, Marcelino F, Valentão P, Andrade PB, Seabra R, Estevinho L, Bento, Pereira JA. Phenolic compounds and antimicrobial activity of olive (*Olea europaea* L. Cv. Cobrançosa) leaves. Molecules 2007;12(5) 1153-1162.

[148] Sudjana AN, D'Orazio C, Ryan V, Rasool N, Ng J, Islam N, Riley TV, Hammer KA. Antimicrobial activity of commercial *Olea europaea* (olive) leaf extract. International Journal of Antimicrobial Agents 2009;33(5) 461-463.

[149] Micol V, Caturla N, Pérez-Fons L, Más V, Pérez L, Estepa A. The olive leaf extract exhibits antiviral activity against viral haemorrhagic septicaemia rhabdovirus (VHSV). Antiviral research 2005;66(2) 129-136.

[150] Silva S, Gomes L, Leitao F, Coelho AV, Boas LV. Phenolic compounds and antioxidant activity of *Olea europaea* L. fruits and leaves. Food Science and Technology International 2006;12(5) 385-395.

[151] Somova LI, Shode FO, Ramnanan P, Nadar, A. Antihypertensive, antiatherosclerotic and antioxidant activity of triterpenoids isolated from *Olea europaea*, subspecies africana leaves. Journal of Ethnopharmacology 2003;84(2) 299-305.

[152] Susalit E, Agus N, Effendi I, Tjandrawinata RR, Nofiarny D, Perrinjaquet-Moccetti T, Verbruggen M. Olive (*Olea europaea*) leaf extract effective in patients with stage-1 hypertension: Comparison with Captopril. Phytomedicine 2011;18(4) 251-258.

[153] Eidi A, Eidi M, Darzi R. Antidiabetic effect of *Olea europaea* L. in normal and diabetic rats. Phytotherapy research 2009;23(3) 347-350.

[154] Jones J. The Encyclopedia of Medicinal Plants. London: Dorling Kindersley; 2001.

[155] Bombardelli E, Morazzoni P. *Urtica dioica* L. Fitoterapia 1997;68(5) 387-402.

[156] Lanfranco G. Mediċina popolari ta' l-imgħoddi fil-Gżejjer Maltin. Valletta: Klabb Kotba Maltin; 2001.

[157] Collier HOJ, Chesher GB. Identification of 5-hydoxytrytapmine in the sting of the nettle (*Urtica dioica*). British Journal of Pharmacology and Chemotherapy 1956;11(2) 186-189.

[158] Wang MY, Wei YF, Li XB. Chemical constituents of antirheumatism fraction from *Urtica fissa*. Chinese Traditional and Herbal Drugs 2006;37(9) 1300.

[159] Ji TF, Liu CH, Wang AG, Yang JB, Su YL, Yuan L, Feng XZ. Studies on the chemical constituents of *Urtica dioica* L. grown in Tibet Autonomous Region. Journal of Chinese medicinal materials 2007;30(6) 662-664.

[160] Ilies DC, Tudor I, Radulescu V. Chemical composition of the essential oil of *Urtica dioica*. Chemistry of Natural Compounds 2012 1-2.

[161] Ullah R, Hussain I, Ahmad S. Diocanol; One new phenol derivative isolated and characterized from *Urtica dioica*. Arabian Journal of Chemistry 2013. http://www.sciencedirect.com/science/article/pii/S1878535213000786 [Accessed 15 August 2014]

[162] Lerner DR, Raikhel NV. The gene for stinging nettle lectin (*Urtica dioica* agglutinin) encodes both a lectin and a chitinase. Journal of Biological Chemistry 1992;267(16) 11085-11091.

[163] Guil-Guerrero JL, Rebolloso-Fuentes MM, Isasa ME. Fatty acids and carotenoids from Stinging Nettle (*Urtica dioica* L.). Journal of Food Composition and Analysis 2003;16(2) 111-119.

[164] Peumans WJ, De Ley M, Broekaert WF. An unusual lectin from stinging nettle (*Urtica doica*) rhizomes. Federation of European Biochemical Societies Letters 1984);177(1) 99-103.

[165] Rossi B, Attard E. The Haemagglutination potential of extracts from *Urtica dubia* plant parts. Journal of Natural Remedies 2011;11(1) 76-78.

[166] Harata K, Schubert WD, Muraki M. Structure of Urtica dioica agglutinin isolectin I: dimer formation mediated by two zinc ions bound at the sugar-binding site. Acta Crystallographica Section D: Biological Crystallography 2001;57(11) 1513-1517.

[167] Gülçin I, Küfreviolu ÖÍ, Oktay M, Büyükokurolu ME. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). Journal of Ethnopharmacology 2004;90(2) 205-215.

[168] Mittman P. Randomized double-blind study of freeze-dried *Urtica dioica* in the treatment of allergic rhinitis Planta Medica 1990;56 44–47.

[169] Testai L, Chericoni S, Calderone V, Nencioni G, Nieri P, Morelli I, Martinotti E. Cardiovascular effects of *Urtica dioica* L.(Urticaceae) roots extracts: *in vitro* and *in vivo* pharmacological studies. Journal of Ethnopharmacology 2002;81(1) 105-109.

[170] Longstaff M, Powell KS, Gatehouse JA, Raemaekers R, Newell CA, Hamilton W. Production and purification of active snowdrop lectin in *Escherichia coli*. European Journal of Biochemistry 1998;252(1) 59-65.

[171] Rivera D, Inocencio C, Obón C, Alcaraz F. Review of food and medicinal uses of *Capparis* L. Subgenus *Capparis* (Capparidaceae). Economic botany 2003;57(4) 515-534.

[172] Youssef SA. Medicinal and non-medicinal uses of some plants found in the middle region of Saudi Arabia. Journal of Medicinal Plants Research 2013;7(34) 2501-2517.

[173] Rodrigo M, Lazaro MJ, Alvarruiz A, Giner V. Composition of capers (*Capparis spinosa*): influence of cultivar, size and harvest date. Journal of Food Science (1992);57(5) 1152-1154.

[174] Khanfar MA, Sabri SS, Abu Zarga MH, Zeller KP. The chemical constituents of *Capparis spinosa* of Jordanian origin. Natural Product Research 2003;17(1) 9-14.

[175] Yang T, Wang CH, Chou GX, Wu T, Cheng XM, Wang ZT. New alkaloids from *Capparis spinosa*: Structure and X-ray crystallographic analysis. Food chemistry 2010;123(3) 705-710.

[176] Romeo V, Ziino M, Giuffrida D, Condurso, C, Verzera A. Flavour profile of capers (*Capparis spinosa* L. from the Eolian Archipelago by HS-SPME/GC–MS. Food Chemistry 2007;101(3) 1272-1278.

[177] Tlili N Nasri N, Saadaoui E, Khaldi A, Triki S. Carotenoid and tocopherol composition of leaves, buds, and flowers of Capparis spinosa grown wild in Tunisia. Journal of Agricultural and Food Chemistry 2009;57(12) 5381-5385.

[178] Tlili N, Nasri N, Saadaoui E, Khaldi A, Triki S. Sterol composition of caper (*Capparis spinosa*) seeds. African Journal of Biotechnology 2010;9(22) 3328-3333.

[179] Lam S., Han Q, Ng T. Isolation and characterization of a lectin with potentially exploitable activities from caper (*Capparis spinosa*) seeds. Bioscience reports 2009;29 293-299.

[180] Parnis MJ. The Chemistry and Pharmacology of local *Capparis spinosa* L. MSc thesis. University of Malta; 2004.

[181] Purvis MJ, Collier DC, Walls D. Laboratory techniques in botany. London: Butterworths; 1964.

[182] Lillie RD. HJ Conn's Biological Stains. 9. Baltimore: Williams and Wilkins; 1976.

[183] Steinberg DM, Sokoll LJ, Bowles KC, Nichols JH, Roberts R, Schultheis SK, O'Donnell CM. Clinical evaluation of Toxi Prep™: a semiautomated solid-phase extraction system for screening of drugs in urine. Clinical chemistry 1997;43(11) 2099-2105.

[184] Deshpande SS, Cheryan M, Salunkhe DK, Luh, BS. Tannin analysis of food products. Critical Reviews in Food Science & Nutrition 1986;24(4) 401-449.

[185] Arena A, Bisignano G, Pavone B, Tomaino A, Bonina FP, Saija A, Cristani M, D'Arrigo M, Trombetta D. Antiviral and immunomodulatory effect of a lyophilized extract of *Capparis spinosa* L. buds. Phytotherapy research 2008;22(3) 313-317.

[186] Feng X, Lu J, Xin H, Zhang L, Wang Y, Tang K. Anti-arthritic active fraction of *Capparis spinosa* L. fruits and its chemical constituents. Yakugaku Zasshi 2011;131(3) 423-429.

[187] Yang T, Wang C, Liu H, Chou G, Cheng X, Wang Z. A new antioxidant compound from *Capparis spinosa*. Pharmaceutical Biology 2010;48(5) 589-594.

[188] Eddouks M, Lemhadri A, Michel JB. Hypolipidemic activity of aqueous extract of *Capparis spinosa* L. in normal and diabetic rats. Journal of Ethnopharmacology 2005;98 345–350

[189] Eddouks M, Lemhadri A, Michel JB. Caraway and caper: potential anti-hyperglycaemic plants in diabetic rats. Journal of Ethnopharmacology 2004;94 143–148

[190] Panico AM, Cardile V, Garufi F, Puglia C, Bonina F, Ronsisvalle G. Protective effect of *Capparis spinosa* on chondrocytes. Life Sciences 2005;77 2479–2488

[191] Trombetta D, Occhiuto F, Perri D, Puglia C, Santagati NA, De Pasquale A, Saija A, Bonina F. Antiallergic and antihistaminic effect of two extracts of *Capparis spinosa* L. flowering buds. Phytotherapy Research 2005;19 29–33.

[192] Ali-Shtayeh MS, Abu Ghdeib SI. Antifungal activity of plant extracts against dermatophytes. Mycoses 1999;42 665–672.

[193] Jacobson RL, Schlein Y. Lectins and toxins in the plant diet of *Phlebotomus papatasi* (Diptera: Psychodidae) can kill *Leishmania major* promastigotes in the sandfly and in culture. Annals of Tropical Medicine and Parasitology 1999;93 351–356.

[194] Mahasneh AM. Screening of some indigenous Qatari medicinal plants for antimicrobial activity. Phytotherapy Research 2002;16 751–753.

[195] Alkofahi A, Batshoun R., Owais W. and Najib N. Biological Activity of some Jordanian medicinal plant extracts. Part II. Fitoterapia 1985;2 163-169.

[196] Chan ELP, Ahmed TM, Wang M, Chan JCM. History of medicine and nephrology in Asia. American Journal of Nephrology 1994;14(4-6) 295-301.

[197] Ling M, Piddlesden SJ, Morgan BP. A component of the medicinal herb ephedra blocks activation in the classical and alternative pathways of complement. Clinical & Experimental Immunology 1995;102(3) 582-588.

[198] Schaneberg BT, Crockett S, Bedir E, Khan IA. The role of chemical fingerprinting, application to *Ephedra*. Phytochemistry 2003;62 911–918.

[199] Oliver AL, Anderson BN, Roddick FA. Factors effecting the production of L-phenylacetylcarbinol by yeast, a case study. Advances in Microbial Physiology 1999;41 1-45.

[200] Wolff RL, Christie WW, Pédrono F, Marpeau AM, Tsevegsüren N, Aitzetmüller K, Gunstone FD. Delta 5-olfenic acids in the seed lipids from four *Ephedra* species and

their distribution between the alpha and beta positions of triacylglycerols. Characteristics common to coniferophytes and cycadophytes. Lipids 1999;34 855-864.

[201] Kobaisy M, Tellez MR, Khan IA, Schaneberg BT. Essential oil composition of three Italian species of *Ephedra*. Journal of Essential Oil Research 2005;17(5) 542-546.

[202] Attard E, Vella K. Effects of Ephedrine and *Ephedra fragilis* Crude Extracts on Human Peripheral Lymphocytes. Pharmacognosy Research 2009;1(2) 38-42.

[203] White LM, Gardner SF, Gurley BJ, Marx MA, Wang PL, Estes M. Pharmacokinetics and cardiovascular effects of ma-huang (*Ephedra sinica*) in normotensive adults. The Journal of Clinical Pharmacology 1997;37(2) 116-122.

[204] Weinberger MM Use of ephedrine in bronchodilator therapy. Pediatric Clinics of North America 1975;22(1) 121.

[205] Limberger RP, Jacques ALB, Schmitt GC, Arbo MD. Pharmacological Effects of Ephedrine. In: Natural Products Berlin Heidelberg: Springer; 2013. p1217-1237.

[206] Aragones EN. A preliminary phytochemical investigation of *Ephedra viridis* Coville found in Oregon. PhD thesis. Oregon State University; 1954.

[207] Senchina DS, Hallam JE, Kohut ML, Nguyen NA, Perera MA. Alkaloids and athlete immune function: caffeine, theophylline, gingerol, ephedrine, and their congeners. Exercise Immunology Review 2014;20 68-93.

[208] Maroyi A. Garden Plants in Zimbabwe: Their ethnomedicinal uses and reported toxicity. Ethnobotany Research & Applications 2012;10 45-57.

[209] Nigussie AS. An Ethnobotanical Study of Medicinal Plants in Farta Wereda, South Gonder Zone of Amhar Region Ethiopia. PhD thesis. Addis Ababa University; 2010.

[210] Khyade MS, Takate YA, Divekar MV. Plants Used as an antidote against snakebite in Akole Taluka of Ahmednagar District (MS), India. Journal of Natural Remedies 2011;11(2) 182-192.

[211] Tiwari L, Pande PC. Indigenous veterinary practices of Darma valley of Pithoragarh district, Uttaranchal. Indian Journal of Traditional Knowledge 2006;5(2) 201-206.

[212] Andersson C, Wennström P, Gry OJ. Nicotine alkaloids in Solanaceous food plants. Copenhagen: TemaNord; 2003.

[213] da Silva FR, Erdtmann B, Dalpiaz T, Nunes E, Ferraz A, Martins TL, Dias JF, da Rosa DP, Porawskie M, Bona S, da Silva J. Genotoxicity of *Nicotiana tabacum* leaves on *Helix aspersa*. Genetics and Molecular Biology 2013;36(2) 269-275.

[214] Lisko JG, Stanfill SB, Duncan BW, Watson CH. Application of GC-MS/MS for the Analysis of Tobacco Alkaloids in Cigarette Filler and Various Tobacco Species. Analytical Chemistry 2013;85(6) 3380-3384.

[215] Slyn'ko NM, Tatarova LE, Shakirov MM, Shul'ts, EE. Synthesis of N-aryloxyalkylanabasine derivatives. Chemistry of Natural Compounds 2013;49(2) 294-301.

[216] Zammit M, Shoemake C, Attard E, Azzopardi LM. The Effects of Anabasine and the Alkaloid Extract of *Nicotiana glauca* on Lepidopterous Larvae. International Journal of Biology 2014;6(3) 46-53.

[217] Wagner H, Bladt S. Plant Drug Analysis: A Thin Layer Chromatography Atlas. Second Edition Berlin Heidelberg: Springer-Verlag; 1996.

[218] Saunders JA, Blume DE. Quantitation of major tobacco alkaloids by high performance liquid chromatography. Journal of Chromatography 1981;205 147-154.

[219] Keeler RF, Crowe MW, Lambert EA. Teratogenicity in swine of the tobacco alkaloid anabasine isolated from Nicotiana glauca. Teratology 1984;30(1) 61-69.

[220] Plumlee KH, Holstege DM, Blanchard PC, Fiser KM, Galey FD. Nicotiana glauca toxicosis of cattle. Journal of Veterinary Diagnostic Investigation 1993;5(3) 498-499.

[221] Puripattanavong J., Songkram C., Lomlim L., Amnuaikit T. Development of Concentrated Emulsion containing Nicotiana tabacum Extract for Use as Pesticide. Journal of Applied Pharmaceutical Science 2013;3(11) 16-21.

[222] Shao YM, Dong K, Zhang CX. The nicotinic acetylcholine receptor gene family of the silkworm, Bombyx mori. BioMed Central genomics 2007;8(1) 324.

[223] Glennon RA, Dukat M. Central nicotinic receptor ligands and pharmacophores. Pharmacochemistry Library 2000;31 103-114.

Chapter 2

Phytochemicals in Antitumor Herbs and Herbal Formulas

Mariana Albulescu

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/60422

1. Introduction

Cancer is an uncontrolled proliferation of anaplastic cells, which tend to invade surrounding tissues and metastasize to other tissues and organs. A hundred years ago, cancer was not a common disease, but today, it is one of the leading causes of death worldwide, andone of three persons is having a lifetime risk of developing cancer. The incidence of cancer has risen alarmingly in the last few decades: in 2008, 12.7 million people were diagnosed with cancer globally, and about 7.6 million people died of it [1]; in 2012, 14.1 million cases of cancer were diagnosed, with 8.2 million deaths; in 2030, more than 21 million new cancer cases are estimated and 13 million deaths are expected. Considering the above data, it confirms the hypothesis that cancer seems to be a civilization disease. The major type of cancer diagnosed among females is breast cancer, accounting for 23% of the total cancer cases; in males, 17% of the total cancer cases are caused by lung cancer [2]. More than 60% of all cancer deaths occur in low- and middle-income countries, many of which lack the medical resources and health systems to support the disease burden [3]. However, cancer mortality surprisingly increased in U.S. population as well, and cancer metastasis attributes to approximately 90% of cancer-related deaths [4].

After years of intense biomedical research on understanding the mechanisms of tumor genesis and biology of cancer, usual cancer treatment still consists in surgery, radiation, and chemotherapy, each having its own limitations: surgery and radiation therapy could be effective especially for the primary tumor; chemotherapy with serious side effects associated with severe toxicity to normal cells is commonly used for the whole-body treatment of recurrent disease. Considerable research activity is devoted to the discovery of more potent treatments, while minimizing their toxic side effects and to discover selective drugs that can kill malignant tumor cells without affecting normal cells [5, 6].

© 2015 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Recently, there is a greater global interest in nonsynthetic, natural medicines derived from natural sources due to better tolerance and minimum adverse drug reactions as compared to synthetic medicines. Many cancer patients prefer complementary and alternative medicine; herbal medicines were by far the most commonly used group of treatments because they are believed by the general public to be safe, cause less side effects, and less likely to cause dependency [7].

When we are considering natural antitumor compounds, generally we refer to secondary metabolites. Secondary metabolites are the products of metabolism not essential for normal growth, development, or reproduction of an organism. Many of them have proved invaluable as antibacterial or antifungal agents, anticancer drugs, cholesterol-lowering agents, immunosuppressant, antiparasitic agents, herbicides, diagnostics, and tools for research [8]. Nowadays, the increased interest in the obtaining of considerable amounts of secondary metabolites has led to intensive research in the field of cell cultures technology. A lot of efforts have been put into plant cell, tissue, and organ culture as an alternative method to whole plant cultivation for the production of pharmacologically important plant secondary metabolites [9].

Getting plant metabolites is not the only problem; the delivery of biologically active substances in the human body is also subjected to innovative research such as nanotechnology that has been used for tumor diagnosis and to design and development of targeted drug delivery [10-11].

2. What is cancer?

Cancer is ultimately the result of cells that uncontrollably grow and do not die. Normal cells in the body follow an orderly path of growth, division, and death. There are three major types of cell death:

a. Apoptosis—a naturally occurring programmed and targeted cause of cellular death that may occur in multicellular organisms. Biochemical events lead to characteristic cell changes (morphology) and death [12].

b. Autophagy—the basic catabolic mechanism that involves cell degradation of unnecessary or dysfunctional cellular components through the actions of lysosomes. Autophagy allows the degradation and recycling of cellular components [13].

c. Necrosis—a form of cell injury that results in the premature death of cells in living tissue by autolysis, caused by factors external to the cell or tissue, such as infection, toxins, or trauma that result in the unregulated digestion of cell components. Necrosis is almost always detrimental to the organism and can be fatal [14].

Cancer results from a multistage carcinogenesis process that involves three distinguishable but closely connected stages: initiation (normal cell → transformed or initiated cell), promotion (initiated cell → preneoplastic cell), and progression (preneoplastic cell → neoplastic cell). The malignant tumor has the abilities to invade surrounding tissues (metastasis), migrate around

http://dx.doi.org/10.5772/60422

the body in the blood or the lymphatic system, and set up secondary foci of cancerous growths at distant sites. Metastasis is responsible for 90% of the deaths caused by cancer [1].

Every living cell in the human body has the potential to become cancerous. A generic name is usually given to a group of cancers, depending on the type of cells of their origin, which include carcinoma, sarcoma, leukemia, lymphoma, and myeloma.

- Carcinoma: a type of cancer that develops from epithelial cells. About 80% of the human cancers are carcinomas.
- Sarcoma: cancer arising from a mesenchymal cell (connective tissue cell). Sarcoma may arise from the bone, cartilage, muscle, fat, and fibrous tissue.
- Myeloma: a cancer of plasma cells, a type of white blood cell normally responsible for producing antibodies.
- Leukemia: cancer arising from a blood forming cell (hematopoietic stem cell).
- Lymphoma: cancer arising from a cell of the lymphatic tissue (lymphocytes) [15].

When cancer begins, it invariably produces no symptoms. Signs and symptoms only appear as the mass continues to grow or ulcerates. General symptoms may include unintentional weight loss, fever, being excessively tired, and changes to the skin. The symptoms of metastatic cancers depend on the location of the tumor and can include enlarged lymph nodes, enlarged liver or enlarged spleen, which can be felt in the abdomen, pain or fracture of affected bones, and neurological symptoms [16].

3. What causes cancer?

Cancer results from a mutation in the chromosomal DNA of a normal cell under the action of carcinogens. The mutated cell proliferates indiscriminately (pathological mitosis), usually forming a mass known as neoplasm or malignant tumor. Cancer can be triggered by both internal factors (nutritional imbalance in the diet, aging, immune conditions, hormones, and mutations occurring in metabolism) and external factors (tobacco, alcohol, chemicals, infectious agents, and radiation). These causal factors may act together or in sequence to initiate or promote the development of cancer [1, 2, 15, 17].

3.1. Carcinogens

A carcinogen is any substance, radionuclide, or radiation that is an agent directly involved in causing cancer. Carcinogens include a wide range of possibilities: chemical and physical pollution, unhealthy lifestyle, stress, aging, viruses, bacteria, heredity, etc.

3.1.1. Chemical carcinogens

Most of the human cancers are caused by certain chemical agents.

- *Tabacco*. Among the dangerous factors leading to cancer, cigarette smoking (also second-hand tobacco smoking) is essential. Smoke contains several carcinogenic pyrolytic products that bind to DNA and cause many genetic mutations. Tobacco smoke generates more than 2000 chemical compounds, and more than 45 are known or suspected as chemical carcinogens; nicotine is one of the major cancer promoters [18].
- *Polluted environment* is another major source of chemical carcinogens. Industrial toxic chemicals and heavy metals having carcinogenic activity may enter and get accumulated in the human body and damage DNA [2].
- *Contaminated food*. The food containing residues of pesticides, insecticides, and herbicides is one of the three major causes of cancer. Most of the food additives including preservatives (butylated hydroxytoluene), sweeteners (saccharin and cyclamates), and synthetic colorants have carcinogenic activity [15].
- *Chlorine*, used to purify drinking water, may produce certain carcinogenic compounds in the treated water, including chloroform and trichloroethylene, which have carcinogenic activity [15].
- *Reactive oxygen species* (ROS—superoxide, hydrogen peroxide, hydroxyl radical) are also major causes of DNA, protein, and lipid damages, which lead to cancer or aging [19].

3.1.2. Physical carcinogens

Ionizing radiation emitted by radioactive substances ruptures the DNA strands, leading to mutations in the genes. Ultraviolet radiation induces skin cancer. The electromagnetic fields generated by electrical appliances, power lines, and cell phones emit 30-100 times higher electromagnetic fields than the maximum permissible limits. Studies have revealed that prolonged exposure to electromagnetic fields causes mutations in the genes [15].

3.1.3. Biological carcinogens

Certain viruses are suspected to cause cancer in human: for example, the Epstein-Barr virus is linked to Burkitt's lymphoma; testicular tumors and leiomyoma in children; viruses of hepatitis B and hepatitis C are known to enhance risk of the hepatocellular carcinoma; human papilloma virus is a major risk factor of the cervical and of anal cancers [15].

3.1.4. Lifestyle

The World Cancer Research Fund and the American Institute for Cancer Research assess that 30-40% of all cancers could be prevented by appropriate diet and physical activity to avoid overweight and obesity. Many lines of evidence demonstrate that a diet based on abundant and various foods of plant origin protects against epithelial cancers, particularly those of the gastrointestinal tract [2].

http://dx.doi.org/10.5772/60422

3.1.5. Age factor

The incidence of cancer is three times higher in women as compared to men during 30 to 50 years of age, whereas men have a greater risk of cancer as compared to women during 60 to 80 years of age. Cancer is generally considered as the disease of middle-aged and elderly people, but some cancers are known to affect the children [15].

3.1.6. Genes (hereditary factor)

Extensive evidence indicates that only 5-10% of cancers are genetically determined. It is believed that members of those families, who are predisposed to a particular cancer, have one or more activated oncogenes in their genome; therefore, fewer additional mutations are required in such persons to develop the cancer. Genetic mutations are commonly seen in the breast and the ovarian cancers, especially in cancers occurring below 30 years [15].

4. How do natural remedies work?

The plants act on several fronts in the fight against cancer, such as nourishing the body with minerals, vitamins, enzymes, and micronutrients; increasing the immune system of the body; inducing antioxidant action and protecting the body from oxidative stress; enhancing detoxification functions of the body; alkalizing the body fluids; inhibiting cancer-activating enzymes; promoting production of protective enzymes; stimulating DNA repair mechanism; and modulating the activity of specific hormones and enzymes to inhibit growth of cancer.

4.1. Immunomodulatory properties of plant

The immune system is a complex defense network that protects the host from disease and has a large impact on antitumor resistance. Most of the anticancer drugs currently used in chemotherapy are cytotoxic to normal cells, leading a low immune function of organism and can result in development of the tumor, and difficulty of tumor patients' recovery [20].

A major research interest has focused on the immunomodulatory properties of plant-derived medicines. Several studies have reported that many flavonoids, such as quercetin, catechins, resveratrol, green tea polyphenols, grape seed proanthocyanidins, silymarin, and curcumin, have anti-inflammatory and immunomodulatory properties [2].

4.2. Chemoprevention

Chemoprevention is defined as the use of natural or synthetic chemical agents to reverse, suppress, or prevent carcinogenic progression to invasive cancer. Many chemopreventive agents are phytochemicals (secondary metabolites) and nonnutritive plant chemicals that have protective or disease-preventive properties [2, 21]. Several commonly used herbs such as coriander, cumin, garlic, ginger, mint, oregano, and turmeric have been identified by the National Cancer Institute as possessing cancer-preventive properties.

4.3. Impact on cancer induction: antimutagenesis

During the initiation phase, natural chemopreventive agents can inhibit the absorption of a carcinogen into the organism through the antioxidant activity, prevent inflammatory-induced damage or enhance neutralization, and remove carcinogens through metabolic enzymes [3].

4.4. Anti-inflammatory activity

Long-term inflammation leads to the development of dysplasia [22]. Many phenolic substances, including phenolic acids (e.g., gallic, ellagic, caffeic, chlorogenic), flavonoids (e.g., genistein, kaempferol, quercetin, daidzein, isorhamnetin, naringenin), anthocyanins (e.g., pelargonidin), and catechins, have potent anti-inflammatory properties, affecting different stages of the inflammation. Flavonoid aglycones have been shown to exert higher activity than adequate glycosides [2].

4.5. Antioxidant properties

Oxidative stress has been proven to be one of the main factors that lead to the formation of cancer. Oxidative stress is defined as a discrepancy between production of free radicals and reactive metabolites, so-called reactive oxygen species [ROS, e.g., hydrogen peroxide (H_2O_2), superoxide anion ($O\cdot_2^-$), and hydroxyl radical ($\cdot OH$)], and their eradication by defending mechanisms, referred to as antioxidants. Excessive oxidative stress in the body for extended periods of time activates inflammatory pathways, which cause the transformation of normal cells into cancer cells, support the survival of cancer cells, and finally lead to cancer cell proliferation [23].

Many herbs are known to contain large amounts of phenolic antioxidants having the capacities to quench lipid peroxidation, prevent DNA oxidative damage, and scavenge reactive oxygen species (ROS) [24].

4.6. Pro-oxidative activity

The pro-oxidant behavior of phenolics is observed in the presence of transition metal ions such as copper or iron. Many studies suggest that the antitumor activity of some polyphenols (e.g., resveratrol, gallic acid, delphinidin, baicalin, quercetin, epicatechin, and epigallocatechin-3-gallate) is also a consequence of their pro-oxidant properties. It seems that while the antioxidant activity lowers the risk of cancer induction by protecting normal cells from oxidative injury, pro-oxidative properties of polyphenolics are more relevant for apoptosis induction and destruction of existing tumor cells [2].

4.7. Modulation of activity of xenobiotic-metabolizing enzymes

A carcinogen is a xenobiotic, and generally, biotransformation involves modification, conjugation, and excretion and is strictly involved in the detoxification of carcinogens. Many plant-derived phenolic compounds such as flavonoids, including kaempferol, daidzenin, genistein,

http://dx.doi.org/10.5772/60422

diosmetin, and theaflavin, have been reported to affect the activity of metabolizing enzymes leading to faster carcinogen detoxification [2].

4.8. Changing multidrug resistance

Multidrug resistance is a significant challenge in the treatment of infectious diseases and cancer. The antitumor treatments currently in use often fail at some stage of the sickness because many types of cancers develop resistance to chemotherapeutic drugs. Some plants can change the resistance of tumor cells to antitumor drugs [25].

4.9. Suppression of angiogenesis

Angiogenesis is the process of formation of new vessels (vascularization) from the preexisting microvascular network. This is as an essential step in tumor dissemination and formation of metastases. Many plant-derived phenolics have been reported to restrain angiogenesis and, consequently, cancer invasion and metastasis [2].

4.10. Apoptosis induction

The activation of apoptosis in preneoplastic cells is one of the crucial mechanisms of cancer chemoprevention. Polyphenolic compounds have been shown to induce selective promotion of apoptosis in cancerous or precancerous cells, by affecting different cellular mechanisms [2].

5. Which are the phytochemicals with antitumor activity?

Plant secondary metabolites and their semisynthetic derivatives are playing today an important role in anticancer drug therapy. In this chapter, the main classes of natural compounds are presented, which have been identified to contain substances with antitumor activity.

5.1. Terpenes

Terpenes are a large and diverse class of organic compounds, often strong-smelling, produced by a variety of plants, particularly conifers. Many terpenes are aromatic hydrocarbons and thus may have had a protective function. The difference between terpenes and terpenoids is that terpenes are hydrocarbons, whereas terpenoids contain additional functional groups. Terpenes are derived biosynthetically from units of isoprene, which has the molecular formula C_5H_8. The basic molecular formulae of terpenes are multiples of $(C_5H_8)_n$, where n is the number of linked isoprene units.

- **Diterpenes are** composed of four isoprene units and have the molecular formula $C_{20}H_{32}$. Taxanes are diterpenes produced by the plants of the genus *Taxus* and are widely used as chemotherapy agents although present difficulties in formulation as medicines because they are poorly soluble in water. The principal mechanism of action of the taxane class of drugs is inhibition of the process of mitosis [26]. Taxane agents include taxol (or paclitaxel) and docetaxel (Figure 1).

Figure 1. Taxol (or paclitaxel, left) and docetaxel (right).

Taxol, originally isolated in 1971 from the bark of Pacific Yew, *Taxus brevifolia*, is a strong anticancer drug approved by the U.S. FDA to treat a variety of tumors, including breast, ovarian, and AIDS-related Kaposi's sarcoma, among others. Among the conifers apart from the genus *Taxus*, only two other species have been reportedly claimed to produce taxanes: *T. brevifolia* and *Taxus baccata* or the European yew.

- **Triterpenes and triterpenoids**

Triterpenes consist of *six isoprene* units and have the molecular formula $C_{30}H_{48}$. Triterpenes are widespread in nature and are highly abundant in medicinal plants, especially in the leaves, bark, fruits, and seeds of the herbs. The pentacyclic triterpenes can be classified into lupane, oleanane, or ursane groups. Triterpenoids are structurally diverse organic compounds, more than 20,000 known members, widespread in nature. Several triterpenoids, such as ursolic and oleanolic acid, betulinic acid, and lupeol, possess antioxidative and anti-inflammatory, antidiabetic properties, and have been suggested to be potentially promising anticancer agents. Triterpenoids exist in free form or combined with sugar into glycosides—the triterpenoid saponins. In anticancer mechanisms, triterpenoids are multitargeted agents that induce apoptosis, inhibit cell proliferation, suppress angiogenesis, cause mitochondrial dysfunction, and modulate genes and proteins [27].

- **Boswellic acid** (Figure 2), a triterpene isolated from Boswellia serrata (native in India and Pakistan), has been found to possess potent anti-inflammatory and anticancer activity. Boswellic acid have been used to treat Crohn disease, ulcerative colitis, bronchial asthma, endotoxin-induced hepatitis, and arthritis. Boswellic acid is a mixture of four major pentacyclic triterpene acids. Beta-boswellic acid (Figure 2), keto-beta-boswellic acid, and acetyl-keto-beta-boswellic acid have been indicated in apoptosis of cancer cells, in particular, brain tumors and cells affected by leukemia or colon cancer, melanoma, hepatoma, and prostate cancer [28].

- **Oleanolic acid** (Figure 3) is a triterpenoid widely found in several dietary and medicinal plants. Oleanolic acid is abundant in ginseng root and in olive plant (*Olea europaea*), which is the primary commercial source for the compound. Oleanolic acid can be easily obtained in high yield from olive pulp remaining after crushing of the olive fruit and also from olive leaves.

http://dx.doi.org/10.5772/60422

Figure 2. β-Boswellic acid.

Oleanolic acid

Ursolic acid

Betulinic acid

Figure 3. Structure of oleanolic, ursolic acid and betulinic acid.

Oleanolic acid exists in nature as free acid but also serves as an aglycone of triterpenoid saponins, linked with one or more sugar moieties to form glycosides. Effects of oleanolic acid on cancer cells have been demonstrated in chemoprevention and therapy of breast, colorectal, non-small cell lung, epithelial ovarian, pancreatic, prostate cancer, and melanoma. Oleanolic acid inhibited the proliferation of cancer cells and induced apoptosis via ROS-mediated mitochondrial mechanism [29].

- **Ursolic acid** (Figure 3), an isomer of oleanolic acid, is found in combination with oleanolic acid and has similar pharmacological properties. Ursolic acid is easily obtained in very high purity by methanol extraction of rosemary leaf [29].

- **Betulinic acid** (Figure 3) is a naturally occurring pentacyclic triterpenoid found in the bark of several species of plants, principally the white birch (*Betula pubescens*, *Betulaceae*), but also the ber tree (*Ziziphus mauritiana*), self-heal (*Prunella vulgaris*), rosemary (*Rosmarinus officinalis*), and *Pulsatilla* chinensis. Betulinic acid exhibits various biological activities such as anti-HIV, antimalarial, antibacterial, and anti-inflammatory properties, as well as a more recently discovered potential as an anticancer agent by inhibition of topoisomerase and apoptosis in the tumors [3]. A major inconvenience for the future clinical development of betulinic acid and analogues resides in their poor solubility in aqueous media such as blood serum and polar solvents used for bioassays. In order to solve the solubility and to enhance

pharmacological properties, many derivatives were synthesized and evaluated for cytotoxic activity [30-33].

- **Triterpenic saponins**

Saponins are a group of naturally occurring plant glycosides, characterized by their strong foam-forming properties in aqueous solution. Originally named for soapwort plants (*Saponaria* spp.), saponins consist of a hydrophobic aglycone linked to a hydrophilic carbohydrate. The presence of saponins has been reported in more than 100 families of plants, out of which at least 150 kinds of natural saponins have been found to possess significant anticancer properties. Saponins are common in a variety of higher plants and usually found in roots, tubers, leaves, blooms, or seeds. The amphipathic nature of the class gives them activity as surfactants that can be used to enhance penetration of macromolecules such as proteins through cell membranes. Saponins have also been used as adjuvant in vaccines [34]. Based on the carbon skeletons, saponins were classified into triterpenes and steroids. Their glycone parts were mostly oligosaccharides, arranged either in a linear or branched fashion, attached to hydroxyl groups through an acetal linkage. The considerable variety of aglycones, carbohydrates, and their different attachments results in many different saponins. Modern research found that saponins have antitumor effect on many cancer cells at least by cell cycle arrest and apoptosis. Meanwhile, saponins in combination with conventional tumor treatment strategies result in improved therapeutic success [35].

- **Oleananes** are the most common saponins in nature. Their antitumor effect worked through various pathways, such as anticancer, antimetastasis, immunostimulation, chemoprevention, etc. Some of the most studied triterpene saponins are soybean saponin, saikosaponins, avicins, and tubeimoside [35].
- **Cycloartane saponins** could be used as chemotherapeutic agent in the treatment of tumors. For example, total Astragalus saponins possess antitumor properties in human colon cancer cells. In addition, Astragalus saponins could be used as an adjuvant in combination with other orthodox chemotherapeutic drugs to reduce the side effects of the latter compounds [35].

5.2. Phenols and polyphenols

Phenolic phytochemicals represent the largest category of phytochemicals widespread in different fruits and vegetables and one of the major groups of secondary metabolites in plants. Numerous phenolic compounds have been reported to demonstrate selective activity, destroying cancer cells without damaging normal cells. Many clinical trials have been made on cancer prevention of breast, colon, gastric, reproductive, head and neck, and prostate cancers by using plant polyphenols such as epigallocatechin-3-gallate, curcumin, resveratrol, genistein, and quercetin [2, 36].

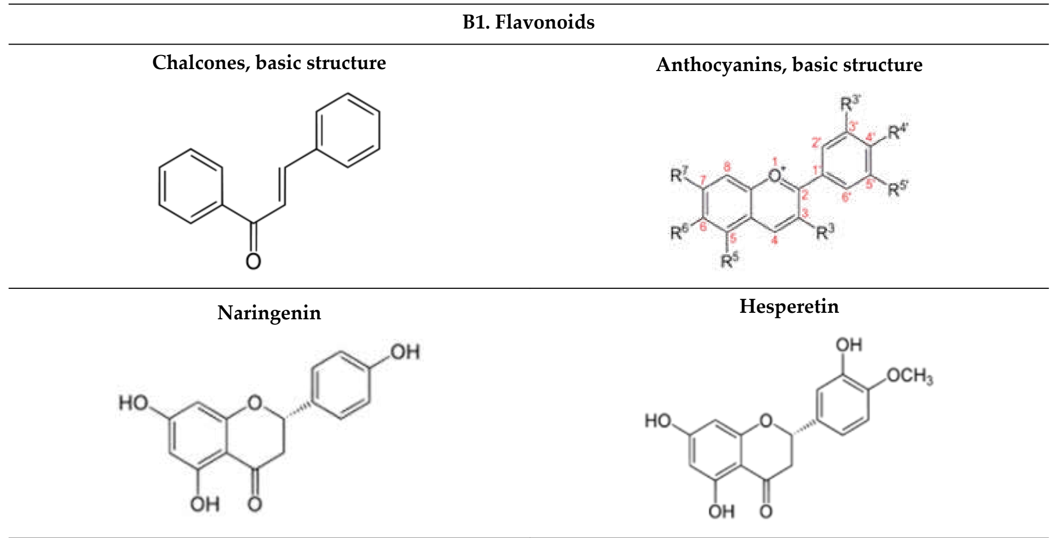
Most of the studies with plant polyphenols showed that cancer-preventing mechanisms include antioxidant activity (the strength of this property is influenced by the number and location of hydroxyl groups, the size and shape of molecules, and steric properties), radical scavenging activity, inactivation of carcinogenic substances, antiproliferation, cell cycle arrest,

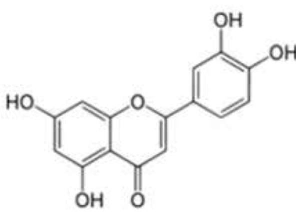
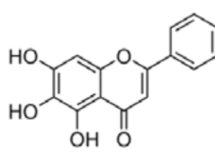
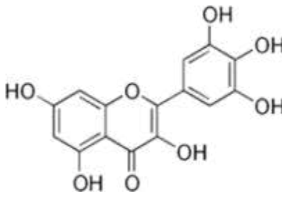
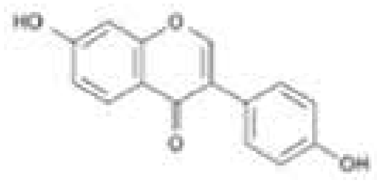
induction of apoptosis and differentiation, inhibition of angiogenesis, modulation of tumor suppression genes, and others [36].

Plant polyphenols are divided into two major groups (Table 1)—flavonoids (chalcones, flavanols, flavones, flavanones, isoflavones, and anthocyanins) and nonflavonoids (phenolic acids, e.g., caffeic acid, gallic acid; stilbenes, e.g., trans-resveratrol, tannins, lignins) [21]. Flavonoids and phenolic acids account for the majority (60% and 30%, respectively) of total dietary polyphenols. The average daily intake of flavonoids alone is 1-2 g [2].

A. Flavonoids		**B. Nonflavonoids**	
Chalcones and dihydrochalcones	Butein, cardamonin, phloretin	**Stilbenoids**	Resveratrol
Anthocyanines	Cyanidin, pelargonidin, delphinidin	**Hydroxycinnamic acids**	Caffeic acid
Flavanones	Naringenin, hesperitin, baicalein	**Phenolic acids**	Gallic acid, protocatechuic acid, ellagic acid
Flavanols	Catechins, gallic esters of catechins, teaflavons	**Curcuminoids**	Curcumin
Flavones	Wogonin, luteolin, apigenin, diosmin	**Tannins**	Hydrolysable and condensed tannins
Flavonols	Quercetin, kaempferol myricetin		
Isoflavones	Genistein, daidzein		

Table 1. Main classes of plant polyphenols



(+) - Catechin	**(-) - Epicatechin**
Epigallocatechin gallate	**Apigenin**
Luteolin 	**Wogonin**
Baicalein 	**Quercetin**
Myricetin 	**Kaempferol**
Genistein	**Daidzein** 

http://dx.doi.org/10.5772/60422

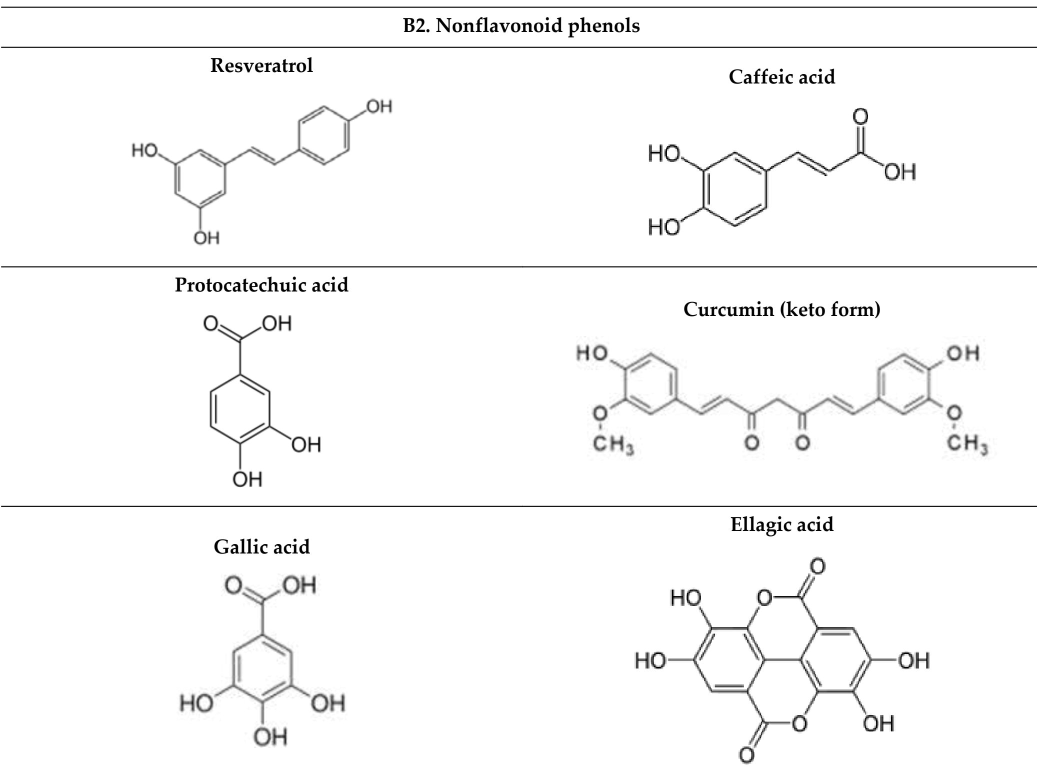


Table 2. Chemical structure of some plant polyphenols with antitumor activity

Flavonoids commonly share the same generic structure, the flavan nucleus consisting of two aromatic rings linked by a pyran ring. Differences in the location of the right phenolic ring to pyran ring make it possible to distinguish between flavonoids (2-phenylbenzopyrans) and isoflavonoids (3-phenylbenzopyrans). 2-Phenylbenzopyran group may be further divided into 3-hydroxyflavonoids (flavonols, flavanols, and anthocyanidins) and flavonoids without substituent at C3 (flavanones and flavones). Flavones differ from flavanones by a C2-C3 double bond [19].

5.2.1. Flavonoids as anticancer agents

- **Chalcones as anticancer agents**

Chalcone (Table 2) is an aromatic ketone that forms the central core for a variety of important biological compounds, which are known collectively as chalcones or chalconoid. They are a group of plant-derived polyphenolic compounds belonging to the flavonoids family even if the chroman ring is not yet formed (they are precursors of flavonoid structure). Some of the most significant chalcones identified from plants include flavokawin, butein, xanthoangelol, 4-hydroxyderricin, cardamonin, 2′,4′-dihydroxychalcone, and naringenin chalcone (phloretin). These chalcones have been linked with immunomodulation, antibacterial, antiviral, anti-inflammatory, antioxidant, and anticancer activities [36].

- **Anthocyanins as anticancer agents**

Anthocyanins (also named anthocyans; Table 2) are water-soluble vacuolar pigments that may appear red, purple, or blue depending on the pH. Anthocyanins occur in all tissues of higher plants, including leaves, stems, roots, flowers, and fruits. Dietary intake of anthocyanins has been estimated at up to 200 mg/day, which is higher than other flavonoids. More than 550 different anthocyanins are discovered until now. Anthocyanins are derived from anthocyanidins by adding pendant sugars and are mostly 3-glucosides of the anthocyanidins. From various studies, it is found that some plants or their parts containing anthocyanins have anticancer property, and their analogues may be helpful in synthesizing newer effective anticancer agents in future. The number of hydroxyl groups and presence of sugar moiety is crucial for the specific modulatory actions of anthocyanins. Numerous *in vitro* and *in vivo* studies showed that anthocyanins can affect basic cell functions related to cancer development. They may inhibit the formation and growth of tumors by induction of cell cycle arrest and apoptosis, thus eliminating damaged cells or tumor cells [36].

- **Flavanones as anticancer agents**

The consumption of citrus fruits and juices has been widely investigated for its possible role in the prevention of cardiovascular disease and cancer. These beneficial effects are mainly attributed to flavanones, the typical polyphenols of citrus species. Major flavanones in plant species include hesperetin, naringenin, eriodictyol, isosakuranetin, and their respective glycosides. Hesperetin and its derivatives are characteristic flavanones of sweet orange, tangelo, lemon, and lime, while naringenin and its derivatives are those of grapefruit and sour orange. The major citrus flavanones can be effective in fighting carcinogenesis by minimizing DNA damage (protecting effect of naringenin against UV-induced damage of DNA), tumor development, and proliferation [19].

Naringenin (Table 2) is found in high concentrations in citrus fruit while low concentrations are also found in tomatoes and their products. Naringenin can be found as aglycone and/or as glycosides, such as naringin (naringenin-7-neohesperidoside) and narirutin (naringenin-7-rutinoside). Naringenin was successfully investigated for its cell antiproliferation effect on colon cancer cell line. In a comparative study, flavanones showed a significant antiproliferative activity against lung, colon, breast, prostate, and melanoma cancerous cell lines [19].

Hesperetin (Table 2) and its glycosides are also mainly present in citrus fruit. The aglycone is less dominant in nature than the glycosides. The most widely distributed glycosides of hesperetin are hesperidin (hesperetin-7-rutinoside) and neohesperidin (hesperetin-7-neohesperidoside), which are conjugates with rutinose and neohesperidose, respectively [19]. Recent study shows that hesperetin exhibits a potential anticancer activity against human cervical cancer cell lines *in vitro* through the reduction in cell viability and the induction of apoptosis. Altogether, these data sustain our contention that hesperetin has anticancer properties and merits further investigation as a potential therapeutic agent [37].

- **Flavanols as anticancer agents**

Flavan-3-ols (flavanols) possess two benzene rings (called the A- and B-rings) and a dihydropyran heterocycle (the C-ring) with a hydroxyl group on carbon 3 and have been shown to

have the ability to scavenge free radicals, reduce the rate of LDL oxidation, inhibit lipid peroxidation, modify enzymes that activate or detoxify, and participate in the modulation of the immune response in several biological systems. The hydroxylation pattern of the B-ring appears to have a critical influence on their activities, particularly in the inhibition of protein kinase, which can induce antiproliferate activities [38]. **Catechins** belong to flavan-3-ols and there are two chiral centers on the molecule on carbons 2 and 3 (they are four diastereoisomers). Two of the isomers are in *trans* configuration and are called catechin, and the other two are in *cis* configuration and are called epicatechin. The most common catechin isomer is the (+)-catechin (Table 2). The most common epicatechin isomer is (**-**)-epicatechin (Table 2). Catechins and epicatechins are found in cocoa (108 mg/100 g), prune juice (25 mg/100 ml) and broad bean pod (16 mg/100 g), açaí oil (67 mg/kg), argan oil, barley grain, peaches, green tea, and vinegar [39-42].

The results of a recent study show that catechin present in the extract of *Ligaria cuneifolia,* a hemiparasite species that belongs to Argentine flora, can reduce proliferation and induces apoptosis of lymphoma cell line [38]. The recent data show that catechins also affect the molecular mechanisms involved in angiogenesis, regulation of cell death and multidrug resistance in cancer. Catechins present antioxidant activity by scavenging free radicals, chelating redox active transition-metal ions, inhibiting redox active transcription factors, inhibiting pro-oxidant enzymes, and inducing antioxidant enzymes [43].

Tea (*Camellia sinensis*), one of the most popular beverages in the world, is manufactured as black (78%), green (20%), or oolong tea (2%) [43]. Catechins are the major group of bioactive compounds found in green tea, meaning about 25% of the dry weight of fresh tea leaf [44], which contains characteristic polyphenolic compounds, such as epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epicatechin (EC). The chemopreventive properties of this beverage are mostly connected with EGCG that EGCG and includes induction of apoptosis, promotion of cell growth arrest, inhibition of cell proliferation and transformation, inhibition of angiogenesis, and metastasis [2]. Tea polyphenols inhibited in experimental studies the growth of lung, mammary, and stomach human cancer cell lines [45].

Also, red wine containing higher levels of catechin showed significant reducing powers, thereby confirming the antioxidant potential of red wine due to catechin content. It is obvious that drinking green tea or red wine helps in improving the general well-being in humans.

Epigallocatechin gallate (EGCG, Table 2), also known as epigallocatechin 3-gallate, is the ester of epigallocatechin and gallic acid. EGCG has been shown to inhibit the growth of different human hepatoma cell lines at concentrations of 50-100 μg/ml and increased tumor necrosis [21]. Various clinical studies have revealed that treatment by EGCG inhibits tumor incidence and multiplicity in different organ sites such as liver, stomach, skin, lung, mammary gland, and colon. Preclinical research data in recent studies show promising results, and EGCG has great potential in cancer prevention because of its safety, low cost, and bioavailability [36].

Theaflavins are antioxidant polyphenols that are formed from the condensation of flavan-3-ols in tea leaves during the enzymatic oxidation (fermentation) of black tea [46]. Theaflavin-3-

gallate, theaflavin-3′-gallate, and theaflavin-3-3′-digallate are the principle of theaflavins. Previous research studies led in mice showed beneficial inhibitory effects of topically applied black tea theaflavins (e.g., theaflavin-3,3′-digallate) providing a good promise for chemoprevention (e.g., decreased occurrence of melanoma and nonmelanomas such as squamous cell carcinoma and basal cell carcinoma) [47].

- **Flavones as anticancer agents**

Apigenin (Table 2) is a flavonoid present in various fruits, vegetables, and spices like onions, parsley, oranges, thyme, celery, and sweet red pepper. It possesses several chemopreventive properties, including induction of apoptosis, inhibition of cancer cells proliferation, and involvement in angiogenesis [2].

Luteolin (Table 2) is most often found in leaves, but it is also seen in celery, parsley, broccoli, onion, carrots, peppers, cabbages, apples, thyme, dandelion, chamomile tea, carrots, green pepper, olive oil, peppermint, rosemary, oranges, and oregano. The chemopreventive mechanism of action of luteolin includes induction of apoptosis, inhibition of proliferation of cancer cells, and suppression of angiogenesis and metastasis. Luteolin has been reported to inhibit growth of tumors in human skin, hepatoma, and ovarian cancer cells; in lung and breast cancer, luteolin has been shown to inhibit the invasion of cancer cells [2]. Among 68 plant polyphenols examined for their inhibitory ability against hepatocellular carcinoma cells, luteolin was one of the most potent [21].

Wogonin, baicalein (Table 2), and baicalin (baicalein 7-*O*-glucuronide), all naturally occurring flavonoid extracted from *Scutellaria baicalensis Georgi* (*Lamiaceae*), were reported to have potential antitumor effects in several studies. These phytochemicals are not only cytostatic but also cytotoxic to various human tumor cell lines *in vitro* and inhibit tumor growth *in vivo*. Numerous studies demonstrated that wogonin had a cytostatic effect due to the apoptotic cell death on various tumor cell lines (such as sarcoma, hepatoma cells, breast cancer, and nasopharyngeal carcinoma cells) and antimetastatic ability as well [48, 49].

- **Flavonols**

Quercetin (Table 2) is the most abundant flavonoid in foods. It is present in different fruits (apples, berries, and grapes), vegetables (onions, broccoli), as well as in tea and red wine, mostly in glycosylated forms. The daily intake of quercetin has been estimated at 30 mg. Quercetin is probably the most studied flavonoids, and antioxidant properties are known as outstanding. This flavonol was found to have antiproliferative activity in many situations, such as adenomatous polyposis [36] and breast cancer [2].

Myricetin (Table 2) is the next flavonol occurring in fruits, vegetables, or red wine exerting anticancer activity. In lung cancer, this compound has been observed to block invasion and migration of human lung adenocarcinoma cells. According to the study with human leukemia cell line, myricetin induced apoptosis in these cells [2].

Kaempferol (Table 2) is another flavonol present in tea, broccoli, grapefruit, brussels sprouts, apples, and other plants. This compound exerts antioxidant, anti-inflammatory, and anticancer effects on several types of cancer. Kaempferol has been reported to inhibit the invasion of the

human invasive breast carcinoma cell line, angiogenesis, and human ovarian cancer cells. The available data indicate that kaempferol could be a potential chemopreventive agent against various cancers such as lung, colon, prostate, liver, pancreas, and skin [2].

- **Isoflavones as anticancer agents**

Isoflavone (3-phenyl-4*H*-1-benzopyr-4-one) differs from flavone in location of the phenyl group. Most members of the *Fabaceae* family contain significant quantities of isoflavones. Genistein and daidzein (Table 2), the most prevalent compounds of isoflavonoid class, were found in various legumes including soybean, green bean, alfalfa sprout, mung bean sprout, cowpea, kudzu root, and red clover sprout. Due to the estrogen-like structure of isoflavones found in soybeans and the known role of estrogens in breast carcinogenesis, most soy research has focused on the hormonal activity of these compounds [50]. Some studies showed that daidzein can inhibit hepatoma cell growth and induce apoptosis [21]. Regarding genistein, the major isoflavone in soy, laboratory studies have demonstrated different mechanisms of action, including antiproliferative activity and induction of apoptosis in both animal and human cell lines. In addition, genistein possesses antioxidant activity and the ability to scavenge free radicals, protecting the body against oxidative DNA damage. The chemopreventive activity of genistein has been investigated mainly against breast and prostate cancer.

- **Influence of glycosilation in flavonoid family**

The dietary flavonoids in nature almost all exist as their O/C-glycosides, such as glucoside, galactoside, rhamnoside, arabinoside, and rutinoside. The most abundant flavonoid glycosides in plants are flavone and flavonol O-glycosides. The glycosidation are found mainly as their 3 or 7 O-glycosides, although the 5, 8, and 4′ O-glycosides were also reported in some cases. Regarding the antioxidant and antitumoral properties, it was illustrated that the flavonoid aglycones showed higher anticancer potential than their glycosides in cell level; e.g., rutin did not inhibit cell proliferation of any of the cancer cell lines tested; C/O-glycosylation of apigenin significantly weakened the inhibition of cancer cells; genistein and daidzein glycosides exhibited no noticeable activity on human breast carcinoma cell. It was demonstrated that cellulase can remarkably transform baicalin and wogonoside to their aglycones (baicalein and wogonin) with enhance of antiproliferative effects [51]. Generally, glycosylation reduced the antiproliferative activity in flavonoid classes, and a C2-C3 double bond seems important for the antiproliferative activity of flavonoids, and indeed flavones are typically more potent that flavanones [19].

5.2.2. Nonflavonoids as anticancer agents

- **Stilbenoids**

Stilbenoids are hydroxylated derivatives of stilbene with a C6-C2-C6 structure. They are secondary metabolites occurring naturally in various families of plants; grapes and related products are considered the most important dietary sources of these substances. Trans-resveratrol (3,4′,5-trihydroxystilbene, Table 2), a hydroxylated stilbene, is today a well-known chemopreventive substance for cardiovascular diseases, antiaging, antiviral, and several

malignant neoplasms [36]. High amounts of trans-resveratrol can be found in grapes and grape skins used for wine production, raspberries, mulberries, blueberries cranberries, peanuts, and certain types of pine [21]. All anticancer data obtained on preclinical animal studies showed that resveratrol affects all three discrete stages of carcinogenesis (initiation, promotion, and progression) by modulating signal transduction pathways that control cell division and growth, apoptosis, inflammation, angiogenesis, and metastasis [36]. A synthetic stilbene derivative, tamoxifen, is currently used for the treatment of several types of breast cancer in women, and as a hormone treatment for male breast cancer [52].

- **Caffeic acid**

Caffeic acid (Table 2) is a hydroxycinnamic acid found in most plants, including coffee beans, nuts, berries, and grains. Several studies have investigated the antitumor potential and hepatoprotective activity of the caffeic acid derivative, which have been identified in particular in honey and propolis [21]. Data showed that caffeic acid phenethyl ester (CAPE), a component of propolis, induces cell cycle arrest and has antiproliferation effect on glioma cells *in vitro* and *in vivo*. In addition, CAPE inhibited the metastasis of glioma cells [53].

Caffeic acid 3,4-dihydroxy-phenethyl ester (CADPE), a natural polyphenol from *Sarcandra glabra*, has potent *in vitro* anticancer activity through multiple targets. This compound significantly decreased tumor growth in hepatoma and sarcoma tumor-bearing mice, and also significantly inhibited ascites development. CADPE did not show any toxicity *in vivo* and anticancer efficacies were equivalent to those of 5-fluorouracil and cyclophosphamide (drugs in cancer treatment). The tested 59 human cancer cell lines from leukemia and nine different solid tumors, including colon cancer, gastric cancer, breast cancer, CNS cancer, ovarian cancer, melanoma, lung cancer, renal cancer, and prostate cancer, are sensitive to CADPE that suppresses tumor growth and angiogenesis [54].

- **Protocatechuic acid**

Protocatechuic acid (Table 2), a dihydroxybenzoic acid widely distributed in the plant kingdom, is the major metabolite of antioxidant polyphenols found in green tea. Protocatechuic acid has antioxidant and anti-inflammatory activity and has been shown to inhibit HepG2 (human liver carcinoma cell line) cell growth and induce apoptosis [21].

- **Curcuminoids**

A curcuminoid is a linear diarylheptanoid, with molecules such as curcumin (Table 2) or derivatives of curcumin with different chemical groups that confers increase solubility and make them suitable for drug formulation. Curcumin is the principal curcuminoid of the spice turmeric, which is a member of the ginger family. It has been traditionally used for centuries for treating numerous diseases. Over the past few years, a number of studies uncovered several pharmacological properties of curcumin. It has been shown that curcumin inhibited HepG2 cell growth by inducing apoptosis [21]. Recent studies showed substantial evidence that curcumin inhibited proliferation, migration, invasion and metastasis, and induced apoptosis via modulating multiple signaling pathways in head and neck cancer [36].

http://dx.doi.org/10.5772/60422

- **Gallic acid**

Gallic acid (Table 2) is a hydroxybenzoic acid occurring mostly in certain red fruits, black radish, onions, and also fresh tea leaves containing up to 4.5 g/kg. Gallic acid has been shown to exhibit biological activity, including anticancer and chemopreventive potential, capacity to induce apoptosis in human leukemia. Because gallic acid exhibits anti-invasive and antimetastatic activities in various cancer cells, it might be a potential preventive and therapeutic agent against gastric cancer metastasis [2].

- **Ellagic acid**

Ellagic acid (Table 2) is a dimeric derivative of gallic acid occurring mostly in fruits and berries (such as raspberries, strawberries, blackberries, and pomegranates) and a primary constituent of ellagitannins. The data demonstrate that ellagic acid inhibits carcinogenesis, induces apoptosis in pancreatic and leukemia cancer cells, and also can delay the tumor latency and significantly reduce the estrogen-induced mammary tumors in rats. As a consequence, ellagic acid as well as gallic acid may be considered in a promising new class of cancer chemopreventive and therapeutic agents [2, 55].

- **Tannins**

Tannin (also known as tannoid) is an astringent, bitter plant polyphenolic compound that binds to and precipitates proteins and various other organic compounds, including amino acids and alkaloids. The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides, and in plant growth regulation. They showed antimutagenic activity without any obvious toxicity. Pentagalloylglucose and geraniin were among the most active tannins. Geraniin was isolated from the dry leaf of *Geranium thunbergii*. The plant is an official medicine in the Japanese Pharmacopoeia and is used for the treatment of diarrhea and for controlling intestinal function [45]. The grape (*Vitis vinifera*) skin mostly contains the highest amount of condensed tannins, alongside monomeric flavanols and flavonols, phenolic acids, and resveratrol. The grape skin extract induced prostate tumor cell line apoptosis, and the extract from pomace has a significant antiproliferative effect on human colon adenocarcinoma cells [56].

5.3. Polysaccharides

Polysaccharides are biopolymers with linear or branched side chains, composed of monosaccharides linked together through glycosidic bonds. Considering that the repeating units in the polymer backbone are often six-carbon monosaccharides, the general formula can be represented as $(C_6H_{10}O_5)_n$, where $40 \leq n \leq 3000$. Natural polysaccharides can be obtained from various organisms, such as plants, algae, microorganisms, and animals, and exist in a variety of chemical compositions, molecular weights, and structures [1]. Most polysaccharides derived from higher plants are relatively nontoxic and do not cause significant side effects; thus, plant polysaccharides are ideal candidates for therapeutics with immunomodulatory and antitumor effects and low toxicity [20]. Natural polysaccharides isolated from herbal plants have been

shown to possess bioactivities. For example, the immune-stimulatory properties of *Aloe vera* glucomannan have been confirmed and used for treatment of immune-related diseases [23].

The type of linkage between saccharide units—the glycosidic linkage—seems to be important in immunomodulatory and anticancer activities. The literature also demonstrates that most of antitumor polysaccharides contain Β-1,3-glucans, β-1,6-glucans, and α-1,3-glucans [23]. Although today is investigating especially polysaccharides of bacterial, algae, and fungi origins, plants have an important as sources of bioactive polymers especially because they are nontoxic. Here are some examples in the following lines:

- The genus *Actinidia* (*Actinidiaceae*) consists of over 58 species widely distributed in the Asian continent (China, Taiwan, Korea, Japan, southeast Siberia, and south of Indochina). Some *Actinidia* species, such as *A. macrosperma*, are the important in traditional medicine being used as health foods and medical products. In temperate climate zones, roots of *Actinidia eriantha Benth* (a commonly liana plant) have been used for gastric carcinoma, nasopharyngeal carcinoma, breast carcinoma, and hepatitis in traditional Chinese medicine. The water extracts of this drug possess antitumor and immunopotentiating activities. Four polysaccharides were isolated and purified from the roots of *A. eriantha*, and the chemical composition of these polysaccharides could affect their antitumor and immunomodulatory activity [20].

- *Rhizoma arisaematis* comes from the rhizome of *Pinellia pedatisecta Schott*, which has bitter, warm, pungent, and toxic properties. It has be recorded in Chinese Pharmacopoeia as a traditional Chinese medicine, displaying sedative, stomachic, analgesic, anticoagulant, anti-inflammatory, antiemetic, and antitumor activities. The pharmacognostical study identifies the active components of *R. arisaematis* to be beta-sitosterol, total alkaloids, guanosine, gama-aminobutyric acid, dipeptides, and recently a water soluble polysaccharide composed of rhamnose, fucose, arabinose, mannose, galactose and glucose, with molar ratios of 0.4:0.5:0.3:0.6:0.9:5.3. This polysaccharide significantly inhibits the growth of tumor in animal experiments; immunomodulation might be the mechanism of the antitumor activity [57].

- *Rhodiola rosea L.* belongs to the family Crassullaceae that grows in the Arctic and in the mountainous regions of Europe, Asia, and North America. The rhizome and roots has been widely used for a long time in Russian and Chinese folk medicine to increase human physical and mental performance, longevity, and resistance to high-altitude sickness and to treat fatigue, anemia, cancer, bacterial infection, impotence, nervous system disorders, and cardiovascular diseases. Phytochemical studies of *R. rosea* have revealed the presence of glycosides, flavonoids, essential oils, fats, waxes, sterols, organic acids, tannins, proteins, and polysaccharides. A homogeneous polysaccharide (composed of glucose, galactose, manose, and rhamnose with a relative molar ratio of 4.2:2.4:1.6:1.0) from *R. rosea* was tested for its immunomodulation and anticancer activity *in vitro* and *in vivo* experiments on sarcoma cells. The results showed that this polysaccharide could be used as a novel promising immunotherapeutic agent in cancer treatment [58].

- *Astragalus membranaceus* is commonly used in Chinese herbalism, where it is considered to be one of the 50 fundamental herbs. The plant, used especially for treatment of the kidneys and also to avoid senility, has been shown to be effective in immune enhancement and in the treatment of diabetes, viral infections, and cancers as well. The polysaccharides isolated from the radix of *A. membranaceus* are an active anticancer component. Structural analysis indicated that the polysaccharides were mainly composed of α-(1 → 4)-glucan with α-(1 → 6)-linked branches or α-(1 → 3)-glucan with side-chains containing arabinose and xylose [1].

- *Polysaccharides from ginseng* (*Panax genus*) possess preventive and inhibitory effects against tumors by enhancing immunological functions and induction of apoptosis. A ginseng polysaccharide injection has been developed in China as a useful adjuvant for irradiation therapy and chemotherapy for cancer patients [1].

- *Solanum nigrum* is a species in the *Solanum* genus, native to Euroasia and introduced in North and South America, South Africa, and Australia. Parts of this plant can be toxic to livestock humans, and it is considered a weed. The toxicity of *Solanum* species varies widely depending on the species and the plant's growing conditions. *S. nigrum* is a widely used plant in oriental medicine where it is considered to be antitumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic, and antipyretic [59]. Chinese experiments confirm that the plant inhibits growth of cervical carcinoma in mice [60]. Polysaccharides isolated from *S. nigrum L.*, mainly containing galactose and arabinose, have significant antitumor and immunomodulatory activities; the anticancer activity was mediated by increasing cell apoptosis, inducing cell cycle arrest and activating host immune responses [1].

- *Artemisia argyi* (Asteraceae), the Chinese mugwort, is a herbaceous perennial plant native to China, Japan, and far-eastern Siberia. It is used in herbal medicine for conditions of the liver, spleen, and kidney. In Chinese traditional herbal medicine, it is used for the treatment of cancer, microbial infections, inflammatory diseases, diarrhea, hepatitis, malaria, and circulatory disorders. In a recent study, a water-soluble polysaccharide was isolated from *A. argyi*, and its antitumor activity was evaluated *in vivo*. This new hetero-polysaccharide had clear antitumor and immunomodulatory activities [61].

- The *Angelica sinensis* commonly known as "female ginseng" is a herb from the family *Apiaceae*, grows in cool high altitude mountains in China, Japan, and Korea. The root of the plant, a well-known Chinese medicine, has been used as a tonic, hematopoietic, and anti-inflammatory agent for thousands of years. Pharmacological experiments have proved that polysaccharide is one of the major active ingredients in *A. sinensis* possessing antitumor effects on experimental tumor models *in vivo* and inhibitory effects on invasion and metastasis of tumor cells *in vitro* [62].

- Tea plant (*Camellia sinensis*) is mainly cultivated in tropical and subtropical climates, in areas with enough rainfall. The flowers of *Camellia sinensis* have been used for deodorization, skin care, cough suppressant, and expectorant in China. Recent studies have demonstrated that the extract of tea flower had various bioactivities, including antiproliferative and apoptotic effects against human breast cancer. A regular use of green tea protects the body against many cancers including those of the liver, esophagus, stomach, intestine, and lungs. Tea

polysaccharides, one of the main components of tea extracts, have been demonstrated to have immunological, antiradiation, antioxidant, anticancer, and hypoglycemic effects [63].

Although they are not derived from plants, we must remember some polysaccharides that have been demonstrated to have powerful antitumor activity: chitin, the most abundant renewable natural resource after cellulose, a homopolymer of *N*-acetyl-d-glucosamine, is active on bladder human cancer cells and colon carcinoma [64]; a water-soluble polysaccharide from *Inonotus obliquus* has immunomodulatory and antitumor activity [65]; the polysaccharides extracted from *Pleurotus eryngii* have been demonstrated to have multiple functions, such as antitumor, antioxidant, hepatoprotective activity, and enhance immunity [66]; gama-carrageenan sulfated polysaccharides extracted from marine red alga, with different molecular weight, has antitumor and immunostimulating activities [67].

5.4. Alkaloids

Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms.

- Camptothecin (Figure 4) is a naturally occurring pyridoindole (quinoline) alkaloid isolated from the seeds of Chinese plant Camptotheca acuminata and Mappia foetida. Camptothecin and some of its analogs have shown a broad spectrum of antitumor activity against many solid tumors including colorectal, breast, lung, and ovarian cancers. Derivatives of camptothecin such as 18-OH-camptothecin, 11-OH-camptothecin, and 10-OH-camptothecin have been found to possess a strong antileukemic activity [3, 68].

The primary limitations of camptothecin are its extremely low water solubility, and the hydrolysis of the active lactone ring to the inactive carboxylate, which reduces the drug efficacy and can lead to side effects. To overcome these stability and solubility problems of camptothecin, several new approaches have been investigated such as using drug delivery technologies, e.g., incorporation in liposomes, polymer micelles, microemulsions, and microspheres [69]. Specifically, camptothecin nanocrystals were prepared with a sonication-precipitation method with promising results [68].

Figure 4. Camptothecin (left) and harmine (right).

- Harmine (Figure 4), the most representative naturally occurring beta-carboline alkaloid, was originally isolated from *Peganum harmala*, which is being widely used as a traditional herbal drug in the Middle East and North Africa. In Northwest China, the extracts of the seeds of

P. harmala have been used for hundreds of years to treat the gastrointestinal cancers and malaria. Harmine was reported to exhibit a diverse range of pharmacological properties such as hallucinogenic, antitumor, antiviral, and antiparasitic activities. In order to search for novel leading compounds with better antitumor activities and less neurotoxicities, a series of harmine derivatives were designed and synthesized by modification of β-carboline nucleus [70].

5.5. Peptides, proteins, and lectins

- Peptides are short chains of amino acids linked by peptide bonds. They are naturally, abundantly occurring biological molecules. Many plants are an enormously rich source of peptides with potential antitumor effect, e.g., *Violaceae*, *Rubiaceae*, and *Cucurbitaceae* families. The mechanism of anticancer peptides action consists of inhibition of tumor angiogenesis, induction of tumor apoptosis, induction of tumor necrosis, immunomodulation. Studies have shown that peptides from plants exhibit marked inhibitory effects on the proliferation of various tumor cell lines, such as murine leukemia, rat osteoblast-like sarcoma, human nasopharyngeal carcinoma, lung, liver, and mammary gland cancer and ovarian neoplasm [71].

- Studies have shown that most peptides isolated from plants are cyclic peptides, so-called cyclopeptides (which usually consist of less than 14 amino acid residues with no disulfide bond). More than 450 cyclopeptides have been discovered in higher plants. They exhibit more potent biological activities, possibly due to the stable configuration provided by their cyclic structure. Some cyclopetides have been reported to have powerful antitumor activities, for example, cherimolacyclopeptide, a cycloheptapeptide from *Annona cherimola* seeds [72].

- Cyclotides are small disulfide rich peptides isolated from plants, consisting of 28-37 amino acid residues. They have been found in the plants of *Rubiaceae*, *Violaceae*, and *Cucurbitaceae*. Cyclotides are exceptionally stable and are resistant to denaturation by thermal, chemical, or enzymatic treatments and have a wide range of biological activities, including anti-HIV, antitumor, antimicrobial, hemolytic, neurotensin antagonism, trypsin inhibitor, uterotonic, and insecticidal activity [72].

- Lectins are members of a super family of proteins that express the capacity to bind reversibly to a specific carbohydrate. Lectins are present in many plant families and are most abundantly seen in the leguminosae family: 10-15% of the total protein content. Foods with high concentrations of lectins, such as beans, cereal grains, seeds, nuts, and potatoes, may be harmful if consumed in excess in uncooked or improperly cooked form. Adverse effects may include allergies, autoimmune diseases, or even interfere with the absorption of nutrients, thereby acting as antinutrition molecules.

However, plant lectins attracted increasing attention from cancer biologists due to their possible antitumor properties. A large-scale study in colorectal cancer patients and a control group showed some beneficial effects of consuming plant lectins, but the pathways remain unclear [73].

Astragalus membranaceus lectin displays antiproliferative properties toward human leukemia cells *in vitro*. Mistletoe lectin, which is used as an adjuvant in cancer therapy, is known to activate caspases, enzymes involved in the self destruction of cells [74].

Concanavalin A, a member of the legume lectin family with a mannose/glucose-binding specificity, was reported to induce apoptosis in human melanoma cells and demonstrated potent therapeutic effect in liver cancer [75].

Recently, some antitumoral lectins have been discovered and researched, such as mistletoe (*V. album*) lectins, rice (*Oryza sativa)* bran agglutinin with remarkable antitumour activities, wheat (*Triticum* spp.) germ agglutinin, a typical chitin-binding lectin with strong inhibitory effects on the growth of the pancreatic tumour cells, and garlic (*Allium sativum L*) lectin, isolated from garlic which induced apoptosis at a low concentration [76]. *Phaseolus vulgaris* lectin can also induce apoptotic cell death toward various types of cancers; more interestingly, induces autophagic cell death in hepatocarcinoma [77].

5.6. Quinones

Quinones are secondary metabolites generally having a hexacyclic di-ketone system derived from the oxidation of hydroquinones, and isolated principally from plants. Naturally occurring quinones are widely distributed and include benzoquinones, naphthoquinones, anthraquinones, and polyquinones. Quinones exhibit numerous biological activities, such as neurological, antibacterial, antioxidant, antitumor, and anti-HIV activities that have been proven to be related to the redox properties of their carbonyl functions. Quinones, in general, and naphthoquinones, in particular, are well known for antibacterial, antifungal, and antitumoral activities.

- Lapachol (Figure 5), the most studied substance of natural quinone class, is the main constituent in Pau D'Arco (a natural remedy) and has been extracted from the bark of South American tree, *Tabebuia impetiginosa*. Lapachol possesses strong biological activity against liver, kidney, breast, prostate, cervix cancer, and leukemia. Unfortunately the toxic effect limits the use of lapachol [78].
- Plumbagin (Figure 5) is a naphthoquinone compound that displayed antiproliferative activity on a panel of 60 cancer cell lines. However, plumbagin, identified in the Cameroonian plants *Diospyros crassiflora* and *Diospyros canaliculata,* was suggested as a promising anticancer lead drug [79].

Plumbagin has been shown to have activity against breast, prostate, ovarian, pancreatic, lung, liver, renal, cervical, and skin cancer, in addition to having activity against myeloma and leukemia. It has been shown to inhibit cell proliferation and cell invasion and effectively induces apoptosis and causes cell cycle arrest [80].

- Emodin (Figure 5), isolated from *Rheum emodi*, a Himalayan rhubarb, is also produced by many species of fungi, including *Aspergillus* fungi. The pharmacological studies have demonstrated that emodin when isolated from rhubarb exhibits anticancer effects on several human cancers, including human pancreatic cancer [81].

Figure 5. Chemical structures of quinones with antitumoral activity.

5.7. Isothiocyanates

Cruciferous vegetables are a rich source of glucosinolates (natural chemicals most likely contribute to plant defense against pests and diseases), which are converted to isothiocyanates. Evidence supports that consumption of cruciferous vegetables has substantial chemopreventive activity against various human malignancies, including pancreatic cancer. Benzyl isothiocyanate (Figure 6), an agent that is present in cruciferous vegetables such as watercress, cabbage, cauliflower, mustard, and horseradish, is widely consumed as part of a routine diet. Benzyl isothiocyanate is quite effective in suppressing pancreatic tumor growth by inhibiting various key signaling pathways [82].

Figure 6. Benzyl isothiocyanate (R=benzyl).

6. Herbs and herbal formulas with antitumor activity

6.1. Aloe vera

Aloe vera is one of the most impressive herbs, called by many people “a miracle.” The plant works on multiple plans purifying, strengthening, and healing the body. In a single plant, aloe vera offers potent, natural medicine that nourishes the body with minerals, vitamins, enzymes,

and saccharides; alkalizes the body, helping to balance overly acidic dietary habits; prevents and treats Candida infections; protects the body from oxidative stress; boosts the oxygenation of your blood; reduces inflammation; purifies the blood, repairs "sludge blood"; halts the growth of cancer tumors; stops colon cancer, heals the intestines and lubricates the digestive tract [83].

Scientific research shows strong immunomodulatory and antitumor properties for aloe vera polysaccharides. Acemannan (Figure 7) is the name given to the major carbohydrate fraction obtained from the gel of the aloe vera leaf. This compound has several important therapeutic properties including acceleration of wound healing, immune stimulation, and antiviral and anticancer effects. The research results suggest that acemannan accelerates the destruction of cancer tumors, improves survival time and results in far better recovery from toxic cancer treatments [83, 84]. Acemannan is currently being used for treatment and clinical management of fibrosarcoma in dogs and cats. Administration of acemannan has been shown to increase tumor necrosis and prolonged host survival [85].

= Acetyl group

Figure 7. Acemannan structure

Also, *Aloe vera* amplifies the antioxidant effects of vitamins C and vitamin E probably due to its effect on enhancing blood quality and allowing the blood to more effectively transport oxygen and nutrients to the body's cells [83].

6.2. Catharanthus roseus or Vinca rosea

This contains vinca alkaloids, which were the first phytoconstituents ever used to treat cancer. Intense work on *C. roseus*, a folklore hypoglycemic drug, led to isolation of more than 70 dimeric indole alkaloids, which include vinblastine, vincristine, alstonine, ajmalicine, and reserpine. Vinca alkaloids execute anticancer effect by arresting division of the cancerous cells. Vinblastine is used in the treatment of Hodgkin's disease, non-Hodgkin's lymphoma, and cancers of the kidney and the testis. Vincristine is usually given in combination with other anticancer agents to treat acute lymphocytic leukemia, Wilm's tumour, neuroblastoma,

rhabdomyosarcoma, Ewing's sarcoma, lymphoma, and cancers of the breast, lung, bladder, and the cervix [15].

6.3. Garlic (*Allium sativum*)

Garlic has been used for thousands of years to treat various diseases. Garlic contains approximately 33 sulfur compounds (aliin, allicin, ajoene, allylpropyl disulfide, diallyl trisulfide, sallylcysteine, vinyldithiines, S-allylmercaptocystein, and others), several enzymes (allinase, peroxidases, myrosinase, and others), 17 amino acids (arginine and others), and minerals (selenium, germanium, tellurium, and other trace minerals). Garlic has shown significant therapeutic effect in stomach, colorectal, and breast cancer in humans. Biological effects of garlic are attributed to its characteristic organ sulfur compounds [86]. The constitutive compounds of garlic can selectively inhibit tumor proliferation by a number of factors, e.g., controlling DNA repair mechanisms, chromosomal stability, and cell cycle regulation. Garlic constituents can suppress carcinogen formation, carcinogen bioactivation, and tumor proliferation [87, 88].

6.4. Parsley (*Petroselinum crispum*)

Parsley has a variety of nutrients that protect against developing cancer. It is an excellent source of vitamin C and a good source of beta-carotene, folic acid, vitamin K, flavonoids, and volatile oil components—including myristicin, limonene, eugenol, and alpha-thujene. Parsley's volatile oils—particularly myristicin—have been shown to inhibit tumor formation in animal studies, and particularly tumor formation in the lungs. The flavonoids include apiin, apigenin, crisoeriol, and luteolin [89]. Luteolin have been shown to function as antioxidants that combine with ROS and help prevent oxygen-based damage to cells. Apigenin research studies have associated it with a decreased risk of pancreatic cancer, leukemia, cervical, and ovarian cancer. Apigenin has also been shown to interfere with cancer cell proliferation, exhibiting strong antitumor properties [90].

6.5. Chaparal (*Larrea divaricata Cav.*—Zygophyllaceae)

This contains five species of evergreen shrubs that are native to the Southwestern United States, and it is a herb derived from the common desert shrubs *Larrea tridentata* and *Larrea divaricata*. It is a plant widely used in popular medicine to treat tumors, infections, and inflammatory diseases. Many studies have shown that chaparral possesses antioxidant properties and exhibits cytotoxic properties in a variety of cell types. The principal ingredient in chaparral is nordihydroguaiaretic acid that is an anticancer agent and a potent antioxidant [91]. This herb also has anti-inflammatory, analgesic, expectorant, emetic, and diuretic properties. Chaparral chelates remove heavy metals in the body and offer protection against the harmful effects of radiation, sun exposure, and the formation of tumors and cancer cells. The lignans found in chaparral that are very similar to estrogen; they are isolated from the flowering tops of *Larrea tridentata* and are effective against human breast cancer, human colon cancer, and human melanoma cell lines [92].

6.6. Essiac

Essiac is an herbal formula that was originally known to an American tribe and was renamed Essiac in 1920 by a Canadian nurse, Rene Caisse. Essiac is a tea prepared from a mixture of herbs: burdock root (*Arctium lappa*), sheep sorrel (*Rumex acetosella*), slippery elm bark (*Ulmus rubra*), and turkey rhubarb (*Rheum officinale*), and it has been used in alternative medicine for over 50 years. The phytochemicals found in these ingredients are antioxidants protecting cells against oxidative damage manifest interference with DNA replication and have antibacterial effects. Essiac enhances activity of the immune cells and reduces toxic side effects of chemotherapy and radiotherapy. Essiac has been used in the treatment of malignant melanoma, lymphoma, and cancers of the pancreas, breast, ovary, esophagus, bladder, bile duct, and bone. Despite a lack of clinical studies reporting efficacy, 72% of the patients taking Essiac reported a positive opinion of the product [93].

6.7. Hoxsey herbal formula

Hoxsey herbal formula was discovered incidentally in by Elder Hoxsey, when one of his horses suffering from cancer on the leg, got cured after grazing certain herbs. Later, his grandson Harry Hoxsey tried these herbs on the human cancer. The cancer treatment practiced by Harry M. Hoxsey is one of the longest-lived unconventional therapies of this century. Hoxsey herbal formula contains *Arctium lappa*, *Berberis vulgaris*, *Glycyrrhiza glabra*, *Larrea tridentata*, *Picramnia antidesma*, *Rhamnus purshianus*, *Stillingia sylvatica*, *Trifolium pratense*, and *Xanthoxylum americanum*. Hoxsey herbal formula has been used to treat malignant melanoma, lymphoma and cancer of the skin [94].

6.8. Iscaddor

Iscaddor is a fermented extract of *Viscum album* (mistletoe) that was discovered by the Austrian scientist, Rudolf Steiner, and has been used in the treatment of cancer by European physicians since 1920s. Mistletoe extracts are complex multicomponent mixtures, containing various biologically active substances such as glycoproteins, in particular the mistletoe lectins, polypeptides, amino acids, and oligo- and polysaccharides. More than 20 prospective clinical trials using mistletoe extracts in patients with various malignancies have been reported, and in most of these studies, it was concluded that mistletoe extracts had therapeutic benefit in terms of response rate, overall survival, quality of life, and reduce side effects [95].

7. Conclusion

Conventional chemotherapy, radiotherapy, and surgical treatments of cancer mainly focus on mass cell killing without high specificity and often cause severe side effects and toxicities. Because of these reasons and high mortality rate associated with cancer, many cancer patients seek alternative methods of treatment and herbal medicines have a vital role to play in the prevention and treatment of cancer.

http://dx.doi.org/10.5772/60422

Over the past decade, there has been an increasing demand of drug development against cancer; at the same time there is considerable scientific and commercial interest in the continuing discovery of new anticancer agents from natural product sources, especially herbs. Already a great number of modern drugs in clinical use, having the ability to control cancer cells, are of natural product origins; with advanced knowledge and modern device in isolation and structure elucidation techniques and in molecular science, a more efficient research for finding new plant remedies is possible. There are an impressive number of higher plants, and it is estimated that only 10% of all plants have been examined for their chemical composition and only about 100,000 types of chemical structures have been identified. Thus, there is still a lot to be done in the search for novel natural chemopreventive compounds.

Chemically speaking, it can notice a large structural diversity in herbs: alkaloids, polyphenols, tannins, fatty acids, terpenes, polysaccharides, etc. We can say that nature has cures for serious diseases, in every place all around the globe; most of them are waiting to be discovered and used for the good of people.

Until now, the scientific researchers have investigated mainly particular herbs well known in worldwide alternative medicine ; far less have been investigated the mixtures of plants, e.g., some recognized herbs formulations (e.g., Essiac, Hoxsey, Iscador) that proved to be effective on hundreds of patients. The synergisms arising among the biologically active compounds are waiting to be demonstrated.

In scientific terms, no magic overall formula has been found so far to be applicable to all types of cancer. However, there are enough people who had a longer life or even healed with plants; therefore, medical and herbal research must continue.

Author details

Mariana Albulescu[1,2]

Address all correspondence to: Malbulescu@yahoo.com

1 Faculty of Chemistry, Biology, Geography, Department of Biology-Chemistry, West University of Timişoara, Timişoara, Romania

2 Advanced Environmental Research Laboratories, West University of Timişoara, Timişoara, Romania

References

[1] Zong A, Cao H, Wang F. Anticancer polysaccharides from natural resources: a review of recent research. Carbohydrate Polymers 2012; 90: 1395-1410.

[2] Nowak R, Olech M, Nowacka N. Plant polyphenols as chemopreventive agents. In: Watson RR, Preedy VR, Zibadi S (eds.), Polyphenols in Human Health and Disease. Amsterdam: Elsevier; 2013. pp. 1289-1307.

[3] Pan L, Chai H, Kinghorn AD. The continuing search for antitumor agents from higher plants. Phytochemistry Letters 2010; 3: 1-8.

[4] Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics. CA: A Cancer Journal for Clinicians 2009; 59(4): 225-249.

[5] Gao Y, Xie J, Chen H, Gu S, Zhao R, Shao J, Jia L. Nanotechnology-based intelligent drug design for cancer metastasis treatment. Biotechnology Advances 2014; 32: 761-777.

[6] Khazir J, Mir BA, Pilcher L, Riley DL. Role of plants in anticancer drug discovery. Phytochemistry Letters 2014; 7: 173-181.

[7] Olaku O, White JD. Herbal therapy use by cancer patients: a literature review on case reports. European Journal of Cancer 2011; 47: 508-514.

[8] Vaishnav P, Demain AL. Unexpected applications of secondary metabolites. Biotechnology Advances 2010; 29: 223-229.

[9] Baque MA, Moh SH, Lee EJ, Zhong JJ, Paek KY. Production of biomass and useful compounds from adventitious roots of high-value added medicinal plants using bioreactor. Biotechnology Advances 2012; 30: 1255-1267.

[10] Gao Y, Xie J, Chen H, Gu S, Zhaoa R, Shao J, Jia L. Nanotechnology-based intelligent drug design for cancer metastasis treatment. Biotechnology Advances 2014; 32: 761-777.

[11] Li Z, Jiang H, Xu C, Gu L. A review: using nanoparticles to enhance absorption and bioavailability of phenolic phytochemicals. Food Hydrocolloids 2014; xxx: 1-12. http://dx.doi.org/10.1016/j.foodhyd.2014.05.010.

[12] Douglas RG. Means to an End: Apoptosis and Other Cell Death Mechanisms. New York: Cold Spring Harbor Laboratory Press; 2011.

[13] Lin NY, Beyer C, Gießl A, et al. Autophagy regulates TNFα-mediated joint destruction in experimental arthritis. Annals of Rheumatic Diseases 2012; 72(5): 761-768.

[14] Proskuryakov SY, Konoplyannikov AG, Gabai VL. Necrosis: a specific form of programmed cell death? Experimental Cell Research 2003; 283(1): 1-16.

[15] What's Cancer? Sinovedic Cancer Clinic, http://sinovedic.in/what-s-cancer (accessed 15 September 2014).

[16] Holland-Frei. Cancer Medicine, chapter 1, 6th edition. Hamilton (ON): BC Decker; 2003.

[17] Kuppusamy P, Yusoff MM, Maniam GP, Govindan N. A case study—regulation and functional mechanisms of cancer cells and control its activity using plants and their derivatives. Journal of Pharmacy Research 2013; 6:884-892.

[18] Fowles J, Dybing E. Application of toxicological risk assessment principles to the chemical constituents of cigarette smoke. Tobacco Control 2013; 12(4): 424-430.

[19] Khan KM, Zill EH, Dangles O. A comprehensive review on flavanones, the major citrus polyphenols. Journal of Food Composition and Analysis 2014; 33: 85-104.

[20] Xu H, Yao L, Sun H, Wu Y. Chemical composition and antitumor activity of different polysaccharides from the roots of *Actinidia eriantha*. Carbohydrate Polymers 2009; 78:316-322.

[21] Stagos D, Amoutzias GD, Matakos A, Spyrou A, Tsatsakis AM, Kouretas D. Chemoprevention of liver cancer by plant polyphenols. Food and Chemical Toxicology 2012; 50(6): 2155-2170.

[22] Coussens LM, Werb Z. Inflammation and cancer. Nature 2002; 420:860-867.

[23] Zhang L, Reddy N, Rao Koyyalamudi S. Isolation, characterization, and biological activities of polysaccharides from medicinal plants and mushrooms. In: Atta-ur-Rahman FRS (ed), Studies in Natural Products Chemistry. Vol. 42, chapter 5. Elsevier; 2014. pp. 117-151. http://www.gbv.de/dms/tib-ub-hannover/78161273x.pdf. (accessed 10 Sept 2014).

[24] Yoo KM, Lee CH, Lee H, Moon B, Lee CY. Relative antioxidant and cytoprotective activities of common herbs. Food Chemistry 2008; 106:929-936.

[25] Persidis A. Cancer multidrug resistance. Nature Biotechnology 1999; 17: 94-95.

[26] Hagiwara H, Sunada Y. Mechanism of taxane neurotoxicity. Breast Cancer 2004; 11(1): 82-85.

[27] Yan X-J, Gong L-H, Zheng F-Y, Cheng K-J, Chen Z-S, Shi Z. Triterpenoids as reversal agents for anticancer drug resistance treatment. Drug Discovery Today 2014; 19(4): 482-488.

[28] Agrawal SS, Saraswati S, Mathur R, Pandey M. Antitumor properties of Boswellic acid against Ehrlich ascites cells bearing mouse. Food and Chemical Toxicology 2011; 49:1924-1934.

[29] Shanmugam MK, Dai X, Kumar AP, Tan BK, Sethi G, Bishayee A. Oleanolic acid and its synthetic derivatives for the prevention and therapy of cancer: preclinical and clinical evidence. Cancer Letters 2014; 346:206-216.

[30] Zuco V, Supino R, Righetti SC, et al. Selective cytotoxicity of betulinic acid on tumor cell lines, but not on normal cells. Cancer Letters 2002; 175(1): 17-25.

[31] Pisha E, Chai H, Lee IS, et al. Discovery of betulinic acid as a selective inhibitor of human melanoma that functions by induction of apoptosis. Nature Medicine 1995; 1(10): 1046-1051.

[32] Gauthier C, Legault J, Lebrun M, Dufour P, Pichette A. Glycosidation of lupane-type triterpenoids as potent in vitro cytotoxic agents. Bioorganic and Medicinal Chemistry 2006; 14(19): 6713-25.

[33] Schmidt ML, Kuzmanoff KL, Ling-Indeck L, Pezzuto JM. Betulinic acid induces apoptosis in human neuroblastoma cell lines. European Journal of Cancer 1997; 33(12): 2007-2010.

[34] Skene CD, Sutton PH. Saponin-adjuvanted particulate vaccines for clinical use. Methods 2006; 40(1): 53-9.

[35] Man S, Gao W, Zhang Y, Huang L, Liu C. Chemical study and medical application of saponins as anti-cancer agents. Fitoterapia 2010; 81:703-714.

[36] Valavanidis A, Vlachogianni T. Plant polyphenols: recent advances in epidemiological research and other studies on cancer prevention. In: Atta-ur-Rahman (ed.), Studies in Natural Products Chemistry, Vol. 39, chapter 8. Elsevier; 2013, pp. 270-295. http://dx.doi.org/10.1016/B978-0-444-62615-8.00008-4 (accessed 10 September 2014)

[37] Alshatwi AA, Ramesh E, Periasamy VS, Subash-Babu P. The apoptotic effect of hesperetin on human cervical cancer cells is mediated through cell cycle arrest, death receptor, and mitochondrial pathways. Fundamental and Clinical Pharmacology 2013; 27(6): 581-592.

[38] Papademetrio DL, Trabucchi A, Cavaliere V, Ricco R, Costantino S, Wagner ML, Álvarez E. The catechin flavonoid reduces proliferation and induces apoptosis of murine lymphoma cells LB02 through modulation of antiapoptotic proteins. Revista Brasileira de Farmacognosia 2013; 23(3): 455-463.

[39] Kwik-Uribe C, Bektash RM. Cocoa flavanols—measurement, bioavailability and bioactivity. Asia Pacific Journal of Clinical Nutrition 2008; 17(Suppl 1): 280-283.

[40] Pacheco-Palencia LA, Mertens-Talcott S, Talcott ST. Chemical composition, antioxidant properties, and thermal stability of a phytochemical enriched oil from Acai (*Euterpe oleracea Mart.).* Journal of Agricultural and Food Chemistry 2008; 56(12): 4631-4636.

[41] Charrouf Z, Guillaume D. Phenols and polyphenols from *Argania spinosa*. American Journal of Food Technology 2007; 2(7): 679.

[42] Cheng GW, Crisosto CH, Browning J. Potential, phenolic composition, and polyphenoloxidase activity of buffer extracts of peach and nectarine skin tissue. American Society for Horticultural Science 1995, 120(5): 835-838.

[43] Zanwar AA, Badole SL, Shende PS, Hegde MV, Bodhankar SL. Antioxidant role of catechin in health and disease. In: Watson RR, Preedy VR, Zibadi S (eds.), Polyphenols in Human Health and Disease, chapter 21. Elsevier Press, USA; 2013. p 981.

[44] Balentine DA, Harbowy ME, Graham HN. Tea: the plant and its manufacture; chemistry and consumption of the beverage. In: Spiller GA (ed.), Caffeine. Boca Raton: CRC Press 1998. pp. 35-72.

[45] Okuda T. Systematics and health effects of chemically distinct tannins in medicinal plants. Phytochemistry 2005; 66: 2012-2031.

[46] Leung LK, Su Y, Chen R, Zhang Z, Huang Y, Chen ZY. Theaflavins in black tea and catechins in green tea are equally effective antioxidants. Journal of Nutrition 2001; 131(9): 2248-2251.

[47] Menaa F, Menaa A, Menaa B. Polyphenols nano-formulations for topical delivery and skin tissue engineering. In: Watson RR (ed.), Polyphenols in Human Health and Disease, Vol. 1, chapter 65. Elsevier; 2014. pp. 839-848.

[48] Peng M-X, Zhang H-W, Chen B-A. Main signal pathways underlying the molecular mechanisms of the antitumor effects of wogonin. Chinese Journal of Natural Medicines 2012, 10(6): 401–407.

[49] Li-Weber M. New therapeutic aspects of flavones: the anticancer properties of *Scutellaria* and its main active constituents Wogonin, Baicalein and Baicalin. Cancer Treatment Reviews 2009; 35:57-68.

[50] Doo T, Maskarinec G. Polyphenols and breast cancer prevention: a summary of the epidemiologic evidence. In: Watson RR, Preedy VR, Zibadi S (eds.), Polyphenols in Human Health and Disease, Chapter 100. Elsevier; 2013. pp. 1331-1340.

[51] Xiao J, Chen T, Cao H. Flavonoid glycosylation and biological benefits, Biotechnology Advances 2014. http://dx.doi.org/10.1016/j.biotechadv.2014.05.004.

[52] Csuk R, Albert S, Siewert B, Schwarz S. Synthesis and biological evaluation of novel (E) stilbene-based antitumor agents. European Journal of Medicinal Chemistry 2012; 54:669-678.

[53] Lin WL, Liang WH, Lee YJ, Chuang SK, Tseng TH. Antitumor progression potential of caffeic acid phenethyl ester involving p75(NTR) in C6 glioma cells. Chemico-Biological Interactions 2010 188(3): 607-15.

[54] Guo X, Shen L, Tong Y, Zhang J, Wu G, He Q, Yu S, Ye X, Zou L, Zhang Z, Lian XY. Antitumor activity of caffeic acid 3,4-dihydroxyphenethyl ester and its pharmacokinetic and metabolic properties. Phytomedicine 2013; 20:904- 912.

[55] Castro GD, Quintans LN, Maciel ME, Castro JA. Preventive effects of plant polyphenols in the promotion of mammary cancer and testicular damage induced by alcohol

drinking. In: Watson RR, Preedy VR, Zibadi S (ed.). Polyphenols in Human Health and Disease. San Diego: Elsevier-Academic Press; 2014. pp. 1181-1190.

[56] Enqin Xia, Xiran He, Huabin Li, Shan Wu, Sha Li, Guifang Deng. Biological activities of polyphenols from grapes. In: Watson RR, Preedy VR, Zibadi S. Polyphenols in Human Health and Disease. Elsevier. doi: http://dx.doi.org/10.1016/B978-0-12-398456-2.00005-0.

[57] Chen G, Xu J, Miao X, Huan Y, Liu X, Ju Y, Han X. Characterization and antitumor activities of the water-soluble polysaccharide from *Rhizoma arisaematis*. Carbohydrate Polymers 2012; 90: 67-72.

[58] Cai Z, Li W, Wang H, Yan W, Zhou Y, Wang G, Cui J, Wang F. Antitumor effects of a purified polysaccharide from *Rhodiola rosea* and its action mechanism. Carbohydrate Polymers 2012; 90: 296-300.

[59] Jain R, Sharma A, Gupta S, Sarethy IP, Gabrani R. *Solanum nigrum*: current perspectives on therapeutic properties. Alternative Medicine Review 2011; 16(1): 78-85.

[60] Jian L, Qingwang L, Tao F, Kun L. Aqueous extract of *Solanum nigrum* inhibit growth of cervical carcinoma (U14) via modulating immune response of tumor bearing mice and inducing apoptosis of tumor cells. Fitoterapia 2008; 79(7, 8): 548-556.

[61] Bao X, Yuan H, Wang C, Liu J, Lan M. Antitumor and immunomodulatory activities of a polysaccharide from *Artemisia argyi*. Carbohydrate Polymers 2013; 98:1236-1243.

[62] Jin M, Zhao K, Huang Q, Xu C, Shang P. Isolation, structure and bioactivities of the polysaccharides from *Angelica sinensis (Oliv.) Diels*: a review. Carbohydrate Polymers 2012; 89:713-722.

[63] Xu R, Ye H, Sun Y, Tu Y, Zeng X. Preparation, preliminary characterization, antioxidant, hepatoprotective and antitumor activities of polysaccharides from the flower of tea plant (*Camellia sinensis*). Food and Chemical Toxicology 2012; 50(7): 2473-2480.

[64] Younes I, Hajji S, Frachet V, Rinaudo M, Jellouli K, Nasri M. Chitin extraction from shrimp shell using enzymatic treatment. Antitumor, antioxidant and antimicrobial activities of chitosan. International Journal of Biological Macromolecules 2014; 69:489-498.

[65] Fan L, Ding S, Ai L, Deng K. Antitumor and immunomodulatory activity of water-soluble polysaccharide from *Inonotus obliquus*. Carbohydrate Polymers 2012; 9: 870-874.

[66] Yang Z, Xu J, Fu Q, Fu X, Shu T, Bi Y, Song B. Antitumor activity of a polysaccharide from *Pleurotus eryngii* on mice bearing renal cancer. Carbohydrate Polymers 2013; 95: 615- 620.

[67] Yuan H, Song J, Li X, Li N, Dai J. Immunomodulation and antitumor activity of k-carrageenan oligosaccharides. Cancer Letters 2006; 243:228-234.

[68] Zhang H, Hollis CP, Zhang Q, Li T. Preparation and antitumor study of camptothecin nanocrystals. International Journal of Pharmaceutics 2011; 415:293- 300.

[69] Watanabe M, Kawano K, Toma K, Hattori Y, Maitani Y. In vivo antitumor activity of camptothecin incorporated in liposomes formulated with an artificial lipid and human serum albumin. Journal of Controlled Release 2008; 127:231-238.

[70] Cao R, Fan W, Guo L, Ma Q, Zhang G, Li J, Chen X, Ren Z, Qiu L. Synthesis and structure activity relationships of harmine derivatives as potential antitumor agents. European Journal of Medicinal Chemistry 2013; 60:135-143.

[71] Wu D, Gao Y, Qi Y, Chen L, Ma Y, Li Y. Peptide-based cancer therapy: opportunity and challenge. Cancer Letters 2014; 351: 13-22.

[72] Ma X, Wu C, Wang W, Li X. Peptides from plants: a new source for antitumor drug research. Asian Journal of Traditional Medicines 2006; 1(2): 85-90

[73] Van Buul VJ, Brouns FJPH. Health effects of wheat lectins: a review. Journal of Cereal Science 2014; 59(2): 112-117.

[74] Clement JF, Tabbasum K, Rao CP. Chemico-biological aspects of plant lectins with a preference to legume lectins. In: Atta-ur-Rahman (ed.), Studies in Natural Products Chemistry, Vol. 40, chapter 10. Elsevier; 2013, pp. 359-381.

[75] Lei HY, Chang CP. Lectin of concanavalin A as an anti-hepatoma therapeutic agent. Journal of Biomedical Science 2009; 16(1): 10. doi:10.1186/1423-0127-16-10.

[76] Liu B, Bian HJ, Bao JK. Plant lectins: potential antineoplastic drugs from bench to clinic. Cancer Letters 2010; 287:1-12.

[77] Fu LL, Zhou CC, Yao S, Yu JY, Liu B, Bao JK. Plant lectins: targeting programmed cell death pathways as antitumor agent. International Journal of Biochemistry and Cell Biology 2011; 43: 1442-1449.

[78] Morisson RK, Brown DE, Oleson JJ, Cooney DA. Oral toxicology studies with lapachol. Toxicology and Applied Pharmacology 1970; 17(1): 1-11.

[79] Eyong KO, Kuete V, Efferth T. Quinones and benzophenones from the medicinal plants of africa. In: Kuete V. (ed.), Medicinal Plant Research in Africa, chapter 10, Elsevier; pp. 351-391.

[80] Hook I, Clive Mills, Sheridan H. Bioactive naphthoquinones from higher plants. In: Studies in Natural Products Chemistry. Vol. 41, chapter 5. Elsevier; 2014. pp. 119-160.

[81] Liu A, Chen H, Wei W, Ye S, Liao W, Gong J, Jiang Z, Wang L, Lin S. Antiproliferative and antimetastatic effects of emodin on human pancreatic cancer. Oncology Reports 2011; 26(1): 81-89. doi: 10.3892/or.2011.1257.

[82] Boreddy SR, Srivastava SK. Pancreatic cancer chemoprevention by phytochemicals. Cancer Letters 2013; 334(2013): 86-94. doi: 10.1016/j.canlet.2012.10.020

[83] Adams M. Aloe Vera Miracle. Truth Publishing International Ltd.; 2007. pp. 1-16, http://www.naturalnews.com/specialreports/aloeveramiracle.pdf (accessed 20 September 2014)

[84] Zhang L, Tizard IR. Activation of a mouse macrophage cell line by acemannan: the major carbohydrate fraction from *Aloe vera* gel. Immunopharmacology 1996; 35(2): 119-128.

[85] Harris C, Pierce K, King G, Yates KM, Hall J, Tizard I. Efficacy of acemannan in treatment of canine and feline spontaneous neoplasms. Molecular Biotherapy 1991; 3(4): 207-13.

[86] Omar SH, Al-Wabel NA. Organosulfur compounds and possible mechanism of garlic in cancer. Saudi Pharmaceutical Journal 2010; 18:51-58.

[87] Shukla Y, Kalra N. Cancer chemoprevention with garlic and its constituents. Cancer Letters 2007; 247:167-181.

[88] Yun HM, Ban JO, Park KR, Lee CK, Jeong HS, Han SB, Hong JT. Potential therapeutic effects of functionally active compounds isolated from garlic. Pharmacology and Therapeutics 2014; 142(2): 183-195.

[89] Zheng GQ, Kenney PM, Lam LKT. Myristicin: a potential cancer chemopreventive agent from parsley leaf oil. Journal of Agricultural and Food Chemistry 1992, 40(1): 107-110.

[90] Harrison ME, Power Coombs MR, Delaney LM, Hoskin DW. Exposure of breast cancer cells to a subcytotoxic dose of apigenin causes growth inhibition, oxidative stress, and hypophosphorylation of Akt. Experimental and Molecular Pathology 2014; 97(2): 211-217 doi: 10.1016/j.yexmp.2014.07.006.

[91] Agarwal R, Wang ZY, Bik DP, Mukhtar H. Nordihydroguaiaretic acid, an inhibitor of lipoxygenase, also inhibits cytochrome P-450-mediated monooxygenase activity in rat epidermal and hepatic microsomes. Drug Metabolism and Disposition 1991; 19(3): 620-624.

[92] Lambert JD, Sang S, Dougherty A, Caldwell CG, Meyers RO, Dorr RT, Timmermann BN. Cytotoxic lignans from *Larrea tridentata*. Phytochemistry 2005; 66(7): 811-815.

[93] Leonard SS, Keil D, Mehlman T, Proper S, Shi X, Harris GK. Essiac tea: scavenging of reactive oxygen species and effects on DNA damage. Journal of Ethnopharmacology 2006; 103: 288-296.

[94] WardSpain P. History of Hoxsey Treatment. http://www.tldp.com/issue/166/166hoxs.htm visiting time: 21 November, 2014.

[95] Bar-Sela G, Wollner M, Hammer L, Agbarya A, Dudnik E, Haim N. White-Berry Mistletoe (*Viscum album L.)* as complementary treatment in cancer: does it help? European Journal of Integrative Medicine 2012; 3: 55-62.

Chapter 3

Advances in Studies of *Vernonanthura patens* (Kunth) H. Rob. Growing in Ecuador

P.I. Manzano, M. Miranda, M.F. Quijano and
L. Monzote

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/59866

1. Introduction

Asteracea family (Asteraceae) includes more than 23,000 species and is the group of Angiosperms with the richest biodiversity existing in the world. *Vernonia* (*Vernonanthura*) is the most representative gene among 1000 species [1 - 4].

Various chemical components have been reported for the genus: sesquiterpene lactones of glaucolide type, hirsutinolide, vernomargolide, eudesmanolide, cardinanolide, and elephantopus, reported for most species, with some exceptions [5-11]. Moreover, diterpenes derived from ent-kaurano and kaurano, pentacyclic triterpenoids from oleanane and ursane, phytosterols, and flavonoids have been isolated and identified [12-15], while the antimicrobial and molluscicidal pharmacological activities have been the most investigated [15-17].

Within the genus *Vernonia*, *Vernonanthura patens* has been one of the less studied species. It is a shrub that grows wild in Ecuador and is widely used by people in rural areas, who attribute various medicinal properties among them: analgesic, anti-inflammatory, anticancer, and antileishmanial. However, medicinal use, studies on the chemical composition, and pharmacological activities until 2010 were very few. The first information collected on the chemical composition of the species reported the absence of sesquiterpene lactones [18], which constituted a chemical marker of gender. Nevertheless, in 1986, the aerial parts were studied, isolated and 10 lactones of a species were identified, existing contradictions on the composition [19]. Jakupovic and Schmedia-Hirschmann, With respect to biological activity, less information exists. The first, was the effect demonstrated by a methanol extract of the leaves against *Artemia salina*, the inertness to the potato disc tumor produced by the introduction of *Agrobacterium tumefaciens*, and the lack of cytotoxicity against cell lines V79 [20]. The antimalarial activity of

© 2015 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

aqueous extract of leaves has been described and a mean inhibitory concentration (IC_{50}) of 38.7 ± 10.1 μg/ml against *Plasmodium falciparum* has been reported [21], *whereas* others authors have reported that an aqueous extract of leaves, got antileishmanial activity against strains of *Leishmania amazonensis* with an IC_{50} >100 μg/ml, being these the only reports found for the species [22].

1.1. Studies in the species *V. patens* in the Ecuadorian coast

In an earlier chapter of this book, has been published the results for the fractionation of a methanol extract of the species, the study of fractions as antifungal against strains of *Fusarium oxysporum* and *Penicilium notatum*, and the chemical characterization by gas chromatography/ mass spectrometry of the fraction that showed that activity. The hexane fraction presented antifungal activity against these strains, and 33 compounds were possible to identify. Three pentacyclic triterpenoids (lupeol, epilupeol, and lupeol acetate), presented as major components, were also isolated and characterized for fractions[23]

Unlike other species of *Vernonanthura* and the own *V. patens* living in other regions of South America, the one that grows in Ecuador presents as major components pentacyclic triterpenoids and no sesquiterpene lactones. These results forced to perform a genetic characterization of the genes from the plastid rbcL and matK of leaf chloroplast DNA fraction, and it confirmed that the species was *V. patens* [24, 25]

This research group has continued with studies to verify the identity of the species, obtaining the identification of 53 compounds from leaves of a methanolic extract within the terpene compounds are highlighted (oxygenated sesquiterpenoids and triterpenoids), aliphatic hydrocarbons, fatty acids, and their methyl and ethyl esters and sugars [26, 27].

In others studies, two types of waxes of leaves and fat fractions of stems and flowers were isolated and were identified 29 fatty acids and 8 triterpenoids as components of the lipid fractions of these organs, which had not been previously reported (Tables 1 and 2) [28]. Figure 1 presents some of the chemical structures identified in this species.

Regarding biological studies, the antileishmanial evaluation of ethanolic extract of the leaves and stems was performed, proving the traditional use of the leaves of the species as antileishmanial. The ethanol extract of stems was highly toxic. Leaves extract showed a higher selectivity index than pentamidine, used as reference drug [29].

	Compounds	Percent relative abundance			
		H_SC_B	H_SC_N	T_SC	F_SC
1	Nonanedioic acid dimethyl ester	0.37	-	0.01	-
2	Tetradecanoic acid	0.31	0.26	0.03	0.23
3	Pentadecanoic acid	0.31	0.24	0.06	0.15
4	Acid, 9-hexadecenoic (ISOM)	0.33	0.45	0.04	-

	Compounds	Percent relative abundance			
		H_SC_B	H_SC_N	T_SC	F_SC
5	Hexadecanoic acid	74.24	57.74	13.31	47.50
6	Hexadecanoic acid ethyl ester	-	-	-	0.26
7	(Z)-9-hexadecenoic acid	0.22	-	-	-
8	2-Hexadecenoic acid	-	0.29	-	-
9	Heptadecanoic acid	0.21	0.27	0.06	0.11
10	8,11-Octadecadienoic acid	-	-	-	7.52
11	7, 8,11-Octadecatrienoic acid	-	6.1	-	-
12	11-Octadecenoic acid	1.15	2.6	-	-
13	8-Octadecenoic acid	-	-	0.40	-
14	Octadecanoic acid	2.36	1.71	0.43	1.32
15	Nonadecanoic acid	0.05	-	-	-
16	9,12-Octadecadienoic acid	-	1.82	1.41	0.56
17	9,11-Octadecadienoic acid	-	0.33	0.18	-
18	9,12,15-Octadecatrienoic acid	-	0.31	-	2.14
19	9,13,15-Octadecatrienoic acid	-	0.72	-	
20	Eicosanoic acid	3.26	2.66	0.17	0.88
21	Heneicosanoic acid	0.38	0.34	-	-
22	Docosanoic acid	3.86	2.88	0.40	3.70
23	Tricosanoic acid	1.2	0.90	0.11	0.56
24	Tetracosanoic acid	3.69	2.70	0.54	7.24
25	Pentacosanoic acid	0.93	0.71	0.11	0.46
26	Hexacosanoic acid	2.79	2.17	0.95	7.32
27	Heptacosanoic acid	0.24	0.17	-	0.19
28	Octacosanoic acid	0.87	0.89	0.79	2.25
29	Triacontanoic acid	0.10	0.33	0.37	0.47

H_SC_B = saponifiable white wax fraction leaves; H_SC_N = saponifiable orange wax fraction leaves; T_SC = saponifiable wax fraction stems, F_SC = saponifiable wax fraction flowers.

Table 1. Compounds identified in the fatty acid fractions of leaves, stems and flowers of *Vernonanthura patens*

All these studies have contributed to knowledge of the species growing in the Ecuadorian coast and serve as reference for other studies.

Pico	Compound	Relative abundance %			
		H_IC_B	H_IC_N	T_IC	F_IC
1	β-Amyrin	2.45	15.52	28.5	19.8
2	α-Amyrin	-	-	-	66.83
3	Lupeol	97.5	88.48	-	0.49
4	α-Amyrin + lupeol	-	-	55.59	-
5	Glutinol	-	-	2.70	-
6	Taraxasterol acetate	-	-	1.93	-
7	Taraxasterol	-	-	-	7.9
8	Neoganmacer 22(29)-en-3.ol	-	-	-	4.9

H_IC_B and H_IC_N, unsaponifiable leaves fractions; T_IC, unsaponifiable stems fractions; F_IC, unsaponifiable flowers fractions

Table 2. Compounds identified in the unsaponifiable fractions *Vernonanthura patens*

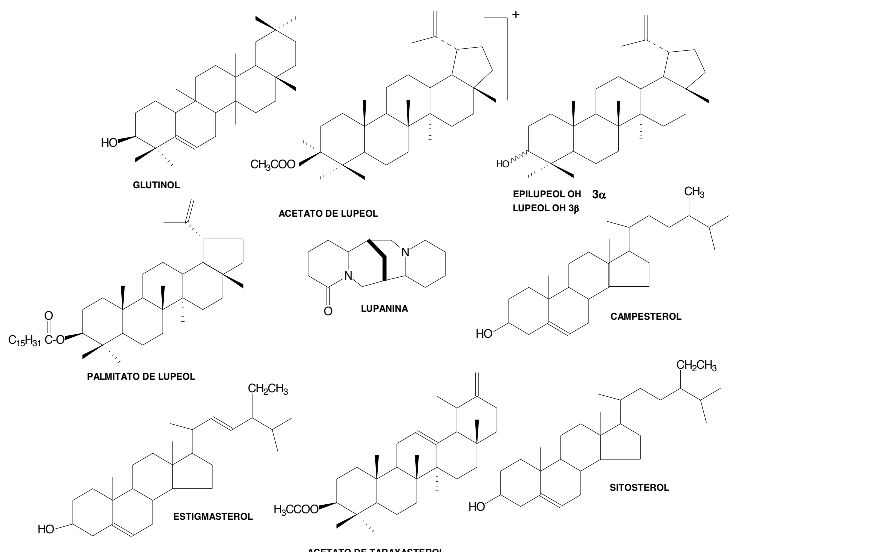


Figure 1. Some compounds isolated from *Vernonanthura patens.*

2. Materials and methods

Leaves, flowers, and stems of the species in the phenological stage of flowering were used and collected around the Biotechnology Research Center of Ecuador located at Km. 30.5 via Perimeter province of Guayas, Ecuador. A sample of the plant material was taken for botanical

identification, which was botanized at the National Herbarium of Ecuador (QCNE), Quito, with the key CIBE37a.

2.1. Extraction

The extraction from the aerial parts of the species (leaves, flowers, and stems) was performed in water and ethanol by triplicate in an ultrasonic bath VWR of 35 KHz power [30]. In all cases, 10 g of sample was extracted in the solvent (water or ethanol), in the following time intervals: 5, 15, 30, 45, and 60 min.

2.2. Determination of antioxidant activity

The determination of antioxidant activity was performed based on the stability of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical to alcoholic and aqueous extracts obtained from the aerial parts. A total of 800 μl of 0.1 N DPPH and 200 μl of the extracts were taken, and the absorbance was measured at 517 nm after 30 min using 200 μl of ethanol and 800 μl of DPPH as control. Determinations were performed in triplicate.

2.3. Determination of total polyphenols

The total polyphenol content was measured by the Folin-Ciocalteu method in a spectrophotometer (BioTek), at 760 nm using gallic acid monohydrate (CAS 149-91-7) as patron of the calibration curve. Results were expressed as milligrams of gallic acid per gram of sample (mg GA/g sample). A total of 250 μl of sample and 350 μl of Folin-Ciocalteu 1 N were mixed; 5 min after, 350 μl of 20% of sodium carbonate was added. After the 90-min incubation at room temperature, the absorbance was measured at 760 nm. Determinations were performed in triplicate.

2.4. Chromatographic profile of the extracts by HPLC

Chromatographic profiles by high-resolution liquid chromatography (HPLC) were performed in all extracts obtained at different extraction times to determine whether the extraction time produces chemical changes in the extracts. HPLC analysis was performed with Perkin Elmer Series 2000 HPLC with TotalCrom Software operating system. The phenolic compounds were detected at 280 nm with a flow rate of 1 ml/min. C18 column was used at a temperature of 30°C. Separations were carried out in a pumping system by varying the proportion of 2.5% (v/v) acetic acid in water (mobile phase A) and 70% methanol in water (mobile phase B). The solvent gradient elution program was as follows: 10% to 26% B (v/v) in 10 min, to 70% B at 20 min, and finally to 90% B at 25 to 31 min. The injection volume for all samples was 10μL.

2.5. Statistical analysis

A factorial design 2 × 3 × 5 was used involving the categorical factors: extraction solvent (water and alcohol), plant organ (leaves, flowers, and stems), and extraction time (5, 15, 30, 45, and 60 min), with antioxidant activity and total polyphenol content as response variables.

2.6. Antileishmanial activity

This test was performed with aqueous extracts obtained as described.

2.6.1. Aqueous extract

Extraction was carried out by decoction of plant materials in proportion of 10% in water during 20 min. The aqueous extract was evaporated to 87ºC and 400 mmHg. Finally, the concentrated extract was lyophilized to 120×10^{-3} mbar and 47ºC below zero.

2.6.2. Reference drug

Pentamidine (Richet, Buenos Aires, Argentina) diluted in sterile distilled water was used for *L. amazonensis*. *L. amazonensis* (MHOM/77BR/LTB0016) was kindly provided by the Department of Immunology, Oswaldo Cruz Foundation (FIOCRUZ), Brazil. Parasites were routinely isolated from mouse lesions and maintained as promastigotes at 26ºC in Schneider's medium (Sigma, St. Louis, MO, USA) containing 10% heat-inactivated fetal bovine serum (HFBS) (Sigma, St. Louis, MO, USA), 100 mg of streptomycin/ml, and 100 U penicillin/ml. The parasites were not used after the tenth passage.

2.6.3. Antileishmanial activity

The activity of the extracts against intracellular amastigotes was evaluated as described previously. The peritoneal macrophages were harvested and plated at 106/ml in 24-well Lab-Tek (Costarâ, USA) and incubated at 37ºC under an atmosphere of 5% CO_2 for 2 h. Nonadherent cells were removed by washing with prewarmed phosphate-buffered saline (PBS). Stationary-phase *L. amazonensis* promastigotes were added at a 4:1 parasite/macrophage ratio, and the cultures were incubated for further 4 h. The cell monolayers were washed three times with prewarmed PBS to remove free parasites. Then 999 ml of RPMI completed medium and 1 ml of the different products dissolved in DMSO were added in duplicate for further 48 h. The cultures were then fixed in absolute methanol, stained with Giemsa, and examined under a light microscope. The number of intracellular amastigotes was determined by counting the amastigotes in 100 macrophages per each sample. In addition, the percentage of infected macrophages was calculated. The results were expressed as percent of reduction of the infection rate in comparison to that of the controls, where the infection rates were obtained by multiplying the percentage of infected macrophages by the number of amastigotes per infected macrophages [31]. The IC_{50} value was determined from the linear regression of concentration–response curves.

The IC_{50} of the extracts for the viability of mouse peritoneal macrophages was determined. Twenty-two macrophages were collected from peritoneal cavities of normal BALB/c mice in ice-cold RPMI 1640 medium (Sigma, St. Louis, Mo, USA) supplemented with antibiotics and seeded at 30,000 cells/well. The cells were incubated for 2 h at 37ºC in 5% CO_2. Nonadherent cells were removed by washing with PBS, and then 1 µl of product solution was added to 200 µl medium containing 10% HFBS and antibiotics. Macrophages treated with 1 µl DMSO were included as controls. The cytotoxicity was determined using the colorimetric assay with 3-[4,5-

dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (Sigma, St. Louis, MO, USA). MTT solutions were prepared at 5 mg/ml in saline solution, filtered, and sterilized at the moment of use, and 15 μl was added to each well. After incubation for an additional 3 h, the formazan crystals were dissolved by addition of 100 μl DMSO. The optical density was determined using an EMS Reader MF Version 2.4-0, at a test wavelength of 560 nm and a reference wavelength of 630 nm [32]. The IC50 was obtained by fitting a sigmoidal Emax model to dose–response curves. Selectivity indexes were calculated by dividing the IC50 for peritoneal macrophage of BALB/c mice by the IC50 for *L. amazonensis* amastigotes. The IC50 of the extracts for the viability of mouse peritoneal macrophages was determined [31].

3. Results and discussions

3.1. Determination of antioxidant activity

The percentage of antioxidant activity by the method of DPPH of the aqueous and the alcoholic extracts of different plant organs, which were obtained at different times of ultrasonic extraction, were determined and differences in time of extraction were observed.

The highest percentage of antioxidant activity was obtained by employing an extraction time of 5 min for the aqueous extracts of leaves and flowers. However, the highest percentage of activity was obtained at 15 min of extraction for the aqueous extract of stems.

Alcoholic extracts of leaves and stems showed no activity at any time of extraction; in contrast, a greater activity at 45 and 60 min of extraction was observed in flowers of *V. patens* with no differences of significance between these times ($p > 0.05$) (Table 3, Figure 2).

Aerial part of the plant	Extraction time				
	5 min	15 min	30 min	45 min	60 min
Leaves	80.98 ± 1.7 Aa	48.36 ± 6.69 Ba	51.68 ± 3.14 Ba	54.16 ± 4.95 Ba	44.72 ± 4.33 Ba
Flowers	82.85 ± 3.79 Aa	73.85 ± 5.83 ABb	62.63 ± 2.69 BCb	68.05 ± 8.77 BCa	56.26 ± 5.38 Cb
Stems	35.10 ± 9.18 Ab	78.76 ± 6.09 Bb	59.14 ± 5.65 Cab	61.35 ± 2.84 Ca	54.60 ± 2.97 Cb
Flowers (ethanol)	34.24 ± 6.36 Ab	28.07 ± 3.29 Ac	69.34 ± 3.09 Bb	79.42 ± 3.29 BCb	80.82 ± 2.60 Cc

Mean values in the same column or row followed by the same capital or lowercase letters, respectively, are not significantly different ($p > 0.05$).

Table 3. Antioxidant activity (%) of aerials parts of *Vernonanthura patens*

There is only one antecedent of antioxidant activity for the *Vernonia* genre, for the species *Vernonia tweedieana*, with no previous studies for *V. patens* [33].

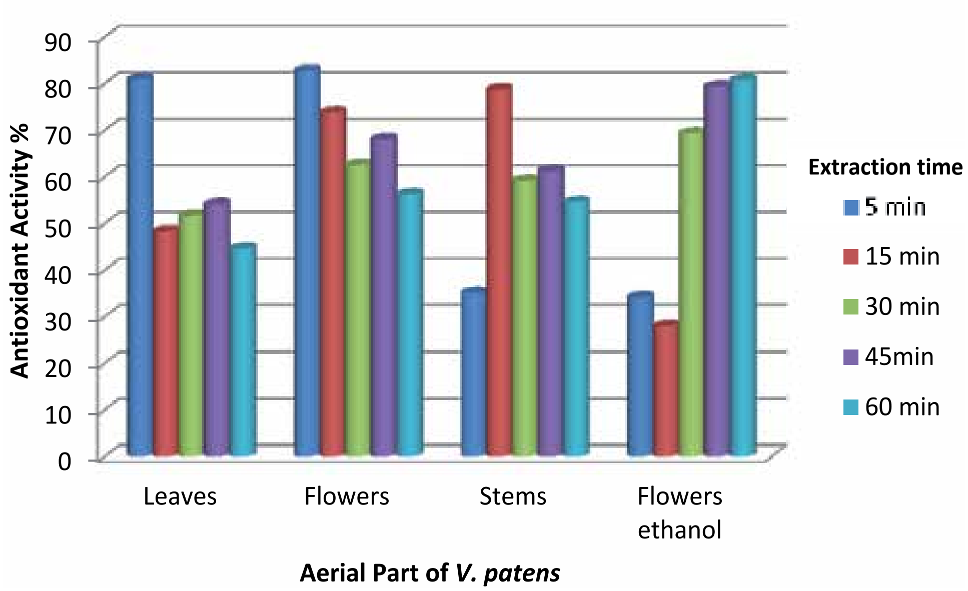


Figure 2. Antioxidant activity of aerials parts of *Vernonanthura patens* according to extraction time.

3.2. Determination of total polyphenols

The part of the plant, the extraction time, and the solvent employed have influence in the content of polyphenols.

No polyphenol could be quantified when ethanol was used as solvent, possibly because this solvent removes other colored chemical compounds such as chlorophyll, carotene, and other pigments that interfere with the spectrophotometric determination of polyphenols in the extract.

When water is used as solvent, there is a direct relationship between the extraction time and the concentration of polyphenols; that is, the longer the extraction, the higher the concentration independent of the plant organ used (Figure 3).

In all cases, the highest percentage of total polyphenols was obtained at 45 min of aqueous extraction without significant differences ($p > 0.05$) with 60 min of extraction. At this time of extraction (45 min), the leaves of the species exhibited the highest percentage of polyphenols, followed by flowers. Stems exhibited the lowest percentage of polyphenol (Table 4).

There are several reports regarding the presence of phenolic compounds in the different plant organs for the *Vernonanthura* (*Vernonia*) genre. Flavonoids have been designated as constituents of gender [34]. Flavonoids, tannins, and phenolics are reported for leaves of *V. cinerea* [35], whereas others authors have reported the presence of flavonoids [14]. For flowers of the same species, flavonoids, tannins, and phenolic compounds have been identifiedas well [35]. Flavonoids and tannins has been detected for leaves of *Vernonia amigdalina*, [36, 37].

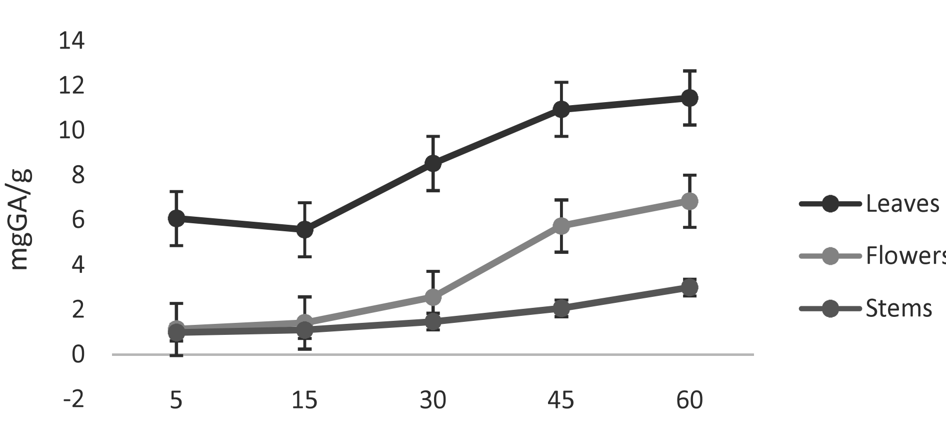


Figure 3. Polyphenol concentration *vs* time for different plant organs.

Aerial part of the plant	Extraction time				
	5 min	**15 min**	**30 min**	**45 min**	**60 min**
Leaves	6.07 ± 1.7 Aa	5.57 ± 0.22 Aa	8.53 ± 1.2 Aa	10.95 ± 0.91 Ba	11.46 ± 1.94 Ba
Flowers	1.12 ± 0.12 Ab	1.41 ± 0.17 Ab	2.55 ± 0.67 Ab	5.74 ± 0.06 Bb	6.85 ± 0.96 Bb
Stems	0.98 ± 0.06 Ac	1.09 ± 0.14 Ac	1.47 ± 0.22 ABc	2.06 ± 0.48 BCc	2.99 ± 0.49 Cc

Mean values in the same column or row followed by the same capital or lowercase letters, respectively, are not significantly different ($p > 0.05$)

Table 4. Total polyphenol content (mg GA/g) of aerial parts of *Vernonanthura patens* using water as solvent.

Phenolic compounds, tannins, and flavonoids among other compounds in the leaves, stems, and flowers of the species *V. patens* of Ecuadorian coast have been reported [38]. This may explain the total polyphenol content found in different plant organs studied, although in not a very high proportion.

3.3. Chromatographic profile of the extracts by HPLC

Considering the fact that the extraction method using ultrasound sometimes causes changes in the chemical composition of the extracts, a chromatographic profile was performed to the aqueous extracts obtained at 5 and 45 min of extraction.

Some changes in the chromatographic profile of the aqueous extract of leaves at 45 min are observed (Figure 4), especially in the baseline, although the highest chromatographic peak intensity observed at about 56.9 min has no variation.

Chromatographic profile changes are minor in the aqueous extract of the flowers; a major chromatographic peak around 56.6 min is also seen (Figure 5).

Nevertheless, the aqueous extract of the stems suffered no change in the chromatographic profile at different extraction times analyzed (5, 15, and 45 min). The retention time of the major peak was found at 56.9 min (Figure 6).

Only the study for alcoholic extracts of flowers was done because it was the only one that showed antioxidant activity. Minimal changes were observed in the chromatographic profile of 5 and 45 min extraction, which may be due to the transmission of the ultrasonic waves in the ethanol solvent, which are lower than when water is used [38, 39]. A chromatographic peak around 57 min was also observed in this extract (Figure 7).

The results lead us to believe that in all plant organs of the species obtained with water and the alcoholic extract of the flowers, there is a major component or a mixture thereof, which eluted at a similar retention time by HPLC, in the studied conditions, which may or may not be responsible for the antioxidant activity found for these compounds. However, it is important to note that no correlation between the polyphenol content of the extracts and the antioxidant activity was found. In all cases, the higher the percentage of antioxidant activity, the lower the percentage of total polyphenolics (Figure 8).

These results indicate that the antioxidant activity of aqueous extracts of the species is not due solely to the presence of polyphenolic compounds that remain to determine the chemical composition of these extracts to determine which one or more compounds are influential in this activity.

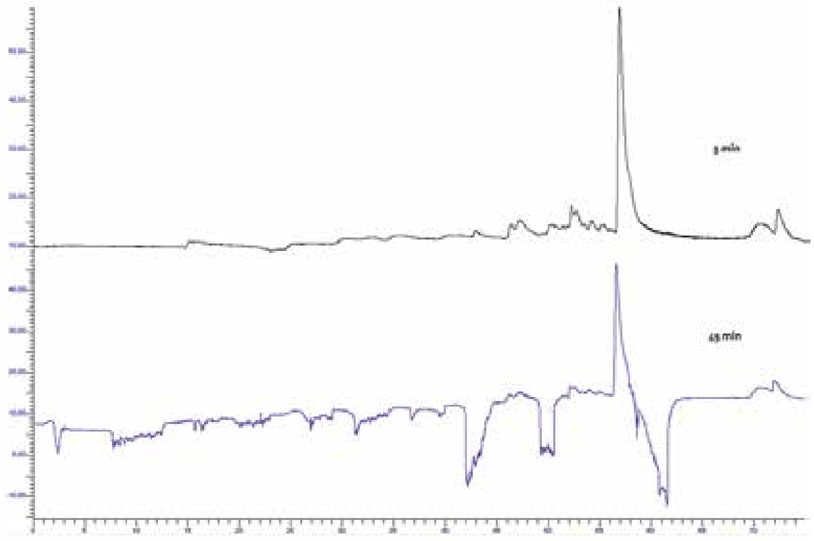


Figure 4. HPLC chromatographic profile of the aqueous extract of the leaves of *Vernonanthura patens* obtained with 5 and 45 min of ultrasound extraction

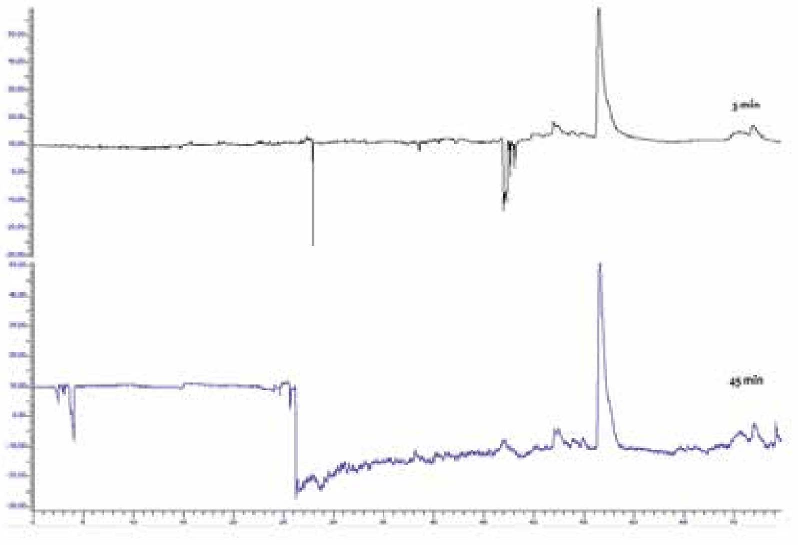


Figure 5. HPLC chromatographic profile of the aqueous extract of the flowers of *Vernonanthura patens* obtained with 5 and 45 min of ultrasound extraction

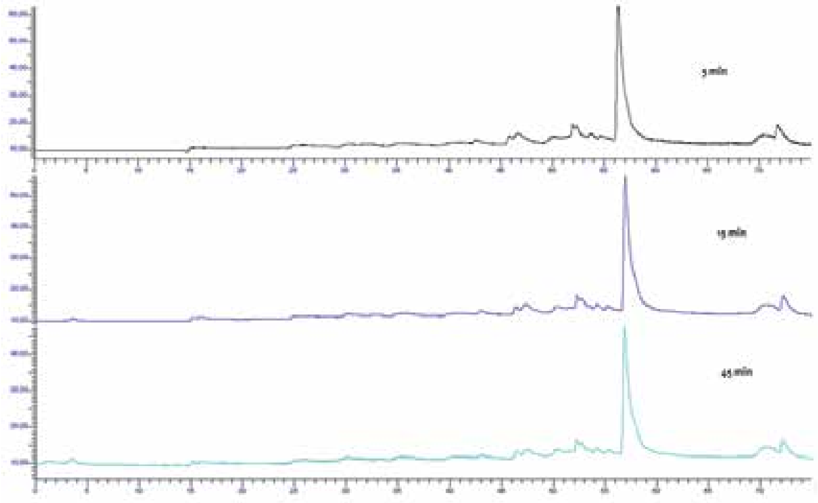


Figure 6. HPLC chromatographic profile of the aqueous extract of the stems of *Vernonanthura patens* obtained with 5, 15, and 45 min of ultrasound extraction

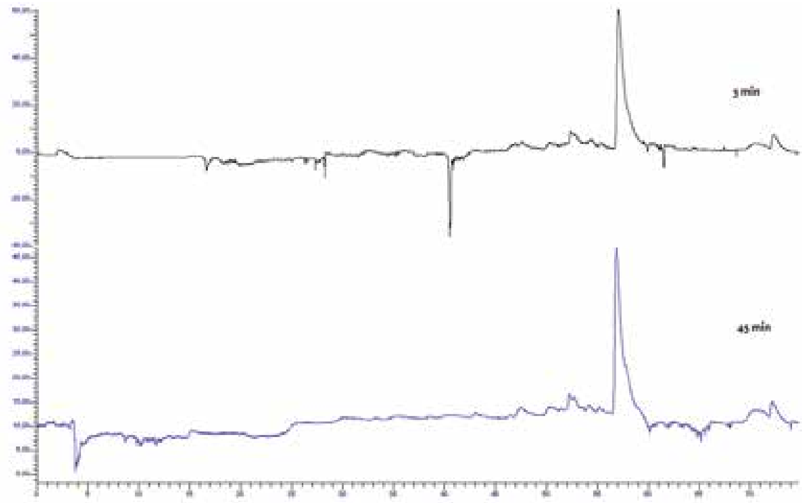


Figure 7. HPLC chromatographic profile of the ethanolic extract of the flowers of *Vernonanthura patens* obtained with 5 and 45 min of ultrasound extraction

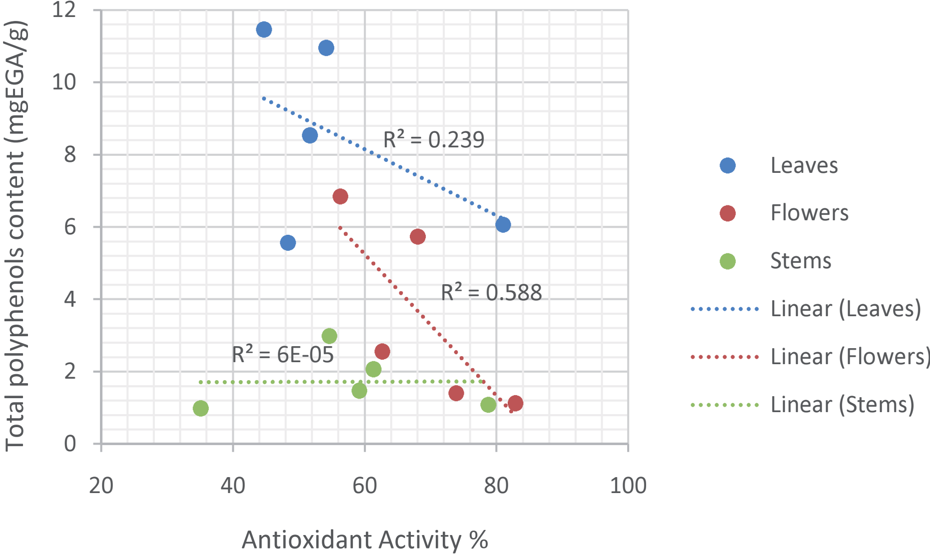


Figure 8. Total polyphenol content *vs* antioxidant activity of the leaves, flowers, and stems of *Vernonanthura patens*

3.4. Evaluation of antileishmanial activity

Aqueous extracts of leaves and stems showed activity and selectivity against *L. amazonensis* (Table 5). The activity and selectivity present in the aqueous extract of the stems showed a

different behavior to that reported for the ethanol extract of this plant organ [28]. The evaluation was reported as not detectable because it caused a minimal toxicity in the concentrations tested, destroying the cell monolayer.

The results obtained for the aqueous extract support the traditional use of the species and are highly relevant as these extracts would enhance the usefulness of a possible low-cost source for the development of an effective herbal drug for the treatment against Leishmania.

Extracts	Promastigote L. amazonensis (IC_{50} μg/ml) $\overline{X}(S)$	Cytotoxicity macrophages (CC_{50} μg/ml)	SI
Leaves	18.8 ± 0.2	200	11
Stems	23.7 ± 0.1	200	8
Pentamidine (positive control)	1.3 ± 0.1	11.7	9

Table 5. Antileishmanial activity and cytotoxicity of aqueous extracts of *Vernonanthura patens* against *L. amazonensis*

IC_{50}, half maximal inhibitory concentration, is expressed as the concentration of extract (mg/ml) that inhibits 50% of the parasite growth. CC50, median cytotoxic concentration, is expressed as the concentration of extract (mg/ml) causing 50% of parasite mortality. $\overline{X}(S)$, mean (standard deviation). SI, selectivity index: CC50 macrophages / IC50 Leishmania. SI ≤ 5 more selective for the cell, SI ≥5 more selective for parasite.

4. Conclusion

The extraction time ultrasound influences the concentration of polyphenolic compounds in all organs tested, when water is used as solvent. Ethanol extracts are unable to determine the concentration of phenolic compounds due to the possible interference of colored compounds extracted with this solvent. Aqueous extracts of the leaves and flowers and the alcoholic extract of flowers showed higher antioxidant activity than the aqueous extracts of the stems. Antioxidant activity in the alcoholic extracts of leaves and stems was not observed.

The HPLC chromatographic profiles of all extracts tested showed a majority chromatographic peak between 56 and 57 min, which could correspond to a mixture of compounds responsible for the antioxidant activity found.

No correlation between antioxidant activity and polyphenol content was found, so presumably they are not solely responsible for the antioxidant activity found.

Aqueous extracts of the leaves and stems of the species *V. patens* showed higher activity and selectivity than the alcoholic extracts, against *L. amazonensis*, corroborating the traditional use of the species.

5. Future directions

The results obtained in the chemical and biological study of the species *V. patens* show its potential as antimicrobial, antioxidant, and particularly antileishmanial, antiparasitic diseases of high incidence in Ecuador and other countries. However, inclusion in the therapeutic still requires further studies among which may be mentioned toxicology to demonstrate the safety of their preparations and as a development of a suitable dosage form to the pathology to be treated, aspects that should motivate future research of our work group.

Acknowledgements

This study was supported by grants from SENESCYT, Prometheus program, and ESPOL (Ecuador).

Author details

P.I. Manzano[1*], M. Miranda[2], M.F. Quijano[2] and L. Monzote[3]

*Address all correspondence to: pmanzano@espol.edu.ec

1 Escuela Superior Politécnica del Litoral, Facultad de Ciencias Naturales y Matemáticas (ESPOL-FCNM), Guayaquil, Ecuador

2 Escuela Superior Politécnica del Litoral, Centro de Investigaciones Biotecnológicas del Ecuador (ESPOL-CIBE), Guayaquil, Ecuador

3 Institute of Tropical Medicine Pedro Kouri, Havana, Cuba

References

[1] Rodríguez A. (2005). *Asteraceae*. In: Hammel BE, Zamora N, Grayum MH (eds.). Plant Manual of Costa Rica. Missouri Bot. Gard. Press, St. Louis & Inst. Nac. de Biodiversidad, Santo Domingo de Heredia, Costa Rica.

[2] Stevens PF. (2006). «Asteraceae». Angiosperm Phylogeny Website. Version 7, Mayo.

[3] Jeffrey C. (2007). Compositae: Introduction with Key to Tribes. 7 in Families and Genera of Vascular Plants. Flowering Plants, Eudicots, *Asterales*. Springer-Verlag, Berlin: J. W. Kadereit and C. Jeffrey. Vol. VIII: 61–68.

[4] Bremer K, Anderber A. (1994). *Asteraceae:* Cladistics & Classification. Universidad de Michigan, Timber Press. ISBN-10: 0881922757. ISBN-13: 978-0881922752.

[5] Borkosky S, Bardón A, Catalán C, Díaz J, Herz W. (1997). Glaucolides, hirsutinolides and other sesquiterpene lactones from *Vernonanthura pinguis*. Phytochemistry, 44(3): 465–470

[6] Bazon J, Callegari J, Vichnewski W, Dias D, Nagamiti K, Cunha W, Herz W. (1997). Cadinanolides and other constituents from *Vernonia fruticulosa* and *Vernonanthura discolor*. Phytochemistry, 44(8): 1535–1536.

[7] Hernández Z, Catalán C, Hernández L, Ramírez D, Joseph-Nathan P. (1999). Sesquiterpene lactones from *Stevia alpina* var. Glutinosa. Fitoquimica, 51(1): 79–82.

[8] Kotowicz C, Bardóna A, Catalán C, Cerda-García-Rojas C, Joseph-Nathan P. (1998). Glaucolides and hirsutinolides from *Vernonanthura squamous*. Phytochemistry, 47(3): 425–428

[9] Pollora GC, Bardón A, Catálan C, Gedris E, Herz W. (2004a). Elephantopus-type sesquiterpene lactones from a *Vernonanthura* species, *Vernonanthura nebularum*. Biochemical Systematics and Ecology, 31(4): 397–405.

[10] Pollora GC, Bardón A, Catalán C, Griffin Cl, Herz W. (2004b). Elephantopus-type sesquiterpene lactones from a second *Vernonanthura* species, *Vernonanthura lipeoensis*. Biochemical Systematics and Ecology, 32(6): 619–625.

[11] Kos O, Castro V, Murillo R, Poveda L, Merfort I. (2006). Ent-kaurane glycosides and sesquiterpene lactones of the hirsutinolide type from *Vernonia triflosculosa*. Phytochemistry, 67: 62–69

[12] Latha R, Geetha T, Varalaskshmi P. (1998). Effect of *Vernonia cinerea* less flower extract in adjuvant-induced arthritis. General Pharmacology, 31(4): 601–606.

[13] Abeysekera A, de Silva K, Silva S, Sirimanne V, Labadie R, Berg A, Van Den S, W Van Der. (1999). Inhibition of chemiluminescence generated by zymosan-activated polymorphonuclear leucocytes by phenolic constituents of *Vernonia cinerea*. Fitoterapia, 70: 317–319.

[14] Iwalewa O, Iwalewa J, Adeboye O. (2003). Analgesic, antipyretic, anti-inflammatory effects of methanol, chloroform and ether extracts of *Vernonia cinerea* Less leaf. Journal of Ethnopharmacology, 86: 229–239.

[15] Portillo A, Vila R, Freixaa B, Ferrob E, Parellac T, Casanovad J, Cañiguerala S. (2005). Antifungal sesquiterpene from the root of *Vernonanthura tweedieana*. Journal of Ethnopharmacology, 97(1): 49–52

[16] Bardón A, Borkoskya S, Ybarra M, Montanaroa S, Cartagena E. (2007). Bioactive plants from Argentina and Bolivia. Fitoterapia, 78(3): 227–231.

[17] Borkosky S, Ponce S, Gabriela Juárez G, González M, Bardón A. (2009). Molluscicidal sesquiterpene lactones from species of the tribe Vernonieae (Compositae). Chemistry and Biodiversity, 6(4): 513–519.

[18] Mabry TJ, Abdel-Baset Z, Padolina WG. (1975). Systematic implications of flavonoides and sesquiterpene lactones in species of *Vernonia*. Biochemical Systematics and Ecology, 2(3–4):185–192.

[19] Jakupovic J, Schmedia-Hirschmann G. (1986). Hirsutinolides, glaucolides and sesquiterpene lactones in species of *Vernonia*. Phytochemistry, 25(2): 145–158.

[20] Gupta M, Monge A. (1986). Screening of Panamanian medicinal plants for brine shrimp toxicity, crown gall tumor inhibition, cytotoxicity and DNA intercalation. International Journal of Pharmacognosy, 34: 19–27.

[21] Fournet A, Barrios A. (1994). Leishmanicidal and trypanocidal activities of Bolivian medicinal plants. Journal of Ethnopharmacology, 42: 19.

[22] Valadeau C, Pabon A, Deharo E, Albán-Castillo J, Estévez Y, Lores F, Rojas R, Gamboa D, Sauvain M, Castillo D, Bourdy G. (2009). Medicinal plants from the Yanesha (Peru): evaluation of the leishmanicidal and antimalarial activity of selected extracts. Journal of Ethnopharmacology, 123(3): 413–422

[23] Manzano Santana PI, Silva Osorio M, Sterner O, Peralta Garcìa EL. (2012). Phytochemical Studies of Fractions and Compounds Present in *Vernonanthura patens* with Antifungal Bioactivity and Potential as Antineoplastic, Phytochemicals—A Global Perspective of Their Role in Nutrition and Health, Dr Venketeshwer Rao (Ed.). ISBN: 978-953-51-0296-0, InTech.

[24] Santos E, Manzano P, Navarrete O, Hidalgo L, Miranda M, Peralta E. (2012a). *Vernonanthura patens* maturase K (matK) gene, partial cds; chloroplast. National Center for Biotechnology Information. GenBank: JX276535.1. http://www.ncbi.nlm.nih.gov/nuccore/JX286590.1

[25] Santos E, Manzano P, Navarrete O, Hidalgo L, Miranda M, Peralta E. (2012b). *Vernonanthura patens* ribulosa-1,5-bifosfato carboxilasa/oxigenasa subunidad grande (rbcL) de genes, cds parcial; cloroplasto National Center for Biotechnology Information. GenBank: JX276536.1. http://www.ncbi.nlm.nih.gov/nuccore/JX276535.1; http://www.ncbi.nlm.nih.gov/nuccore/JX276536.1

[26] Manzano Santana P, Miranda Martínez M, Abreu Payrol J, Silva M, Olov S, Peralta E. (2013a). Pentacyclic triterpenoids with antimicrobial activity from the leaves of *Vernonanthura patens* (Asteraceae). Emirates Journal of Food and Agriculture, 25(7). doi: 10.9755/ejfa.v25i7.15989.

[27] Manzano Santana PI, Orellana León T, Miranda Martínez M, Abreu Payrol J, Ruíz O, Peralta García EL. (2013b). Some pharmacognostic parameters of *Vernonanthura patens* (Kunth) H. Rob. (Asteraceae) endemic to Ecuador. Cuban Journal of Medicinal Plants, 18(1).

[28] Manzano Santana PI, Miranda Martínez M, de Oca Porto RM, Orellana León T, Abreu Payrol J, Peralta García EL. (2013c). Chemical study of lipid compounds of the leaves, stems and flowers *Vernonanthura patens* (Kunth) H. Rob. (Asteraceae). Cuban Journal of Medicinal Plants, 18(4).

[29] Manzano Santana PI, García M, Mendiola J, Fernández-Calienes A, OrellanaT, Miranda M, Peralta E, Monzote L. (2014). In vitro anti-protozoal assessment of *Vernonanthura patents* extracts. Pharmacology Online 1, 1–6.

[30] Lee L, Lee N, Kim Y, Lee C, Hong S, Jeon Y, Kim Y. (2013). Optimization of ultrasonic extraction of phenolic antioxidants from green tea using response surface methodology. Korea Food Research Institute, Vol. 18, pp. 13530–13535.

[31] Monzote L. (2010). Therapeutic potential of essential oil of *Chenopodium ambrosoides* and some of its constituents against leishmania. PhD thesis in Pharmaceutical Sciences, Institute of Tropical Medicine "Pedro Kouri," Havana.

[32] Sladowski D, Steer SJ, Clothier RH, Balls M. (1993). An improve MTT assay. Journal of Immunological Methods, 157(1–2): 203–207.

[33] Basso R. Secondary metabolites in *Vernonia tweedieana* Baker. Master in Pharmaceutical Sciences. Federal University of Santa Maria. Center for Health Sciences. Postgraduate Program in Pharmaceutical Sciences. Dissertation, 2006.

[34] Carvalho M, Costa P, Abreu H. (1999). Flavones from *Vernonia diffusa*. Journal of the Brazilian Chemical Society, 10(2): 163–166.

[35] Latha R, Geetha T, Varalaskshmi P. (1998). Effect of *Vernonia cinerea* less flower extract in adjuvant-induced arthritis. General Pharmacology, 31(4): 601–606.

[36] Awe S, Makinde J, Olajide O. (1999). Cathartic effect of the leaf extract of *Vernonia amygdalina*. Fitoterapia, 70(2): 161–165.

[37] Akinpelu D. Antimicrobial activity of *Vernonia amigdalina* leaves. Fitoterapia, 70(4): 432–434.

[38] Manzano Santana PI, Miranda M, Abreu Payrol J, Silva M, Hernández V, Peralta E. (2013d). Gas chromatography-mass spectrometry study from the leaves fractions obtained of *Vernonanthura patens* (Kunth) H. Rob. International Journal of Organic Chemistry, 3: 105–109. doi:10.4236/ijoc.2013.32011 Published Online June 2013 (http://www.scirp.org/journal/ijoc).

[39] Soares MI, Péres VF, Dariva C, Zini CA, Abad FC, Martinez MM, Caramão EB. (2006). Optimization of the sonication extraction method of *Hibiscus tiliaceus* L. flowers. Ultrasonics Sonochemistry, Apr 13(3): 242–250.

[40] Peres VF, Saffia J, Melecchi MI, Abadc FC, de Assis Jacques R, Martinez MM, Oliveira EC, Caramao EB. (2006). Comparison of soxhlet, ultrasound-assisted and pressurized liquid extraction of terpenes, fatty acids and Vitamin E from *Piper gau*dichaudianum Kunth. Journal of Chromatography A, 1105(1–2): 115–118.

Chapter 4

Phytochemical Profile of Honey

Aldina Kesić, Nadira Ibrišimović-Mehmedinović and Almir Šestan

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/60087

1. Introduction

During the process of cellular respiration in living organisms some reactive oxygen species (ROS) are constantly forming, sometimes as a result of exogenous sources such as pollution, radiation and ionizing radiation and drugs [1, 2, 3, 4]. They can harm vitally important structures, such as cell membranes, destroying deoxyribonucleic acid (DNA) that is an essential core component of every cell and damaging the respiratory enzymes and genetic material, thus creating the preconditions for the emergence of degenerative and malignant diseases [5, 6, 7, 8].

Production of prooxidant species, especially ROS, is in equilibrium with the antioxidant protection of the organism under normal conditions.

Increased production of pro-oxidants and / or reduced antioxidant protection of the organism can lead to tissue damage and disease. This situation is called oxidative stress, which is the cause or contributing factor in the pathology of many diseases [9].

So, if there is a genetic predisposition or exposure to external factors such as cigarette smoke, sun light, pollution, etc., the balance of prooxidants / antioxidants may be impaired (Figure 1).

Oxidative stress, induced mainly by ROS is now recognized as a major cause of several human health disorders (Figure1) [10, 11]. Antioxidants of both endogenous and exogenous sources have the ability to donate electrons and are effective in mitigating the damaging effects of ROS on cellular components [12]. Honey is a complex mixture of more than seventy different compounds present in varying proportions [13,14]. Many of these phytochemicals such as polyphenols and flavonoids present in honey have antioxidant properties [15,16, 17,18,19]. In view of the importance of the antioxidant phytochemicals present in honey, we undertook a

© 2015 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

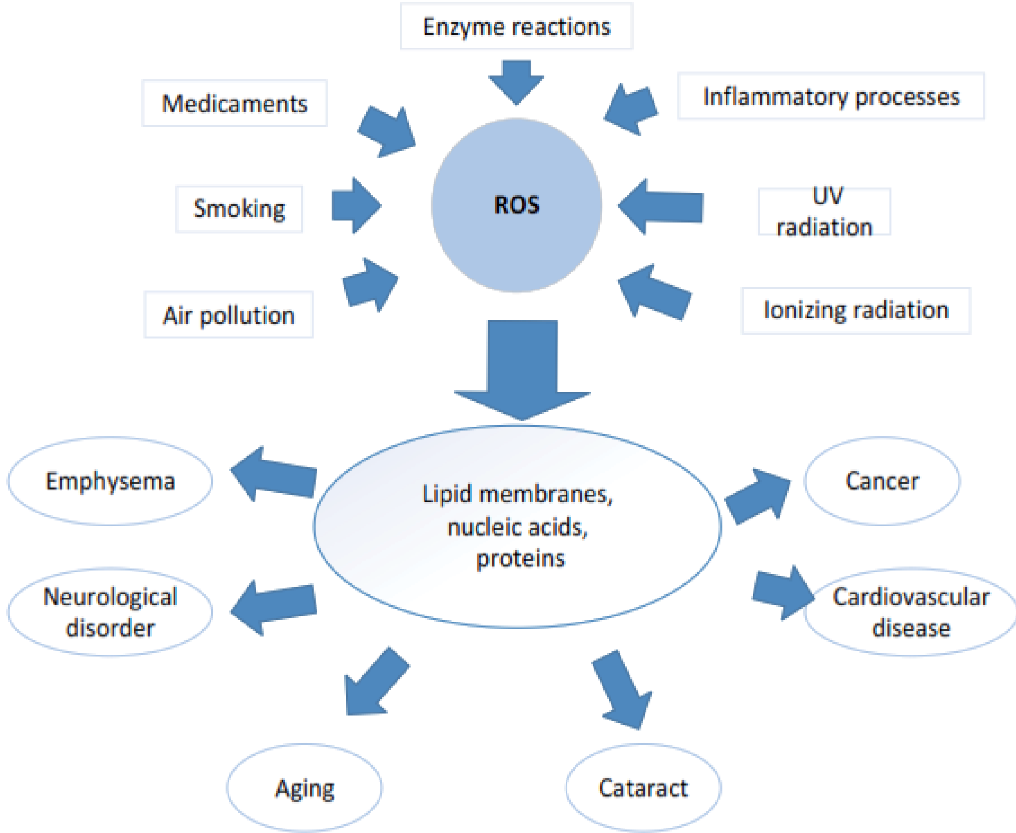


Figure 1. ROS: Occurrence and major consequences on the human body.

study to characterize the photochemical profiles of several honey samples from different botanical and geographical origins.

2. Methods and materials

The study included 60 samples of honey from different botanical and geographical origin. Samples of honey were collected in cooperation with the Union of Beekeepers of Tuzla Canton, Bosnia and Herzegovina. Information about the botanical and geographical origin of the samples was collected from the producers themselves. The analysis of total antioxidants in the samples analyzed, and an analysis of the content of polyphenols were made [20, 21, 22]. Then, statistical analysis of the data was performed and examined the correlation between the total antioxidant capacity of honey and polyphenol content. The total content of antioxidants in the samples was determined by Ferric Reducing Antioxidant Power (FRAP) method [23, 24, 25]. Total polyphenols in the samples were determined using the Folin-Ciocalteu (FC) method [26].

http://dx.doi.org/10.5772/60087

2.1. Determination of total antioxidant capacity of honey using the FRAP method

In order to determine the antioxidant capacity, a lot of methods based on different mechanisms of antioxidant defensce systems were developed, such as the removal or inhibition of free radicals or chelating metal ions, which would otherwise lead to the formation of free radicals.

The method used in our study is an indirect FRAP method. The FRAP is a method that was described in 1996 by Benzie and Strain, but in 1999 was further modified [25, 27]. This is a simple, quick test method based on the reduction of iron from ferric Fe3+ into the ferrous Fe2+ form in the presence of antioxidants, where at low pH level, a deep- blue colored complex of ferro tripyridyltriazine is developed, which has an absorption maximum at 593 nm (Figure 2.). The reaction is not specific. The results are expressed as the μmol Fe2+ equivalent of (Fe) / mL sample.

Figure 2. Ferrum reduction reaction - 2,4,6-tripyridyl-s-triazine (TPTZ).

Analysis results were determined by measuring absorbance of standard solutions, which have been incorporated in the calibration line.

The concentration of antioxidants in the measured sample on the basis of the obtained value is calculated according to the formula:

$$X = \frac{Y - 0{,}0243}{0{,}0011}$$

where:

X – antioxidant concentration

Y – sample average absorbance

The results are expressed in μmol Fe^{II}/L 10 % of honey solution.

3. Determination of total polyphenols using the FC method

The content of total polyphenols in the honey samples was determined using the FC method. The FC method is one of the oldest indirect methods that is sensitive to phenolic and polyphenolic compounds. This method has been standardized and widely used in the determination of polyphenols.

4. Results and discussion

The components of honey, with antioxidant properties, are phenolic acids and flavonoids, enzymes, ascorbic acid, organic acids, amino acids, proteins and some micro biogenic elements [28, 29]. Honey characterization helps us to understand its antioxidant characteristics, thereby, its use as natural foodstuff, i.e., as sources of antioxidant human nutrition.

Our results have shown that antioxidant activity of honey from Bosnia and Herzegovina is in the range of 4.7 μMFeII/L to 1606.54 μMFeII/L.

According to Jerkovic and co-workers´ research, antioxidant activity of honey from the Croatian territory was from 101.5 μMFe to 955.9 μMFe [30].

With the analysis of total antioxidant activity along with the concentration of polyphenols, we came to the conclusion that there has been a significant correlation between these two parameters.

This conclusion is confirmed by research scientist Al-Mamary et-al. [31], who showed that antioxidant activity of several types of honey originating from different countries depends on the concentration of phenolic groups.

In addition, researches and other antioxidant activities of honey that support this assertion were also included [32, 33, 34, 35].

Our results are also in correlation with the results of the Slovenian scientists [26] who, with their studies, confirmed the polyphenol content of 4.48 mg/100 g in locust honey and 24.14 mg/100g in forest honey. Italian scientists [35] confirmed the contents of polyphenols in honey in the value of 3 mg/100g to 17.5 mg/100g. These results show slightly lower values of polyphenols in Italian honey samples compared to the honey from the territory of Bosnia and Herzegovina.

A high correlation coefficient between the antioxidant activity of honey and total polyphenol content has also been confirmed in our studies (Figure 3):

With statistical analysis of the given results, we got Pearson's correlation coefficient that is equal to 0.957. This means that 95.7% of honey samples with a stronger antioxidant activity have higher polyphenol content.

The chemical composition of honey largely depends on botanical origin as an important factor. According to the botanical origin, analyzed honey samples are divided into several groups:

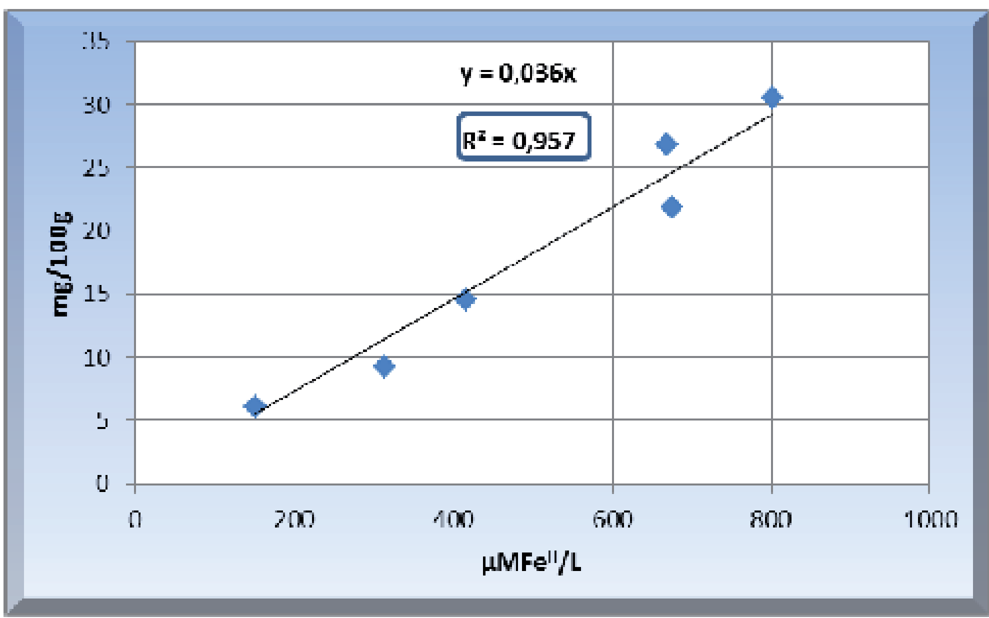


Figure 3. Correlation between total antioxidant activity of honey and polyphenol content

meadow, acacia, forest, mixed, lime, chestnut honey, mountain honey, hawthorn, heather, sage and rosemary. Different antioxidant activity is shown in honey samples of different botanical origin. Antioxidant activity of honey samples divided according to botanical origin grows according to the following sequence: hawthorn <acacia <lime <meadow <chestnut <mixed <sage <ling <forest <mountain <rosemary. This relationship is shown in Figure 4:

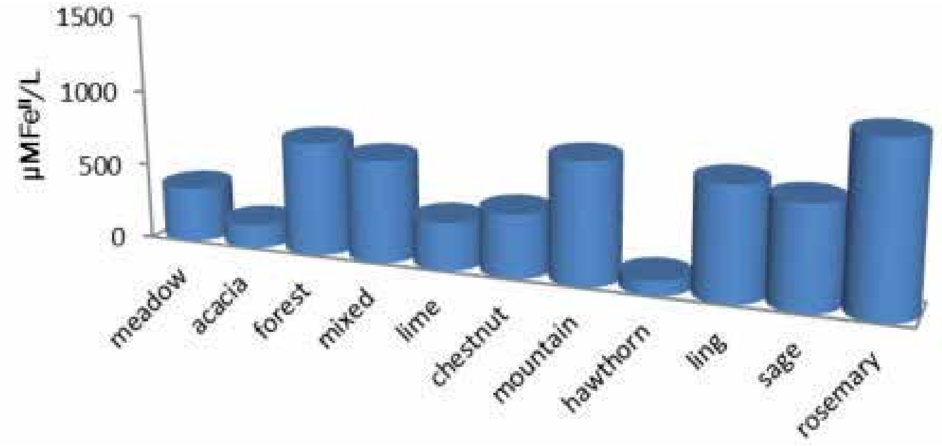


Figure 4. Total antioxidant activity of honey samples of different botanical origin

Rosemary honey has got the highest antioxidant activity. Because of its relatively high content of antioxidants and low content of essential oil and chlorophyll, rosemary is the best source of natural antioxidants. Plants such as rosemary, sage, oregano, thyme, pepper and green tea, contain active substances with strong antioxidant activities. Rosemary extracts have a high concentration of active substances carnosol acid, carnosol, methyl carnosol and rosemary acid. Besides carnosol acid and its derivatives which are the most powerful natural antioxidants, rosemary extract also contains ursolic and oleanolic acid (in its chemical composition triterpenoids).

Rosemary honey showed the highest antioxidant activity since rosemary is a significant source of natural antioxidants. Although the data showed [36] that hawthorn is a significant source of antioxidants, in our analysis, hawthorn honey showed a lower antioxidant activity compared to other analyzed honey samples. Since our study determined the total antioxidant activity of aqueous solutions of honey, there is a possibility that hawthorn contains more antioxidants, that are not soluble in water.

If we divide the analyzed honey samples according to their geographical origin, we can say that the samples from the middle Bosnia are of the richest sources of antioxidants, followed by specimens from North-eastern Bosnia, and slightly weaker source of antioxidants are samples of honey from the north and west of Bosnia and Herzegovina (Figure 5).

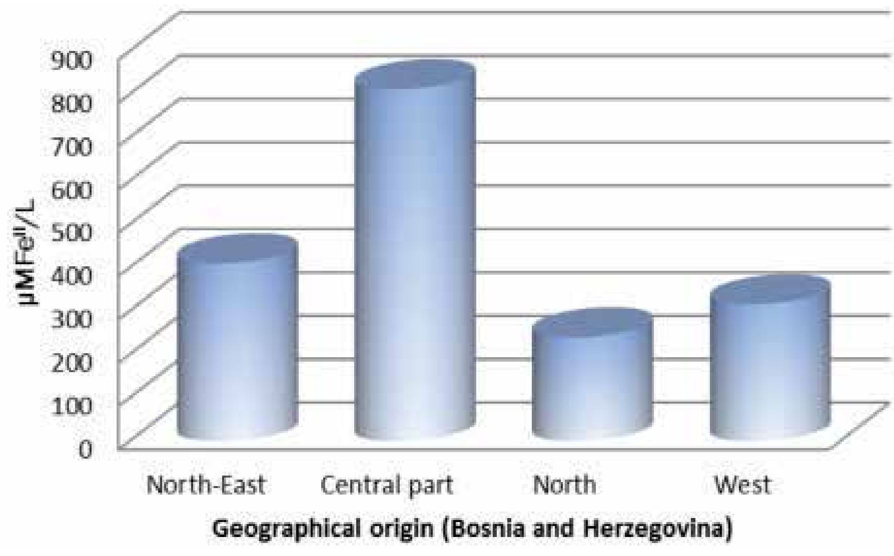


Figure 5. Antioxidant activity of honey samples from different geographic origin

The content of polyphenols in honey is affected by the botanical origin of honey besides having a significant impact on the overall antioxidant activity (Figure 6).

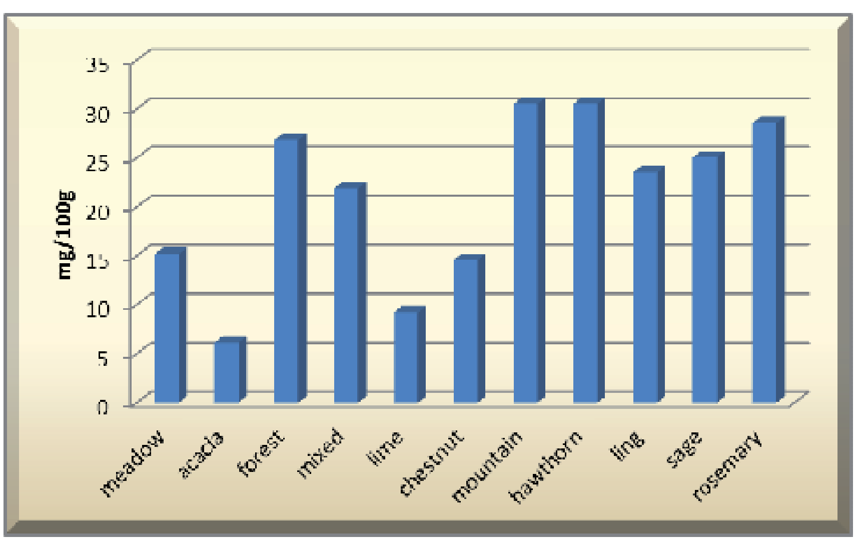


Figure 6. Polyphenol content in honey of different botanical origin.

According to the results of the study, the polyphenol content is increased according to the following order: acacia <lime <chestnut <meadow <mixed <winter savory <sage <forest <rosemary <hawthorn <mountain.

By analyzing these results we can say that the botanical origin significantly affects the polyphenol content and the total antioxidant activity of honey samples. Influence of polyphenol content to total antioxidant activity is shown in Figure 7:

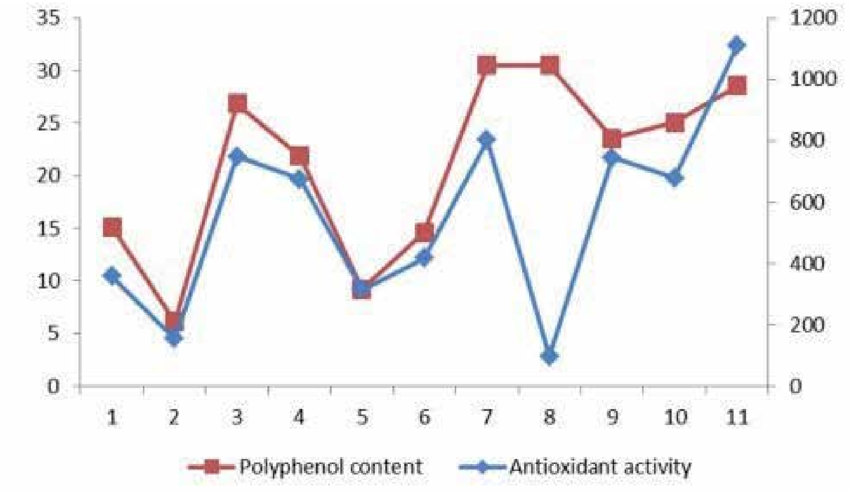


Figure 7. Polyphenol influence on antioxidant activity of honey samples of different botanical origin.

Most samples showed a correlation between polyphenol content and total antioxidant activity.

Exceptions are the samples of honey from the hawthorn where this correlation does not exist. Polyphenols also exhibit antioxidant activity in the presence of copper ions and prooxidant action. So, it can be assumed that the honey samples contain higher amounts of this micro biogenic element, as it affects the overall antioxidant activity.

5. Conclusion

Honey, as a natural food product, is known to be a significant source of antioxidants in the human body. The total antioxidant activity of honey is the result of its complex chemical composition and the complex interactions between different substances. There is a high degree of correlation among the total antioxidant activity and polyphenol content in honey. The chemical composition of honey is largely dependent on its botanical origin. The antioxidant activity of honey samples from different botanical origin is different. Nectarian honey samples are lower than forest honey as a source of antioxidants and have a lower content of polyphenols.

Influence of geographical origin of honey can be linked to the influence of botanical origin. Different plant cover is developed at different geographical locations and, therefore, under different climatic conditions. Therefore, the phytochemical profile present in honey, to a large extent, is a picture of the botanical and geographical origin of honey. The samples of honey from central Bosnia are the richest sources of antioxidants, followed by specimens from northeastern Bosnia - a slightly weaker source of antioxidants are samples of honey from the northern and western parts of Bosnia and Herzegovina.

Author details

Aldina Kesić*, Nadira Ibrišimović-Mehmedinović and Almir Šestan

*Address all correspondence to: aldina.kesic@untz.ba

University in Tuzla, Faculty of Science, Department of Chemistry, Bosnia and Herzegovina

References

[1] Ames B N, Shigenaga M, Hagen T. Oxidants, antioxidants, and the degenerative diseases of aging. Proc. Natl. Acad. Sci. USA. 1993; 90: 7915-7922.

[2] Gutteride JMC, Halliwell B. Free radicals and antioxidants in aging and disease; fact of fantasy. In Antioxidants in nutrition, heath, and disease. Oxford, UK: Oxford University Press. 1994; 111-135.

[3] Šeatović S. Istorijat antioksidanasa. Hemijski pregled. Galenika a.d. Insitut, Centar za bazne tehnologije. Beograd. 2005; 46:109-111.

[4] Briviba K, Sies H. Non enzymatic antioxidant defencesystems. In B. Frei (Ed). Natural antioxidant in human health and disease. New York, Academic Press. 1994; 107-128.

[5] Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properies of phenolic compounds. Trends in Plant Science. 1997; 2(4) 152-159.

[6] Weisburger JH. Mechanisms of antioxidants as exemplified in vegetables tomatoes and tea. Food and Chemical Toxicology. 1999; 37 (9/10): 943-948

[7] Halliwell B. How to characterize a biological antioxidant. Free Radic Res Commun. 1990; (9) 1–32.

[8] Rotillio G, Rossi L, de Martino A, Fereira A M C, Ciriolo MR. Free radicals, Metal ions and Oxidative Stress: Chemical Mechanisms of Damage and Protection in Living systems. Brazil. 1995; 221-227.

[9] Nikolič V. Oksidativna stabilnost olja navadnega rička. Ljubljana. 2006; 21-23.

[10] Al-Mamary M, Al-Meery A, Al-Habori M. Antioxidant activities and total phenolic of different types of honey. Nutrition Research. 2002; 22, 9:1041-1047

[11] Yasuko S, Michael F, Cohen A, Stephen C, Grace B, Hideo Y. Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals in plants Toxicology. 2002; 177: 67–80

[12] Weisburger JH. Mechanisms of antioxidants as exemplified in vegetables tomatoes and tea. Food and Chemical Toxicology, 1999; 37 (9/10): 943-948

[13] Meda A, Lamien CE, Romito M, Millogo L, Nacoulma OG. Determination of total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem. 2005; 91: 571-577.

[14] National Honey Board. Antioxidants and Honey - A "Radical" Discovery. 2004.

[15] http://www.aaccnet.org/FuncFood/content/releases/Honey-antioxodant.htm.

[16] Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. Int. J. Antimicrob. Ag. 2005; 26: 343-356.

[17] Turkmen N, Sari F, Poyrazoglu ES, Velioglu YS. Effects of prolonged heating on antioxidant activity and colour of honey. Food Chem. 2006; 95: 653-657.

[18] Beretta G, Granata P, Ferrero M, Orioli M, Facino RM. Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. Anal. Chem. Acta 2005; 533: 185-191.

[19] Anonymous. Flavonoids in honey. 2004; http://www.chemsoc.org/

[20] Gryglewski RJ, Korbut R, Robak J. On the mechanism of antithrombotic action of flavonoids. Biochemical Pharmacol, 1987; 36: 317-321.

[21] Frankel E. Nutritional benefits of flavonoids. International conference on food factors: Chemistry and cancer prevention, Hamamatsu, Japan. Abstracts. 1995; C 6-2.

[22] Husain SR, Cillard J, Cillard P. Hydroxyl radical scavenging activity of flavonoids, Phytochemistry. 1987; 26: 2489–2491.

[23] Foti M, Piatelli M, Baratta MT, Ruberto G. Flavonoids, coumarins and cinnamic acids as antioxidants in a micellar system. Structure-activity relationship. J. Agric. Food Chem. 1996; 44: 497-501.

[24] Bors W, Heller W, Michel C, Saran M. Flavonoids as antioxidants: determination of radical-scavenging efficiencies, Methods Enzymol. 1990; 186: 343–355.

[25] Halliwel B, Aeschbach R, Loliger J, Aruoma Ol. The characterization of antioxidants. Food Chem Toxicol 1995; 33: 601-17

[26] Jasna B, Urška D, Mojca J, Terezija G. "Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey" Analytical, Nutritional and Clinical Methods Food Chemistry. 2007; 2,105: 822-828

[27] Vraćar LJ. Priručnik za kontrolu svježeg i prerađenog voća, povrća i pećurki i osvježavajućih bezalkoholnih pića. Novi Sad. 2001;

[28] Šenk N. Vrednotenje medu: zveza med barvo medu in antioksidativno aktivnostjo. Univerzitet u Ljubljani, Biotehnički fakultet. 2006; 8-19.

[29] Andrade P, Ferreres F, Amaral MT. Analysis of honey phenolic acidsby HPLC, its application to honey botanical characterization. J. Liq. Chrom. Rel. Technol. 1997; 20(14): 2281-2288

[30] Igor J, Zvonimir M, Oak (Quercus frainetto Ten.) Honeydew Honey—Approach to Screening of Volatile Organic Composition and Antioxidant Capacity (DPPH and FRAP Assay, Molecules), 2010; 15: 3744-3756. doi:10.3390/molecules15053744

[31] Socha R, Juszczak L, Pietrzyk S, Fortuna T. Antioxidant activity and phenolic composition of herbhoneys. Food Chemistry. 2009; 113, 2: 568-574

[32] Al-Mamary M, Al-Meery A, Al-Habori M. Antioxidant activities and total phenolic of different types of honey. Nutrition Research, 2002; 22, 9: 1041-1047

[33] Busserolles J, Gueux E, Rock E, Mazur A, Rayssiguier Y. Substitutinghoney for refined carbohydrates protects rats from hypertriglyceridemicand prooxidative effects of fructose. J Agric Food Chem. 2002; 50: 5870–5877.

[34] Gheldof N, Engeseth NJ. Antioxidant capacity of honey from variousfloral sources based on the determination of oxygen radicalabsorbance and inhibition of in vitro lipoprotein oxidation in humanserum samples. J Agric Food Chem. 2002; 50:3050–3055.

[35] Schramm DD, Karim M, Schrader HR, Holt RR, Cardetti M, KeenCL. Honey with high levels of antioxidants can provide protectionto healthy human subjects. J Agric Food Chem. 2000; 51:1500–1505

[36] Manuela B, Manila C, Augusto A, Maria Piera P, Maria Cristina A. Raw Millefiori honey is packed full of antioxidants. Food Chemistry. 2006; 97, 2: 217-222

[37] Lucía V, de Lorenzo C, Rosa AP. Antioxidant capacity of Spanish honeys and its correlation with polyphenol content and other physicochemical properties; Journal of the Science of Food and Agriculture. 2007; 87, 6: Pages 1069 – 1075.

Section 2

Specific Bioactive Phytochemicals

Chapter 5

Coumarins — An Important Class of Phytochemicals

Maria João Matos, Lourdes Santana,
Eugenio Uriarte, Orlando A. Abreu,
Enrique Molina and Estela Guardado Yordi

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/59982

1. Introduction

Phytochemicals are chemical compounds that occur naturally in the plant kingdom. Some are responsible for the organoleptic properties of the natural sources in which they are present. The term is generally used to refer to those chemicals that may have biological significance, for example carotenoids, flavonoids, coumarins, or chromones, but not all are established as essential nutrients. There may be as many as 4,000 different phytochemicals having potential activity against several diseases such as cancer and metabolic or degenerative diseases.

Among them, coumarins are a family of benzopyrones (1,2-benzopyrones or 2H-1-benzopyran-2-ones) widely distributed in the nature. They represent an important family of naturally occurring and/or synthetic oxygen-containing heterocycles, bearing a typical benzopyrone framework (Figure 1) [1].

Figure 1. Chemical structure of coumarin and the IUPAC numeration of this scaffold.

The name coumarin comes from a French term for the Tonka bean, *coumarou*, seeds of *Dipteryx odorata* (*Coumarouna odorata*) (*Fabaceae/Leguminosae*), one of the sources from which coumarin was first isolated as a natural product in 1820. It has a sweet odor, easy to be recognized as the

© 2015 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

scent of new-mown hay; because of that, coumarin has been used in perfumes since 1882. It is presumed to be produced by plants as a chemical defense to discourage predation [2, 3].

Coumarinic compounds are a class of lactones structurally constructed by a benzene ring fused to α-pyrone ring, and essentially possess - conjugated system with rich electron and good charge-transport properties [4, 5]. The simplicity and versatility of the coumarin scaffold make it an interesting starting-point for a wide range of applications [6-8]. There are coumarins as perfumes, cosmetics, and industrial additives. Some of its derivatives have been used as aroma enhancers in tobaccos and certain alcoholic drinks [9, 10]. But their most relevant role is described in natural products, organic chemistry, and medicinal chemistry [11, 12]. The extraction, synthesis, and evaluation of coumarins have become an extremely attractive and rapidly developing topic [13, 14]. Moreover, a lot of coumarin compounds as medicinal candidates for drugs with strong pharmacological activity, low toxicity and side effects, fewer drug resistance, high bioavailability, broad spectrum, better curative effects, etc., to treat various types of diseases are being actively studied [15]. Several efforts have been made mainly in developing coumarin-based anticoagulant, antioxidant [16], antimicrobial (anti-viral, antifungal, and anti-parasitic) [10, 17], anticancer [18-20], anti-diabetic, analgesic, anti-neurodegenerative, and anti-inflammatory agents [10, 21]. Moreover, the unique and versatile oxygen-containing heterocyclic structure makes coumarin compounds occupy an important place in medicinal chemistry [22, 23]. In addition, studies have been done regarding coumarins as bioactive agents [24], as well as supramolecular medicinal drugs, diagnostic agents and pathologic probes, and biological stains [25]. Particularly, the large - conjugated system in the coumarinic ring, with electron-rich and charge-transport properties, is important in the interaction of this scaffold with molecules and ions. Coumarin-based ion receptors, fluorescent probes, and biological stains are growing quickly and have extensive applications to monitor timely enzyme activity, complex biological events, as well as accurate pharmacological and pharmacokinetic properties in living cells [26, 27].

Coumarin was first synthesized in 1868, and it was used in the pharmaceutical industry as a precursor in the synthesis of a number of synthetic anticoagulant pharmaceuticals, starting with dicoumarol (removed from the current therapy) [28]. So far, some interesting coumarin-based anticoagulant drugs have extensively been used in clinics [29]. Coumarins are a type of vitamin K antagonists [30]. The most notable ones are warfarin, acenocumarol, and phenprocoumon, currently in use in several countries [31, 32]. Warfarin is employed more frequently than acenocoumarol because of its longer half-life (36 h), theoretically providing more stable anticoagulation and avoiding factor VII fluctuations that potentially occur during acenocoumarol treatment (half-life 10 h) [33]. Nowadays, some coumarins proved to be enzymatic inhibitory agents [monoamine oxidase (MAO) inhibitors, acetylcholinesterase (AChE) inhibitors, and butyrylcholinesterase (BuChE) inhibitors] with great potential in neurodegenerative diseases (ND) [34-38]. These studies represent an important tendency in the coumarin's chemistry and biological evaluation [39-41].

Therefore, the coumarin ring is prevalently applied to construct several functional molecules in the medicinal field. A great deal of work has been done directed towards the separation and purification of naturally occurring biological coumarins from a variety of plants, animals, and

http://dx.doi.org/10.5772/59982

microorganisms, as well as towards the artificial synthesis of coumarin compounds with novel structures and properties [42]. Coumarin compounds as medicinal drugs have been increasingly attracting special interest due to their underlying outstanding contributions in the prevention and treatment of diseases, and the related researches and developments have become an extremely attractive highlighted area.

In this context, an overview of the role of coumarins as important phytochemicals and their interesting applications will be presented and discussed. The origin, natural sources, biosynthesis, and applications are going to be presented in this chapter.

2. Natural occurring coumarins

Coumarin (Figure 1) and its derivatives are an important group of natural compounds widely distributed in the natural kingdom [43]. They can be found in the integument of seeds, fruits, flowers, roots, leaves, and stems, although the largest concentration is generally in fruits and flowers [44]. Originally, coumarin was isolated from the seed of *D. odorata*. Coumarins are secondary metabolites of higher plants, few microorganisms (bacteria and fungi), and sponges [45]. The function of this type of end product of secondary metabolism is related to defense mechanisms against herbivores and attacks by microorganisms. These compounds are biosynthesized from phenylalanine via the shikimic acid [46]. Natural coumarins are generally unsaturated lactones and comprise another class of compounds C_6C_3. Almost all the natural coumarins have an oxygenated substituent at position 7 [47], either free as in hydroxylated umbelliferone, or combined (methyl, sugars, etc.) in other derivatives. Structurally, they are considered derivatives of the *ortho*-hydroxy-cinnamic acid.

There are different classifications for the coumarin derivatives. Generally, they can be chemically classified according to the most common cores: simple coumarins, complex coumarins, and various coumarins. More complex coumarins are generally fused with other heterocycles [3]. Therefore, they can be classified as: simple coumarins, furocoumarins, dihydrofurocoumarins, pyranocoumarins (linear and angular), phenylcoumarins, and biscoumarins [1]. As said before, hundreds of coumarins have been identified in natural sources, especially plants [48, 49]. Major coumarin constituents isolated from plants include: simple hydroxycoumarins, furocoumarins and isofurocoumarins, pyranocoumarins, biscoumarins, and dihydroisocoumarins (Figure 2) [1].

Coumarins have been isolated from hundreds of plants species distributed in more than 40 different families. There were isolated more than different 1300 coumarins, well distributed in *Angiospermae*, *Monocotyledoneae* and *Dicotyledoneae* families. Orders with occurrence numbers > 100 are *Araliales*, *Rutales*, *Asterales*, *Fabales*, *Oleales*, *Urticales*, and *Thymelaeales*. Families with occurrence numbers > 100 are *Apiaceae* (*Umbelliferae*), *Rutaceae*, *Asteraceae* (*Compositae*), *Fabaceae* (*Leguminosae*), *Oleaceae*, *Moraceae*, and *Thymelaeaceae*, respectively (Figure 3) [50]. The best known and researched coumarins in the field of phytochemistry, pharmacology, medicinal chemistry, and the food science can be found in these families. Therefore, these are the coumarins that are going to be further addressed in the next sections of this chapter.

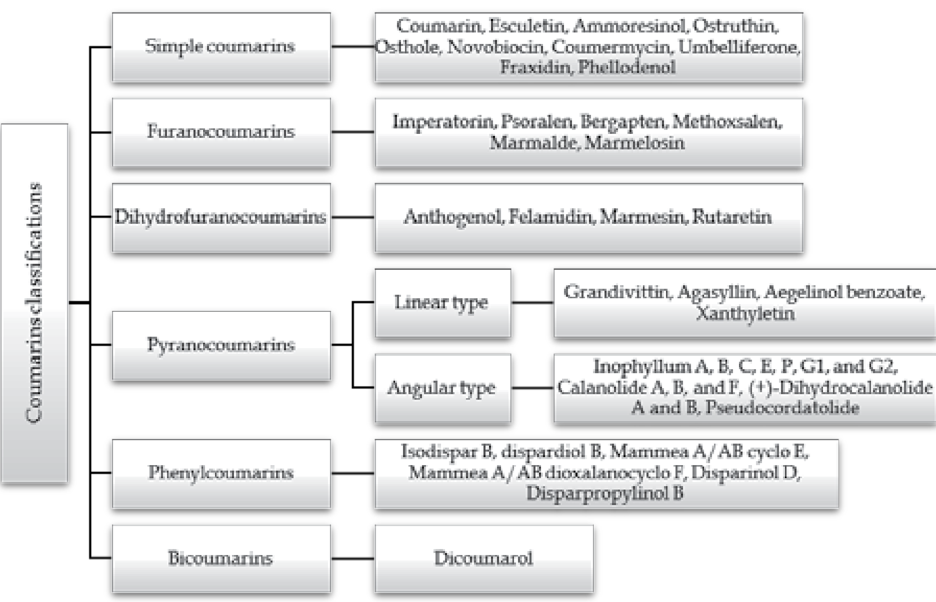


Figure 2. Principal types of coumarins isolated from plants.

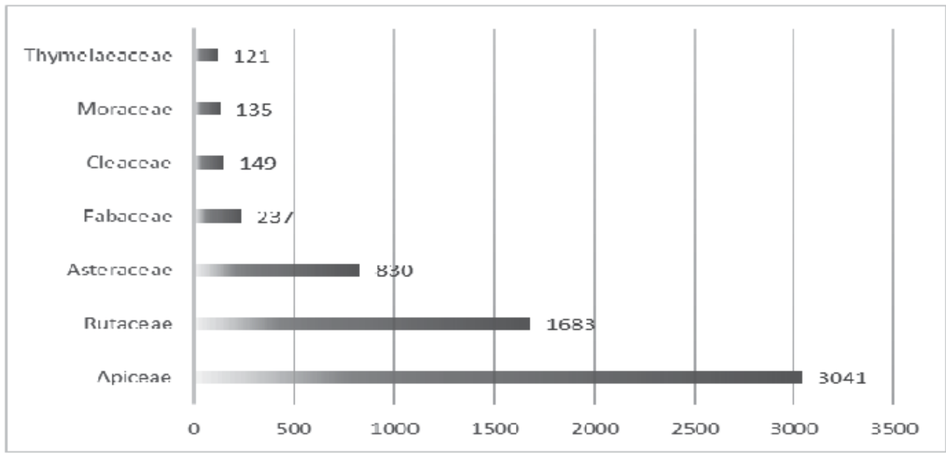


Figure 3. Number of coumarins presented in seven different families of plants[50].

Coumarins usually are in the free state in plants as they are polar structures, and many of them can sublimate. They might also be found in the form of glycosides, including psoralen core-related structures [44]. They are characterized by UV light absorption, resulting in a very characteristic blue fluorescence; they are also very photosensitive as they can be altered by natural light [44]. These features are used in the isolation and analysis, as well as in unusual therapies such as photochemotherapy and the industry of chemical sensors [51, 52].

http://dx.doi.org/10.5772/59982

3. Biosynthesis of coumarins

Simple coumarins are biogenetically derived from shikimic acid, via cinnamic acid. The specificity of the process is the C-2 hydroxylation, producing a break (β-oxidation) of the side chain (i.e. *Salix* spp.), or chain isomerization and subsequent lactonization, generating the umbelliferone. Figure 4 explains the entire process [46, 53].

Figure 4. Biosynthesis of simple coumarins.

Pyrano and furocoumarins (Figure 2) are also biogenetically derived from shikimic acid. These coumarins could be divided in two groups—lineal and angular—depending on the position where the isopentenyl pyrophosphate is condensed to further cyclize and form the heterocycle. The biosynthesis of these complex coumarins could also be the result of the cyclization of a simple coumarin previously prenylated [53].

Among the coumarins classified as "various" is the dicoumarol, which is formed by bacterial fermentation of Yellow Sweet Clover, and was isolated for the first time from decomposed leaves of *Melilotus albus* (*Fabaceae*/*Leguminosae*).

An approximation for the dicumarol biogenesis is the hydroxylation of the 4-position of the coumarin, that then captures a molecule of formaldehyde and is condensed with another molecule of 4-hydroxycoumarin, and finally enolize the keto group forming the dicumarol [46].

From a chemotaxonomic approach, Ribeiro & Kaplan (2002) evidenced that the diversity and structural complexity of the coumarins constitute an example of higher plant evolution. Simple coumarins are the most common in all angiosperms, especially in *Oleaceae* and *Asteraceae*, and their occurrence is of 100% and 98, 68%, respectively [50]. The second most prevalent coumarins are furocoumarins and pyranocoumarins. Coumarin in some families are high (*Thymelaeaceae*, *Rutaceae*, *Apiaceae*, *Fabaceae*, and *Moraceae*). In the case of well-diversified structural types in *Apiaceae* and *Rutaceae*, coumarins are considered as chemotaxonomic markers [50].

Apiaceae is the major source of coumarins (Figure 3) and one of the more diverse, containing five different types of coumarin derivatives (simple coumarins, lineal furocoumarins, angular furocoumarins, lineal pyranocoumarins, and angular pyranocoumarins) [50, 54]. *Rutaceae* is also highlighted in both occurrence and diversification. Generally, the division *Angiospermae* is preferably rich in simple coumarins, followed by the furo and pyranocoumarins [50].

4. Coumarins in medicinal plants

A large number of valuable species used commonly as medicinal plants, aromatic plants, and edible plants for human and animal feeding belongs to coumarin-rich plant families. Among them are species with well-documented biological activity, in which coumarins are part of the active principles. Table 1 shows a selection of plants of these families (first listed seven families with number of occurrence > 100) and some other families with species of particular pharmacological interest on chronic diseases. Coumarins presenting great pharmacological interest have been isolated in different geographical regions from other botanical families. Also shown are the coumarin compounds having species and their yield (if available).

Most of these plants are well known by people and scientists as part of herbal medicine repertories in Europe, Asia, or the Americas [55-58]. From the list, several coumarin-containing species or genera have also ethnomedical records in Cuba and the Caribbean Basin [59, 60]. Among of plant included are species with a great historical record of ethnomedicinal uses, and are present in *traditional medicine systems*: Ayurveda Medicine, Traditional Chinese Medicine and Unani Medicine, or in other recent cultures. Also, renowned species used on conventional therapeutics and modern herbal medicine are included, ie. *Aesculus hippocastanum* (Horse-chestnut), *Passiflora incarnata* (Passion Flower), *Lawsonia inermis* (Henna), *Hypericum perforatum* (Saint John Wort), *Tilia cordata* (Lime Tree) and *Uncaria tomentosa* (Cat's Claw).

Coumarins are also present in several species belonging to different botanical families, which are widespread in the northeastern region of Brazil [61]. Some of them are reported in folk medicine as traditional remedies drugs for the treatment of respiratory diseases [55]. Many pharmacological activities have been ascribed to coumarins such as anticlotting, hypotensive, antimicrobial, anti-inflammatory, and antitumor activities [61].

Recent studies and review manuscripts regarding the coumarin scaffold describe the huge variety of biological activities that may be present in the natural coumarins [8, 18, 62-64]. Venugopala et al. (2013) presented several coumarins displaying activities such as anti-inflammatory, anticoagulant, antibacterial, antifungal, antiviral, anticancer, anti-hypertensive, antitubercular, anticonvulsant, anti-adipogenic, Cytochrome P450 inhibiting, anti-hyperglycemic, antioxidant, and neuroprotective. Several recent reviews summarize and highlight advances in the application of coumarins, especially concerning their antioxidant and anticancer properties [62-70].From *Calophyllum* spp., it is remarkable the antiviral activity of calanolides and other related pyranocoumarins on Epstein-Barr virus and HIV [56]. As active compound of molluscicidal effects on *Biomphalaria glabrata* of *C. brasiliense* extracts were determined (-) mammea A/BB, also found in *C. Verticillatum* [71].

It is the great structural diversity of coumarinic compounds that allows for their several applications, and also allows for the high interest of these derivatives as phytochemicals. The pharmacological and biochemical properties and therapeutic applications of simple coumarins depend upon the pattern of substitution [68].

Family-specie (vernacular name)	Coumarin	Use*	Reference
Apiaceae/ Umbelliferae			
Ammi majus (*Bishop's flower*)	Imperatorin, bergapten, oxypeucedanin, pabulenol, marmesin, xanthotoxin, isopimpinellin and heraclenin.	M	[72]
A. visnaga (Pick-tooth, Toothpickweed)	PyranoCoumarins	M	[58, 73]
Anethum graveolens (Dill)	Aesculetin, bergapten, scopoletin	M, F	[60]
Angelica archangelica (Angelica)	Angelicin, osthol (major constituent in rhizome/ root at 0.2%), bergapten, imperatorin, isoimperatorin (major constituent in fruit), oreoselone, oxypeucedanin, umbelliferone, xantonin, xanthotoxin, xanthotoxol	M, F	[57, 58]
Apium graveolens (Celery)	Apigravin, apiumetin, apiumoside, bergapten, celerin, celereoside, isoimperatorin, isopimpinellin, osthenol, rutaretin, seselin, umbelliferone, 8-hidroxy-5-methoxypsoralen.	M, F	[57, 58]
Coriandrum sativum (Coriander)	Umbelliferone,	M, F	[58]
Cuminum cyminum (Cumin)	Escopoloetina, bergapten	M, F	[58]
Daucus carota subsp. *carota* (Wild Carrot)	8-methoxypsoralen, 5-methoxypsoralen (0.01–0.02 ug/g) in fresh plant, concentration increased in the disease plant.	M	[57]
Foeniculum vulgare (Fennel)	Umbelliferone, esculetin, bergapten, seselin, psoralen	M, F	[58]
Ferula assafoetida (Asafoetida)	Umbelliferone, coumarin-sesquiterpene complexes e.g. asacoumarin A and asacoumarin B.	M	[57]
Petroselinum crispum (Parsley)	Bergapten and oxypeucedanin as major constituent (up to 0.02% and 0.01%, respectively); also 8-metoxypsoralen, imperatorin, isoimperatorin, isopimpinellin, psoralen, xanthotoxin (up to 0.003%).	M, F	[57, 58]
Pimpinella anisum (Aniseed)	Scopoletin, umbelliferone, umbelliprenine, bergapten	M, F	[57, 58]
Trachyspermum ammi /*Carum copticum* (Ajwain)	Coumarins	-	[74]
Rutaceae			

Family-specie (vernacular name)	Coumarin	Use*	Reference
Aegle marmelos (Bael fruit)	Sesquiterpenic coumarin ethers, diterpenic coumarin ethers, triterpenic coumarin ethers, sesterterpenic coumarin ethers, auraptene, epoxyauraptene, marmin.	M, F	[75-77]
Citrus aurantium (Bitter Orange tree)	Volatile Coumarins (0.09%): aurapteno, auraptenol, bergapteno, bergaptol, escoparona, citropteno.	M, F	[58]
C. limonum (Lemon tree)	Escopoletin, umbelliferone, bergamotin, bergapten, bergaptol, citropten	M, F	[58]
C. sinensis (Orange tree)	Herniarin, scopoletin	M, F	[60]
Melicope spp.	Coumarins, chromones, dichromones	M	[56]
Murraya paniculata (*M. exotica*)(Orange Jessamine, Chinese box)	Coumarins	M	[74]
Paramygnya monophylla	Poncitrin, nordentatin	M	[56]
Stauracanthus perforates	Coumarins	M	[78]
Tetradium daniellii (*Euodia daniellii*)	Coumarins	M	[79, 80]
Toddalia aculeata (*T. asiatica*) (Orange climber)	Ulopterol	M	[74, 81]
Zanthoxilum americanum (Northern Prickly Ash)	Xanthyletin, xanthoxyletin, alloxanthoxyletin, 8-(3,3-dimethylallyl)-alloxanthoxyletin.	M	[57]
Z. syncarpum	Coumarins	M	[82]
	Asteraceae/Compositae		
Achillea millefolium (Yarrow)	Coumarins (0.35%)	M	[58]
Ageratum conyzoides (Mexican ageratum)	1-2 benzopirone	M	[83]
Arnica montana (Arnica)	Scopoletin, umbelliferone	M, F	[57, 58]
Chamaemelum nobile (Roman Chamomile)	Scopoletin-7-glucoside	M, F	[57, 58]
Cichorium intybus (Chicory)	Coumarins	M, F	[73]
Conyza sumatrensis (Fleabane)	Osthol	M	[56]
Eupatorium triplinerve (White Snakeroot)	Coumarins	M	[84]

Family-specie (vernacular name)	Coumarin	Use*	Reference
Hieracium pilosela (Mouse Ear)	Coumarins (0.2–0.6%): 7-glucosil- umbeliferone	M	[58]
Lactuca virosa (Wild Lactuce)	Aesculin, cichoriin	M	[58]
Matricaria recutita (Chamomille)	Umbelliferone and its methyl ether, heniarin.	M, F	[57, 58]
Mikania glomerata (Guaco)	Coumarins	M	[85]
Mikania hirsutissima	Coumarins	M	[86]
Fabaceae/Leguminosae			
Dipteryxodorata (*Coumarouna odorata*)(Tonka Bean, Coumaru)	Coumarins (35,000 ppm)	M	[84]
Euchresta formosana	Coumarins	M	[87]
Medicago sativa (Lucerne)	Cumestrol, medicagol, sativole, trifoliole, lucernole, dafnoretin.	M, F	[57, 58]
Melilotus officinalis (Yellow Sweet Clover)	Coumarins (0.4–1%)	M	[84] [58]
Glycyrriza glabra (Liquorice)	Glycyrin, heniarin, liqcoumarin, umbelliferone, GU-7 (3-arylcoumarin derivative)	M, F	[57, 58]
Myroxylon balsamum (Balsam Tolu)	Coumarins	M	[58]
Trigonella foenum-graecum (Fenugreek)	Coumarins	M, F	[57, 58]
Moraceae			
Dorstenia brasiliensis	Coumarins	M	[88]
Morus alba (White Mullberry)	Coumarins	M	[89]
Oleaceae			
Fraxinus excelsior (Common ash)	Fraxoside, esculoside, fraxinol, escopoletoside	M	[57]
Oleae europaea (Olive)	Coumarins	M, F	[90]
Thymelaeaceae			
Daphne feddei	feddeiticin (dicoumarinolignoid), dicoumarin glucosides	-	[91]
D. gnidium (Flax-leaved daphne)	daphnetin, daphnin, acetylumbelliferone, daphnoretin	-	[92]

Family-specie (vernacular name)	Coumarin	Use*	Reference
D. odora (Winter daphne)	daphnetin	-	[93]
D. oleoides	dimeric coumarin glycoside, trimeric coumarin fucosides, daphnetin,	-	[94, 95]
D. pedunculata	3-[(3-hydroxy-4-ethylpropanpicatephenyl)oxy]-6-methoxy-7-hydroxycoumarin	-	[96]
	Achanthaceae		
Justicia pectoralis (Tilo)	Coumarin, umbelliferone	M	[97]
	Araliaceae		
Eleutherococcus senticosus (Eleutherococcus)	Coumarins	M	[58]
	Brassicacae/Cruciferae		
Radicula armoracia (Horseradish root)	Aesculetin	M, F	[57, 58]
	Caryophylacae		
Herniaria glabra (Rupture wort)	(0.1-0.4%) umbelliferone, herniarin	M	[58]
	Caprifoliaceae		
Vivurnum prunifolium (American black haw)	Scopoletin (7-hidroxy-6-methoxicoumarin), scopolin, sculetin	M	[58]
	Clusiaceae/Guttiferae		
Calophyllum brasiliense (Guanandi, Ocuje)	volatile Coumarins, (-) mammea A/BB, brasimarins A, B, and C		[71, 98]
C. cerasiferum	(-) calanolide B	M	[56]
C. cordato-oblongum	Coumarins	M	[56]
Calophyllum inophyllum (Borneo mahogany)	Coumarins		[56]
Calophyllum lanigerum var *austrocoriaceum*	(+)- calanolide A	M	[56]
C. teysmannii var *inophylloide*	(-) calanolide B, sonlattrolide	M	[56]
C. verticillatum	mammea A/BB		[71]
	Connaraceae		
Connarus monocarpus	Bergenin1.5%	M	[56]
	Cupresaceae		

http://dx.doi.org/10.5772/59982

Family-specie (vernacular name)	Coumarin	Use*	Reference
Juniperus communis (Common Juniper)	Umbeliferone	M	[58]
Hippocastanacae			
Aesculus hippocastanum (Horse-chestnut)	Aesculetin, fraxin, scopolin, aesculetosides (glucosides)	M	[57, 58]
Hypericaceae			
Hypericum perforatum (Saint John Wort)	Umbelliferone, escopoletin,	M	[58]
Lamiaceae/Labiadae			
Lavandula angustifolia (Lavender)	Coumarins: 1,500 ppm, 0.25%: hernairin, santonin	M	[58, 84]
L. latifolia (Aspic)	Coumarins: 22 ppm	M	[84]
Lycopus europeus (European Bugle)	Coumarins: 1,200 ppm	M	[84]
Ocimum basilicum (Basil)	Aesculetin, aesculin	M, F	[60]
Salvia officinalis (Garden Sage)	Esculetin	M	[58]
Lauraceae			
Cinnamomum cassia (C. aromaticum) (Chinese cinnamon)	Coumarins	M, F	[57]
C. verum (*C. zeylanicum*) (Cinnamon)	Coumarins (0.65%)	M, F	[57, 58]
Laurus nobilis (laurel, sweet bay)	Coumarins	M, F	[57]
Persea americana (Avocado)	Scopoletin	M	[60]
Lytraceae			
Lawsonia inermis (Henna)	Coumarins	M	[58]
Meliaceae			
Trichilia hirta (Guabán)	Coumarins	M	[99]
Menianthaceae			
Menyanthes trifoliata (Buckbean)	Scoparone, brailin, scopoletin	M	[58]
Monimiaceae			

Family-specie (vernacular name)	Coumarin	Use*	Reference
Peumus boldus (Boldus)	Coumarins 125 ppm	M	[84]
	Passifloraceae		
Passiflora incarnate (Passion Flower)	Scopoletin, umbelliferone	M, F	[57, 58]
	Plantaginaceae		
Plantago major (Large Plantain)	Esculetin	M	[58]
	Poaceae (Graminae)		
Zea mays (Corn)	Coumarins: 2,000 ppm	F	[84]
	Rhamnaceae		
Zizyphus jujube (Jujube)	Coumarins: 3,000 ppm	M, F	[84]
	Rubiaceae		
Galium odoratum (*Asperula odorata*) (Woodruff)	Coumarins: 13,000 ppm	M	[84]
Uncaria tomentosa (Cat's Claw)	Coumarins	M	[100]
	Tiliaceae		
Tilia cordata (Lime tree)	Fraxosides, sculosides	M	[58]
	Urticaceae		
Urtica dioica (Nettle)	Scopoletin	M, F	[57, 58]

* M: Medicine, F: Food.

Table 1. Medicinal and food plant uses of some species from major coumarin-containing families.

5. Natural coumarins, non-nutrients presented in the food

Phytochemicals are defined as bioactive non-nutrient plant compounds presented in fruits, vegetables, grains, and other food plants that have been linked to reducing the risk of major chronic diseases. It is estimated that > 5,000 individual phytochemicals have been identified in fruits, vegetables, and grains, but a large percentage still remain unknown and need to be identified before we can fully understand the health benefits of phytochemicals in whole foods [101].

Phenolics are compounds possessing one or more aromatic rings with one or more hydroxy groups, and generally are categorized as phenolic acids, flavonoids, stilbenes, coumarins, and tannins [102]. Phenolics are the products of secondary metabolism in plants, providing

essential functions in the reproduction and the growth of the plants, acting as defense mechanisms against pathogens, parasites, and predators, as well as contributing to the color of plants [103]. In addition to their roles in plants, phenolic compounds in our diet may provide health benefits associated with reduced risk of chronic diseases. Among the 11 common fruits consumed in the United States, cranberry has the highest total phenolic content, followed by apple, red grape, strawberry, pineapple, banana, peach, lemon, orange, pear, and grapefruit [104]. Some of these fruits as important antioxidant and antiproliferative activities [104]. Among the 10 common vegetables consumed in the United States, broccoli possesses the highest total phenolic content, followed by spinach, yellow onion, red pepper, carrot, cabbage, potato, lettuce, celery, and cucumber [105]. Some of these vegetables proved to display interesting antioxidant and antiproliferative activities [105]. It is estimated that flavonoids account for approximately two thirds of the phenolics in our diet and the remaining one third are from phenolic acids [106].

Epidemiological studies have consistently shown that a high dietary intake of fruits and vegetables as well as whole grains is strongly associated with reduced risk of developing chronic diseases, such as cancer and cardiovascular disease [107-109]. Even if it is not so described in the bibliographic sources, most of the food plants, spice plants, and culinary herbs used regionally or worldwide are coumarin-containing plants, thus its effect on health cannot be ignored. For example, the potentially health-promoting role of popular vegetables and spices proved to be derived from *Apiaceae* [110]. Additionally, the above vegetables and spices also contain several bioactive phytochemicals such as flavonoids (quercetin, rutin) and coumarins (bergapten, isopimpinellin, xanthotoxin), which are reported to have curative, preventive, or nutritive value [84]. The above coumarins have also been found to inhibit multiplication of bacteria, fungi, and viruses [111] and demonstrated anti-allergy [112], anti-inflammation [113], and immunosuppression activities [114].

Table 1 also shows the importance of a number of families containing coumarins in human nutrition. Among other species of interest, the *Apiaceae* family is a prominent food source of coumarins: carrots, celery, parsley, coriander, cumin, fennel, and aniseed are present in the culinary practice around the world and in the food industry (fixative) [110]. *Rutaceae* also proved to contain a great number of coumarins with nutritional and economic interest, particularly the c*itrus* and some other fruits such as Bael [115]. Besides fruits and vegetables, olive oil and beverages such as coffee, wine, and black and green tea are also important dietary sources of coumarins [73].

It is also known that essential oils derived from some plants also contain coumarin derivatives and are used as flavoring in foods. Some essential oils such as Chinese cinnamon oil [116], cinnamon bark oil [117], and lavender oil [118] have important amounts of coumarins. Coumarin's aroma has been described as sweet, aromatic, creamy vanilla bean odor with nut-like tones that are heavy, but not sharp or brilliant [119]. A major source in alcoholic beverages is *Hierochloe odorata*, which is used to flavor a special kind of vodka, produced mainly in Eastern Europe [120].

According to Lake (1999), the main source of coumarin in human diet is the cinnamon. Cinnamon comes from the dried bark of *Cinnamomum verum* and *C. cassia*/*C. aromaticum*, and

is considered a spice. Cinnamon is widely used in various cultures in preparation of desserts, cakes, candy, etc., to decorate and some flavoring dishes. It is also used in some places as a beverage or tea. It is also an ingredient in many curries and other dishes Eastern. Intake levels [tolerable daily intake (TDI)] of coumarin derivatives are 0.1 mg/kg bw [121]. For food and beverages in general, the maximum permissible level is 2 mg/kg [122.

It has been estimated that human exposure to coumarins diet is approximately 0.02 mg/kg/day (Lake, 1999). The theoretical maximum daily intake (TAMDI) of coumarin via food was estimated to be 4.085 mg/day (0.07 mg/kg bw/day) [123].

Evidence has suggested that coumarin is not a genotoxic agent [121, 124]. The International Agency for Research on Cancer [125] has classified coumarin as belonging to group 3 (‘‘not classifiable as to its carcinogenicity in humans”). No epidemiological data relevant to the carcinogenicity of coumarin were available and there was only limited evidence in experimental animals for the carcinogenicity of coumarin [125].

The field of food science is of great interest to develop research related to consumer safety and also those designed to elucidate the potentially health-promoting capacity and biological activity of bioactive components that are part of so-called functional foods. However, the beneficial role of these phytocompounds when in synergy on the original food matrix or when isolated (nutraceutics or food supplement) is currently a hot topic [126].

Due to the structural diversity and versatility of applications of coumarins, not only in food sciences (including diet supplements), it is necessary to continue research related to the safety, and also its bioavailability, interactions with other dietary compounds, and therapeutic and environmental components. It is also important to amplify omics techniques, including epigenetic studies.

Different environmental insults can influence epigenetics and nutrition is one of the major factors that contribute to epigenetic regulation of diseases. Particularly, non-communicable diseases phenotypes can be determined by the role of prenatal or early-age nutrition and epimutations can have transgenerational effects, while some “epi-nutrients” and food products can also stabilize the genome [127, 128]. Therefore, the role of food-based “epi-bioactive” compounds has become an emerging field. Nutri-epigenomics is part of this new era too, since this approach on research has been carried out on chronic or degenerative diseases [129-133].

Besides micronutrients (folate, selenium, retinoic acid, and vitamins D and E) effects on epigenome, investigations on dietary phytochemicals has been carried out to determine their ability to reverse adverse epigenetic marks, mainly in cancer, for instance: polyphenols (resveratrol, curcumin, catechin, ellagitannin); genistein and soy isoflavones; sulfur-containing compounds (sulforaphane, phenylethyl isothiocyanat, phenylhexyl isothiocyanate, diallyldisulfide, allyl mercaptan) from *Allium* spp. (*Alliaceae*); and cruciferous (*Brassicaceae*) vegetables [129, 133].

Histone modifications are one of the major epigenetic mechanisms and its acetylation is mediated by the interplay of histone acetyltransferases (HATs) and histone deacetylases (HDACs). The class III HDACs, called sirtuins (SIRT), has been shown to deacetylate the

transcription factor p53. Given the regulatory functions of p53 in cell metabolism, inhibition of SIRT1 might contribute to inhibition of glycolysis and inhibit cell proliferation and the apoptosis [129, 133, 134].

Dihydrocoumarin (DHC), which is found in *Melilotus officinalis* (*Fabaceae*) (sweet clover) or synthetized, is commonly added as flavoring agent to food and cosmetics. This compound was studied by Olaharski et al. (2005), on different assays to evaluate it as an "epi-bioactive" agent and resulted that DHC inhibited the deacetylase activities of yeast SIRT2p and human SIRT1. DHC exposure in the human TK6 lymphoblastoid cell line also caused concentration-dependent increases in p53 acetylation, cytotoxicity, and flow cytometric analysis, demonstrating that DHC increased apoptosis more than 3-fold over controls [135].

Authors also stated that these findings on DHC could be potentially worrisome, since SIRT1 inhibition may lead to epigenetic alterations, as well as possible stem cell depletion and early tissue senescence, a phenotype associated with senescence and aging. However, on the possibility of deleterious human exposition to epigenetic toxicants that inhibit SIRT deacetylases, this effect can be desirable in cancer treatment mediated chemopreventive potential epigenetic mechanism [36, 129].

6. Extraction techniques and identification of coumarins

There have been a variety of methods described for extraction of coumarins. Generally, coumarins extraction can be performed either on dry or fresh material, with solvents of different polarities, depending on the type of structure. Some coumarins are sparingly soluble in apolar solvents and often they can be crystallized directly by cooling or concentrating the solvent.

Miranda and Cuéllar (2001), in their book entitled "Farmacognosia y Productos Naturales ", raised the possibility of following four variants using the Soxhlet method:

- Soxhlet extracts the dry powdered material with petroleum ether continuously for 3 days. The ether extract is concentrated until 1/5 of the original volume and it is cooled to obtain the crystallization of the extract. Coumarins are presented in the obtained solid.
- The dry material is removed and sprayed with ethanol, continuously using the Soxhlet for 2–3 days, at least. The extract is concentrated under vacuum to an oily residue. This residue is repeatedly washed with portions of hot water. The aqueous washings are combined and concentrated to the minimum volume, acidifying with hydrochloric acid solution 10%. The mixture is refluxed for 30 minutes. If some precipitate appears, it is filtered (hot filtration) and the solution is allowed to cool. The crystals are collected by filtration and found therein the coumarins.
- Available materials are extracted with ethyl ether or by successive macerations with the Soxhlet apparatus. The extract is concentrated to dryness and the residue contains the coumarins.

- The dried and ground plant material is extracted with acetone continuously in a Soxhlet apparatus, for at least 3 days, and the extract is concentrated to dryness giving a residue that contains the coumarins.

Purification and separation of coumarins contained in various extracts could be performed by using chromatographic columns, using as a carrier aluminum oxide and as solvent the eluotropic series: benzene-hexane (1:2.5); benzene; chloroform; chloroform-acetone, in proportions of a linear gradient to pure acetone [44]. For recognition of the described structures some trials were described, within which there are:

- Those that recognize coumarin's phenolic substitutions where Emerson's Reagent is used, developing color.

- The presence of lactone groups can be observed leading to changes of pH in the medium. When coumarins are dissolved in ethanol, solutions change the color when acidified (yellow color disappears).

- The furan ring can be recognized by using the Erlich test. The extract is treated with a solution of dimethylamino-benzaldehyde (5% ethanol), and then acidified by bubbling gaseous hydrochloric acid. The orange color indicates a positive test.

The last test is commonly used for phytochemical screening that is initially performed in plant research. Carrying on this approach, Payo et al., (1996), from 39 Cuban species screened, detected that 51.2% were positive for coumarin test.

Other extraction methods used in coumarins are: microwave, sonication, and supercritical fluid extraction (SFE) [136], these tests also propose capillary electrophoresis for natural products isolation.

Therefore, on the isolation and analysis of coumarins diverse methods have been used: chromatography (paper chromatography, thin layer chromatography, gas chromatography, and high-performance liquid chromatography), titrimetric, and spectrophotometric (colorimetric and polarographic) methods [1].

Due to coumarin-characteristic chromophore groups and its strong UV absorption at around 300 nm, it is routinely possible to be detected by feasible methods such as ultraviolet–visible spectroscopy (UV–vis) [137]. UV–vis detector is used in high performance liquid chromatography (HPLC) and also other hyphenated techniques are employed to characterize and quantify natural products such as Liquid Chromatography (LC)–Photo Diode Array Detector (PDA), coupling of Mass Spectrometry to LC–[137], or Ultra Performance Liquid Chromatography coupled with Mass Spectrometry (UPLC-MS) [138].

A simple spectroscopic technique [35] and HPLC [85] were employed to determine coumarins in the Brazilian medicinal plant "guaco", *Mikania glomerata* (*Asteraceae*). Coumarin HPLC detection is also used in Cuba to standardize a sedative herbal medicine based on *Justicia pectoralis* (*Acathanceae*) [97, 139].

http://dx.doi.org/10.5772/59982

7. Conclusion

Coumarins have been increasingly attracting special interest as phytochemicals due to their underlying outstanding contributions in the prevention and treatment of diseases. Coumarins represent a diverse class of phytochemicals that are ubiquitous in the human diet. Some of the medicinal usages of extracts of plants containing coumarins have been proven in experimental models, which suggested that the extracts possess various pharmacological actions. Several related researches and developments make coumarins an extremely attractive scaffold. The role of coumarins as important phytochemicals and their interesting applications were presented and discussed in this book chapter. The origin, natural sources, biosynthesis, and applications were also described.

Acknowledgements

The authors thank the partial financial support of University of Santiago de Compostela, University of Camagüey Ignacio Agramonte Loynaz and Galician Plan of research, innovation and growth 2011-2015 (Plan I2C, ED481B 2014/086-0)

Author details

Maria João Matos[1], Lourdes Santana[1], Eugenio Uriarte[1], Orlando A. Abreu[2], Enrique Molina[1,2] and Estela Guardado Yordi[1,2*]

*Address all correspondence to: estela.guardado@reduc.edu.cu

1 Faculty of Pharmacy, University of Santiago de Compostela, Spain

2 Faculty of Chemistry, University of Camaguey Ignacio Agramonte Loynaz, Cuba

References

[1] Venugopala KN, Rashmi V, Odhav B. Review on Natural Coumarin Lead Compounds for Their Pharmacological Activity. BioMed Research International. 2013;2013:1-14. DOI:10.1155/2013/963248.

[2] Borges F, Roleira F, Milhazes N, Santana L, Uriarte E. Simple coumarins and analogues in medicinal chemistry: occurrence, synthesis and biological activity. Curr Med Chem. 2005;12(8):887-916. DOI:10.2174/0929867053507315.

[3] Borges F, Roleira F, Milhazes N, Uriarte E, Santana L. Simple coumarins: Privileged scaffolds in medicinal chemistry. Front Med Chem Biol Inter. 2009;4:23-85. DOI: 10.2174/978160805207310904010023.

[4] Murray RDH. Naturally occurring plant coumarins. Progress in the Chemistry of Organic Natural Products. 1997;72:1-119. DOI:10.1007/978-3-7091-6527-0_1.

[5] Murray RDH, Mendez J, Brown SA. The natural coumarins occurrence. In Chemistry and Biochemistry. Chichester, UK: John Wiley and Sons; 1982. DOI: 10.1111/1365-3040.ep11611630.

[6] Matos MJ, Vazquez-Rodriguez S, Santana L, Uriarte E, Fuentes-Edfuf C, Santos Y, et al. Looking for new targets: simple coumarins as antibacterial agents. Medicinal chemistry. 2012;8(6):1140-5. DOI:10.2174/157340641120806114O.

[7] Qian L, Han X, Han H, Chen X, Yuan H. Research progress on coumarin and its derivatives. Guangzhou Huagong 2013;41(1):41-3.

[8] Zheng L, Zhao T, Sun L. Research progress of the pharmacological action and pharmacokinetics of coumarins. Shizhen Guoyi Guoyao. 2013;24(3):714-7.

[9] Fais A, Corda M, Era B, Fadda MB, Matos JM, Quezada E, et al. Tyrosinase Inhibitor Activity of Coumarin-Resveratrol Hybrids. Molecules 2009;14(7):2514-20 DOI: 10.3390/molecules14072514.

[10] Matos MJ, Vazquez-Rodriguez S, Santana L, Uriarte E, Fuentes-Edfuf C, Santos Y, et al. Synthesis and structure-activity relationships of novel amino/nitro substituted 3-arylcoumarins as antibacterial agents. Molecules 2013;18(2):1394-404. DOI:10.3390/molecules18021394.

[11] Chen Z. Coumarins In: Rensheng X, Yang Y, Weimin Z, editors. Introduction to Natural Products Chemistry2012. p. 205-24.

[12] Monga Paramjeet K, Sharma D, Dubey A. Comparative study of microwave and conventional synthesis and pharmacological activity of coumarins Journal of Chemical and Pharmaceutical Research. 2012;4(1):822-50.

[13] Bairagi SH, Salaskar PP, Loke SD, Surve NN, Tandel DV, Dusara MD. Medicinal significance of coumarins. International Journal of Pharmaceutical Research. 2012;4(2): 16-9.

[14] Batra N, Batra S, Pareek A, Nagori BP. Diverse pharmacological activities of 3-substituted coumarins: a review. International Research Journal of Pharmacy. 2012;3(7): 24-9.

[15] Wang H, Lu X, Yao H, Feng J, Liu R. Research progress on application of coumarin and its derivatives. Chemical Industry Times. 2009;23(8):40-3.

[16] Bubols GB, Vianna DR, Medina-Remon A, von Poser G, Lamuela-Raventos RM, Eifler-Lima VL, et al. The antioxidant activity of coumarins and flavonoids. Mini-Reviews in Medicinal Chemistry. 2013;13(3):318-34. DOI:10.2174/1389557511313030002.

[17] Matos MJ, Vina D, Vazquez-Rodriguez S, Uriarte E, Santana L. Focusing on new monoamine oxidase inhibitors: differently substituted coumarins as an interesting scaffold. Current Topics in Medicinal Chemistry (Sharjah, United Arab Emirates) 2012;12(20):2210-39. DOI:10.2174/1568026611212200008.

[18] Kapoor S. The anti-neoplastic effects of coumarin: an emerging concept. Cytotechnology 2013;65(5):787-8. DOI:10.1007/s10616-013-9538-6.

[19] Vazquez-Rodriguez S, Matos MJ, Santana L, Uriarte E, Borges F, Kachler S, et al. Chalcone-based derivatives as new scaffolds for hA3 adenosine receptor antagonists. Journal of Pharmacy and Pharmacology. 2013 65(5):697-703. DOI:10.1111/jphp.12028.

[20] Xia L, Wang Y, Huang W, Qian H. Research advance of anticancer drugs with coumarin structures. Chinese Journal of New Drugs. 2013;22(20):2392-404.

[21] Bansal Y, Sethi P, Bansal G. Coumarin: a potential nucleus for anti-inflammatory molecules. From Medicinal Chemistry Research. 2013;22(7):3049-60. DOI:10.1007/s00044-012-0321-6.

[22] Kontogiorgis C, Detsi A, Hadjipavlou-Litina D. Coumarin-based drugs: a patent review (2008 - present). Expert Opinion on Therapeutic Patents 2012;22(4):437-54. DOI: 10.1517/13543776.2012.678835.

[23] OKennedy R, Thornes RD. Coumarins. Biology, Applications and Mode of Action. New York: John Wiley and Sons; 1997.

[24] Matos MJ, Santana L, Uriarte E, Delogu G, Corda M, Fadda MB, et al. New halogenated phenylcoumarins as tyrosinase inhibitors. Bioorganic & Medicinal Chemistry Letters. 2011;21 (11):3342-5. DOI:10.1016/j.bmcl.2011.04.012.

[25] Song Y, Chen Z, Li H. Advances in coumarin-derived fluorescent chemosensors for metal ions. Current Organic Chemistry. 2012;16(22):2690-707. DOI: 10.2174/138527212804004544

[26] Chen G, Li H, Lan R, Li J. Research progress of coumarin-based fluorescent probes for ions of heavy and transition metals. Huaxue Tongbao. 2013;76(11):1002-10.

[27] Guha S, Dutta M, Das D. Multipurpose applications of coumarin derivatives with special emphasis on the fluorescent probes. Journal of the Indian Chemical Society. 2012;89(12):1603-32. DOI:10.1002/chin.201342230.

[28] Barcellona D, Vannini ML, Fenu L, Balestrieri C, Marongiu F. Warfarin or acenocoumarol: which is better in the management of oral anticoagulants? Thromb Haemost. 2008 100(6):1052-7.

[29] Gomez-Outes A, Suarez-Gea ML, Calvo-Rojas G, Lecumberri R, Rocha E, Pozo-Hernandez C, et al. Discovery of anticoagulant drugs: a historical perspective Current Drug Discovery Technologies. 2012;9(2):83-104. DOI:10.2174/1570163811209020083.

[30] Schalekamp T, de Boer A. Pharmacogenetics of oral anticoagulant therapy. Curr Pharm Des. 2010;16(17):187-203. DOI:10.2174/138161210790112737.

[31] Daly AK. Optimal dosing of warfarin and other coumarin anticoagulants: the role of genetic polymorphisms. Archives of Toxicology. 2013;87(3):407-20. DOI:10.1007/s00204-013-1013-9.

[32] Milatova E, Milata V. Warfarin - its synthesis and properties in a twenty year retrospective. Ceska a Slovenska Farmacie 2013;62(3):111-9.

[33] Beinema M, Brouwers JR, Schalekamp T, Wilffert B. Pharmacogenetic differences between warfarin, acenocoumarol and phenprocoumon. Thromb Haemost. 1998 80(6): 899-902. DOI:DOI: 10.1160/TH08-04-0116.

[34] Anand P, Singh B. A review on cholinesterase inhibitors for Alzheimer's disease. Archives of Pharmacal Research. 2013;36(4):375-99. DOI:10.1007/s12272-013-0036-3.

[35] da Silva I, de Moraes M, Vieira L, Corrêa A, Cass Q, Cardoso C. Acetylcholinesterase capillary enzyme reactor for screening and characterization of selective inhibitors. J Pharm Biomed Anal 2013;73:44-52. DOI:doi:10.1016/j.jpba.2012.01.026.

[36] Huang L, Su T, Li X. Natural Products as Sources of New Lead Compounds for the Treatment of Alzheimer's Disease. Current topics in medicinal chemistry. 2013;13(15):1864-78. DOI:10.2174/15680266113139990142 .

[37] Patil PO, Bari SB, Firke SD, Deshmukh PK, Donda ST, Patil DAF, (),. A comprehensive review on synthesis and designing aspects of coumarin derivatives as monoamine oxidase inhibitors for depression and Alzheimer's disease. Bioorganic & Medicinal Chemistry Letters. 2013;21(9):2434-50. DOI:10.1016/j.bmc.2013.02.017.

[38] Zhou X, Wang X, Wang T, Kong L. Design, synthesis, and acetylcholinesterase inhibitory activity of novel coumarin analogues. Bioorganic & Medicinal Chemistry. 2008;16:8011-21. DOI:10.1016/j.bmc.2008.07.068.

[39] Anand P, Singh B, Singh N. A review on coumarins as acetylcholinesterase inhibitors for Alzheimer's disease. Bioorganic & Medicinal Chemistry Letters. 2012;20(3): 1175-80. DOI:doi:10.1016/j.bmc.2011.12.042.

[40] Helguera AM, Perez-Machado G, Cordeiro MNDS, Borges F. Discovery of MAO-B inhibitors - present status and future directions part I: oxygen heterocycles and analogs. Mini-Reviews in Medicinal Chemistry. 2012;12(10):907-19. DOI: 10.2174/138955712802762301.

[41] Matos MJ, Santana L, Uriarte E, Serra S, Corda M, Fadda MB, et al. Tyrosine-like condensed derivatives as tyrosinase inhibitors. Journal of Pharmacy and Pharmacology. 2012 64(5):742-6. DOI:10.1111/j.2042-7158.2012.01467.x.

[42] Peng XM, Damu GLV, Zhou CH. Current developments of coumarin compounds in medicinal chemistry. Current Pharmaceutical Design. 2013;19(21):3884-930. DOI: 10.2174/1381612811319210013.

[43] Keating GJ, O'Kennedy R. The chemistry and occurrence of coumarins. O'Kennedy RTRD, editor. England: John Wiley & Sons West Sussex; 1997.

[44] Miranda M, Cuellar A. Farmacognosia y productos naturales. La Habana: Félix Varela; 2001.

[45] de Lira SP, Seleghim MHR, Williams DE, Marion F, Hamill P, Jean F, et al. A SARS-coronovirus 3CL protease inhibitor isolated from the marine sponge Axinella cf. corrugata: structure elucidation and synthesis. J Braz Chem Soc. 2007;18 (2). DOI: 10.1590/S0103-50532007000200030.

[46] Dewick PM. Medicinal Natural Products: A Biosynthetic Approach. 2a ed. England: John Wiley & Sons Ltd; 2002. DOI:10.1002/0470846275.

[47] Cai Y, Sun M, Xing J, Luo Q, Corke H. Structure-radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. Life Sci. 2006;78:2872-88. DOI:doi:10.1016/j.lfs.2005.11.004.

[48] Hoult J, Paya M. Pharmacological and biochemical actions of simple coumarins: natural products with therapeutic potential. General Pharmacology. 1996;27(4):713-22. DOI:10.1016/0306-3623(95)02112-4.

[49] Iranshahi M, Askari M, Sahebkar A, Hadjipavlou-Litina D. Evaluation of antioxidant, anti-inflammatory and lipoxygenase inhibitory activities of the prenylated coumarin umbelliprenin. DARU. 2009;17(2):99-103.

[50] Ribeiro CV, Kaplan MA. Tendências evolutivas de famílias produtoras de cumarinas em *angiospermae*. Quim Nova. 2002;25(4):533-8.

[51] Sakuma S, Yamashita S, Hiwatari K, Hoffman R, Pham W. Lectin-immobilized fluorescent nanospheres for targeting to colorectal cancer from a physicochemical perspective. Current Drug Discovery Technologies. 2011 8(4):367-78. DOI: 10.2174/157016311798109407.

[52] Zurita G, Briones M, Uraga E. Fotoquimioterapia en psoriasis: comparación de dos esquemas terapéuticos metotrexato más UVB vs. Puvaterapia. Revista Peruana de Dermatología. 2002;12(2).

[53] Bruneton J. Pharmacognosie -Phytochimie Plantes Médicinales. Tecnique et Documentation. 2e ed. Paris Lavoisier; 1993.

[54] Kontogiorgis C, Hadjipavlou-Litina DJ. Biological Evaluation of Several Coumarin Derivatives Designed as Possible Anti-inflammatory/Antioxidant Agents. Journal of

Enzyme Inhibition and Medicinal Chemistry. 2003;18(1):63-9. DOI: 10.1080/1475636031000069291.

[55] Correa PM. Diciona´rio de plantas u´ teis do Brasil e das exoticas cultivadas. Brazil: Ministério da Agricultura, Instituto Brasileiro de Desenvolvimento, Florestal; 1984.

[56] Lemmens RHMJ, Bunyapraphastara N. Plant resourses of South-East Asia. In: Lemmens RHMJ, Bunyapraphastara N, editors. Medicinal and poisonous plant. Leiden (Holanda): Backhuys; 2003.

[57] Newall C, Anderson L, Phillipson J. Herbal medicines. Aguide for health-care professionals. London: The pharmaceutical Press; 1996.

[58] Peris JB, Stübing G, Vanaclocha B. Fitoterapia aplicada. edicion r, editor. Valencia MICOF; 1995.

[59] Fuentes V, Exposito A. Las enecuestas botanicas sobre plantas medicinales en Cuba. Revista del Jardin Botanico Nacional. 1995;26:77-145.

[60] Robineau L. Famacopea vegetal caribeña. Martinique. F,W.I.: Ediciones Emile Désormeaux; 1997.

[61] Leal LKAM, Ferreira AAG, Bezerra GA, Matos FJA, Viana GSB. Anticonceptive, anti-inflammatory and bronchodilator activities of Brazilian medicinal plants containing coumarin: a comparative study. Journal of Ethnopharmacology. 2000;70 (2):151-9. DOI:10.1016/S0378-8741(99)00165-8.

[62] Kostova I. Synthetic and natural coumarins as cytotoxic agents. Current Medicinal Chemistry - Anti-Cancer Agents. 2005;5(1):29-46. DOI:10.2174/1568011053352550.

[63] Kostova I. Coumarins as Inhibitors of HIV Reverse Transcriptase. Current HIV Research. 2006;4(3):347-63. DOI:10.2174/157016206777709393.

[64] Kostova I. Studying plant derived coumarins for their pharmacological and therapeutic properties as potential anticancer drugs. Expert Opin Drug Discov. 2007;2(12): 1605-18. DOI:10.1517/17460441.2.12.1605.

[65] Fylaktakidou KC, Hadjipavlou-Litina DJ, Litinas KE, Nicolaides DN. Natural and synthetic coumarin derivatives with anti-inflammatory/antioxidant activities. Current Pharmaceutical Design. 2004;10(30):3813-33. DOI:10.2174/1381612043382710.

[66] Galvano F, Piva A, Ritieni A, Galvano G. Dietary strategies to counteract the effects of mycotoxins: A review. Journal of food protection. 2001;64(1):120-31.

[67] Hayes JD, Pulford DJ, Ellis EM, McLeod R, James RF, Seidegard J, et al. Regulation of rat glutathione S-transferase A5 by cancer chemopreventive agents: mechanisms of inducible resistance to aflatoxin B1. Chemico-Biological Interactions. 1998;111-112:51-67. DOI:10.1016/S0009-2797(97)00151-8.

[68] Kostova I, Mojzis J. Biologically active coumarins as inhibitors of HIV-1 (RT, IN and PR). Fut HIV Ther. 2007;1(3):315-29. DOI:10.2217/17469600.1.3.315.

[69] Rietjens IM, Martena MJ, Boersma MG, Spiegelenberg W, Alink GM. Molecular mechanisms of toxicity of important food-borne phytotoxins. Molecular Nutrition & Food Research. 2005;49(2):131-58. DOI:10.1002/mnfr.200400078.

[70] Riveiro ME, De Kimpe N, Moglioni A, Vazquez R, Monczor F, Shayo C, et al. Coumarins: Old Compounds with Novel Promising Therapeutic Perspectives. Curr Med Chem - Anti-Cancer Agents. 2010;17(13):1325-38. DOI:10.2174/092986710790936284.

[71] Gasparotto A, Brenzan M, Piloto I, García-Cortez D, Nakamura C, Dias-Filho V, et al. Estudo fitoquímico e avaliação da atividade moluscicida do Calophyllum brasiliense Camb (Clusiaceae). Quím Nova. 2005;28 (4):575-8. DOI:10.1590/S0100-40422005000400003.

[72] Rizk ET, Hassan SMM. Molluscicidal activity of furanocoumarins isolated from Ammi majus against Biomphalaria alexandrina snails. Egyptian Journal of Pharmaceutical Sciences. 2000;40(1):61-71.

[73] Sonnenberg H, Kaloga M, Eisenbach N, Fromming KK. Isolation and characterization of an angular-type dihydropyranocoumaringlycoside from the fruits of Ammi visnaga (L) Lam (Apiaceae). Zeitschrift für Naturforschung C. 1995;50(9-10):729-31. DOI:10.1515/znc-1995-9-1021.

[74] Shan B, Cai YZ, Sun M, Corke H. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. J Agric Food Chem 2005;53(20):7749-59. DOI:10.1021/jf051513y.

[75] Ahmad MZ, Ali M, Mir SR. New sester- and triterpenic coumarin ethers from the roots of Aegle marmelos (L.) Corr. Journal of Natural Product and Plant Resources 2012;2(6):636-43.

[76] Ahmad MZ, Ali M, Mir SR. New sesqui- and diterpenic coumarin ethers from the roots of Aegle marmelos (L.) Corr. Natural Products Journal. 2012;2(4):252-8. DOI: 10.2174/2210315511202040252.

[77] Mustahil NA, Riyanto S, Sukari MA, Rahmani M, Mohd nor SM, Ali AM. Antileukemic activity of extracts and constituents of Aegle marmelos. Research Journal of Chemistry and Environment. 2013;17(1):62-7.

[78] Setzer WN, Setzer MC, Schmidt JM, Moriarity DM, Vogler B, Reeb S, et al. Cytotoxic components from the bark of Stauranthus perforatus from Monteverde, Costa Rica. Planta Medica. 2000;66(5):493-4. DOI:10.1055/s-2000-8595.

[79] Ramesh B, Pugalendi KV. Antihyperlipidemic and antidiabetic effects of Umbelliferone in streptozotocin diabetic rats. The Yale journal of biology and medicine. 2005;78(4):189-96.

[80] Yoo SW, Kim JS, Kang SS, Son KH, Chang HW, Kim HP, et al. Constituents of the fruits and leaves of Euodia daniellii. Arch Pharm Res (Korea). 2002;25(6):824-30. DOI: 10.1007/BF02976999.

[81] Karunai Raj M, Balachandran C, Duraipandiyan V, Agastian P, Ignacimuthu S. Antimicrobial activity of Ulopterol isolated from Toddalia asiatica (L.) Lam.: A traditional medicinal plant. Journal of Ethnopharmacology. 2012;140 (1):161- 5. DOI:10.1016/j.jep.2012.01.005.

[82] Ross SA, Sultana GN, Burandt CL, El Sohly MA, Marais JP, Ferreira DJ. Syncarpamide, a New Antiplasmodial (+)-Norepinephrine Derivative from Zanthoxylum syncarpum. Journal of Natural Products. 2004;67(1):88-90. DOI:10.1021/np030417t.

[83] Ming LC. Ageratum conyzoides: A tropical source of medicinal and agricultural products. In: Janick J, editor. Perspectives on new crops and new uses. Alexandria, VA.: ASHS Press; 1999. p. 469-73.

[84] Duke JA. Handbook of phytochemical constituents of GRAS herbs and other economic plants. Boca Raton, FL: CRC Press; 2000.

[85] Radünz LL, Melo EC, Barbosa LCA, Rocha RP, Berbert PA. Rendimento extrativo de cumarina de folhas de guaco (Mikania glomerata Sprengel) submetidas a diferentes temperaturas de secagem. Rev bras plantas med. 2012;14 (3):453-7.

[86] Ohkoshi E, Makino M, Fujimoto Y. Studies on the constituents of Mikania hirsutissima (Compositae). Chemical & Pharmaceutical Bulletin. 1999;47(10):1436-8. DOI: 10.1248/cpb.47.1436.

[87] Lo WL, Chang FR, Liaw CC, Wu YC. Cytotoxic coumaronochromones from the roots of Euchresta formosana. Planta Medica. 2002;68(2):146-51. DOI:10.1055/s-2002-20248.

[88] Uchiyama T, Hara S, Makino M, Fujimoto Y. seco-Adianane-type triterpenoids from Dorstenia brasiliensis (Moraceae). Phytochemistry. 2002;60(8):761-4. DOI:10.1016/S0031-9422(02)00180-2.

[89] Oh H, Ko EK, Jun JY, Oh MH, Park SU, Kang KH, et al. Hepatoprotective and free radical scavenging activities of prenylflavonoids, coumarin, and stilbene from Morus alba. Planta Medica. 2002;68(10):932. DOI:10.1055/s-2002-34930.

[90] Ross IA. Olea europapaea L. In: A. RI, editor. Medicinal Plants of the World, Volume 3 Chemical Constituents, Traditional and Modern Medicinal Uses. TotowaNJ: Humana Press Inc; 2005. p. 273-400. DOI:10.1007/978-1-59259-887-8.

[91] Liang S, Shen Y-H, Tian J-M, Wu Z-J, Jin H-Z, Zhang W-D, et al. Three New Dicoumarins from Daphne feddei. HCA. 2009;92(1):133-8. DOI:10.1002/hlca.200800232.

[92] Cottiglia F, Loy G, Garau D, Floris C, Casu M, Pompei R, et al. Antimicrobial evaluation of coumarins and flavonoids from the stems of Daphne gnidium L. Phytomedicine. 2001;8(4):302-5.

[93] Yao L, Wang T, Wang H. Effect of soy skim from soybean aqueous processing on the performance of corn ethanol fermentation. Bioresource Technology. 2011;102(19): 9199-205. DOI:10.1016/j.biortech.2011.06.071.

[94] Riaz M, Malik A. Novel Coumarin Glycosides from Daphne oleoides. HCA 2001;84(3):656-61. DOI:10.1002/1522-2675(20010321)84:3<656::AID-HLCA656>3.0.CO; 2-D.

[95] Yesilada E, Taninaka H, Takaishi Y, Honda G, Sezik E, Momota H, et al. In vitro inhibitory effects of Daphne oleoides ssp. oleoides on inflammatory cytokines and activity-guided isolation of active constituents. Cytokine. 2001;13(6):359-64. DOI: 10.1006/cyto.2001.0838.

[96] Du J-L, Xu W-Z, Cheng X-R, Jin H-Z. A new coumarin from Daphne pedunculata. Journal Chemistry of Natural Compounds. 2013;49(3):426-7. DOI:10.1007/s10600-013-0629-6.

[97] Rodríguez JE, López OD, Gil JM. Método para la cuantificación de cumarina en extracto seco a partir de extractos de Justicia pectoralis Jacq. Revista Cubana de Plantas Medicinales. 2008;13(3).

[98] Oliveira M, Lemos L, de Oliveira R, Dall 'Oglio E, de Sousa Júnior P, de Oliveira Martins D. Evaluation of toxicity of Calophyllum brasiliense stem bark extract by in vivo and in vitro assays. Journal of Ethnopharmacology. 2014 155(1):30-8. DOI: 10.1016/j.jep.2014.06.019.

[99] Hernández E, Batista A, Portuondo D, Tamayo V, Mora N, H. MJ, et al. Immunorestorative in immunosuppressed Balb/c mice and cytotoxic activity of water extract from Trichilia hirta root. BLACPMA 2010;9(6):457-64

[100] Puhan M, Suarez A, Lo Cascio C, Zahn A, Heitz M, Braendli O. Didgeridoo playing as alternative treatment for obstructive sleep apnoea syndrome: randomised controlled trial. BMJ. 2006;332:266-70. DOI:10.1136/bmj.38705.470590.55.

[101] Liu RH. Health benefits of fruits and vegetables are from additive and synergistic combination of phytochemicals. Am J Clin Nutr. 2003;78(3):517S-20S.

[102] Spencer J, Abd el Mohsen M, Minihane A, Mathers J. Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research.. British Journal of Nutrition. 2008;99(01):12-22. DOI:10.1017/S0007114507798938.

[103] Bravo L. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev. 1998;56:317-33. DOI:10.1111/j.1753-4887.1998.tb01670.x.

[104] Sun J, Chu Y-F, Wu X, Liu RH. Antioxidant and antiproliferative activities of fruits. J Agric Food Chem. 2002;50:7449-54.

[105] Chu Y-F, Sun J, Wu X, Liu RH. Antioxidant and antiproliferative activities of vegetables. J Agric Food Chem. 2002;50(23):6910-6. DOI:10.1021/jf020665f.

[106] Tapiero H, Tew K, Ba G, Mathe G. Polyphenols: do they play a role in the prevention of human pathologies? Biomed Pharmacother. 2002;56(4):200-7. DOI:10.1016/S0753-3322(02)00178-6.

[107] Temple NJ. Antioxidants and disease: more questions than answers. Nutrition Research. 2000;20(3):449-59. DOI:10.1016/S0271-5317(00)00138-X.

[108] Willett WC. Diet and health: what should we eat. Science 1994;264(5158):532-7. DOI:10.1126/science.8160011.

[109] Willett WC. Balancing life-style and genomics research for disease prevention. Science 2002;296(5568):695-8. DOI:10.1126/science.1071055.

[110] Jaw-Ming Cherng Q, Wen Chiang A, Lien-Chai Chiang B. Immunomodulatory activities of common vegetables and spices of Umbelliferae and its related coumarins and flavonoids. Food Chemistry. 2008;106 (3):944-50. DOI:10.1016/j.foodchem.2007.07.005.

[111] Hudson JB, Graham EA, Harris L, Ashwood-Smith MJ. The unusual UVA-dependent antiviral properties of the furoisocoumarin, coriandrin. Photochemistry & Photobiology. 1993;57(3):491-6. DOI:10.1111/j.1751-1097.1993.tb02324.x.

[112] Kimura Y, Okuda H. Histamine-release effectors from Angelica dahurica var. dahurica root. Journal of Natural Products. 1997;60(3):249-51. DOI:10.1021/np960407a.

[113] Chen YF, Tsai HY, Wu TS. Anti-inflammatory and analgesic activities from roots of Angelica pubescens. Planta Medica. 1995;61(1):2-8. DOI:10.1055/s-2006-957987.

[114] Kuzel TM, Roenigk HH, Samuelson E, Rosen ST. Suppression of anti-interferon alpha-2a antibody formation in patients with mycosis fungoides by exposure to long-wave UV radiation in the A range and methosalen ingestion. Journal of the National Cancer Institute. 1992;84(2):119-21. DOI:10.1093/jnci/84.2.119

[115] Evans WC. Trease and Evans Pharmacognosy. 16th edition ed: Elsevier Ltd.; 2009.

[116] Choi J, Lee KT, Ka H, Jung T, Jung HJ, Park HJ. Constituents of the essential oil of the Cinnamomum cassia stem bark and the biological properties. Archives of Pharmacal Research. 2001;24(5):418-23. DOI:DOI: 10.1007/BF02975187.

[117] Bourgaud F, Hehn A, Larbat R. Biosynthesis of coumarins in plants: a major pathway still to be unravelled for cytochrome P450 enzymes. Phytochemistry Reviews. 2006;5(2-3):293-308. DOI:10.1007/s11101-006-9040-2.

[118] Rosselli S, Maggio AM, Faraone N. The cytotoxic properties of natural coumarins isolated from roots of Ferulago campestris (Apiaceae) and of synthetic ester derivatives of aegelinol. Natural Product Communications. 2009;4(12):1701-6.

[119] Clark GS. Coumarin. An aroma chemical profile. Perfumer & Flavorist,. 1995;20:23-34.

[120] Nykanen I, editor. The volatile compounds of Hierochloe odorata. Proceedings of the alko symposium on flavour research of alcoholic beverages; 1984 Helsinki, Finland: Foundation for Biotechnical and Industrial Fermentation Research.

[121] Lake BG. Coumarin metabolism, toxicity and carcinogenicity: relevance for human risk assessment. Food and Chemical Toxicology. 1999;37(4):423-53. DOI:10.1016/S0278-6915(99)00010-1.

[122] European Council. Council Directive of 22 June 1988 on the approximation of the laws of the member states relating toflavourings for use in foodstuffs and to source materials for their production. In: Communities OJotE, editor.1988.

[123] AFC. Opinion of the scientific panel on food additives, flavour-ings, processing aids and materials in contact with food (AFC) on a request from the Commission related to Coumarin. EFSA Journal. 2004;104:1-36. DOI:10.2903/j.efsa.2004.104.

[124] Beamand JA, Barton PT, Price RJ, G. LB. Lack of effect of coumarin on unscheduled DNA synthesis in precision-cut human liver slices. Food Chem Toxicol. 1998 36(8): 647-53. DOI:doi:10.1016/S0278-6915(98)00025-8.

[125] IARC. Coumarin. IARC monographs on the evaluation of carcinogenic risks to humans Some Industrial Chemicals. Lyon, France: International Agency for Research on Cancer; 2000. p. 193-226.

[126] Liu RH. Potential Synergy of Phytochemicals in Cancer Prevention: Mechanism of Action. J Nutr. 2004;134(12 Suppl):3479S-85S.

[127] Godfrey KM, Lillycrop KA, Hanson MA, Burdge GC. Epigenetic Mechanisms in the Developmental Origins of Adult Disease. In: H.I. R, R.O.C. O, F. B, editors. Epigenetic Aspects of Chronic Diseases. London Springer London; 2011. p. 187-204 DOI: 10.1007/978-1-84882-644-1_13.

[128] Mazzio EA, Soliman KFA. Epigenetics and Nutritional Environmental Signals. Integrative and Comparative Biology. 2014;54(1):21-30. DOI:10.1093/icb/icu049.

[129] Gerhäuser C. Cancer cell metabolism, epigenetics and the potential influence of dietary components - A perspective. Biomedical Research. 2012;23(1):1-21.

[130] Kontogiorgis CA, Bompou E-M, Ntella M, Berghe W. Natural Products from Mediterranean Diet: From Anti-Inflammatory Agents to Dietary Epigenetic Modulators. Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry. 2010;9(2):101-24. DOI:10.2174/187152310791110652.

[131] Milenkovic D, Vanden Berghe UGent W, Boby C, Leroux C, Declerck K, Szarc vel Szic K, et al. Dietary flavanols modulate the transcription of genes associated with cardiovascular pathology without changes in their DNA methylation state. PLOS ONE. 2014;9(4):e95527. DOI:10.1371/journal.pone.0095527.

[132] Schnekenburgera M, Dicatoa M, Diederich M. Plant-derived epigenetic modulators for cancer treatment and prevention. Biotechnology Advances. 2014;32(6):1123-32. DOI:10.1016/j.biotechadv.2014.03.009.

[133] Vanden Berghe W. Epigenetic impact of dietary polyphenols in cancer chemoprevention: Lifelong remodeling of our epigenomes. Pharmacological Research 2012;65(6): 565-76. DOI:10.1016/j.phrs.2012.03.007.

[134] Maulik N, Gautam M. Nutrition, epigenetic mechanisms, and human disease. Boca Raton, FL: CRC Press Taylor & Francis Group; 2011.

[135] Olaharski A, Rine J, Marshall BL, Babiarz J, Zhang L, Verdin E, et al. The Flavoring Agent Dihydrocoumarin Reverses Epigenetic Silencing and Inhibits Sirtuin Deacetylases. PLoS Genet. 2005;1(6):e77. DOI:10.1371/journal.pgen.0010077.

[136] Delazar A, Nahar L, Hamedeyazdan S, Sarker SD. Microwave-Assisted Extraction in Natural Products Isolation. In: Sarker S, Nahar L, editors. Natural Products Isolation, Methods in Molecular Biology. LLC: Springer Science+Business Media; 2012. p. 103-4. DOI:10.1007/978-1-61779-624-1.

[137] Sarker DS, Nahar L. An Introduction to Natural Products Isolation. In: Sarker DS, Nahar L, editors. Natural Products Isolation, Methods in Molecular Biology: Humana Press; 2012. p. 1-25. DOI:10.1007/978-1-61779-624-1.

[138] Dugrand A, Alexandre O, Thibault D, Alain H, Yann F, Bourgaud F. Coumarin and Furanocoumarin Quantitation in Citrus Peel via Ultraperformance Liquid Chromatography Coupled with Mass Spectrometry (UPLC-MS). Journal of Agricultural and Food Chemistry. 2013;61(45):10677–84. DOI:10.1021/jf402763t.

[139] Rodríguez JE, López OD, Núñez Y, Rodríguez C, Nogueira A. Obtención de extractos secos a partir de extractos acuosos de Justicia pectoralis (tilo). Revista Cubana de Plantas Medicinales. 2013;18(4).

Chapter 6

Quinolines, Isoquinolines, Angustureine, and Congeneric Alkaloids — Occurrence, Chemistry, and Biological Activity

Gaspar Diaz, Izabel Luzia Miranda and
Marisa Alves Nogueira Diaz

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/59819

1. Introduction

The alkaloids are a family of compounds widely found in nature. Therefore, they They are nitrogenous secondary metabolites heterocyclic derivatives of amino acids or by the transamination process, which confers basic character [1].

The term alkaloid, linguistically derived from the Arabic *al-quali* (ash plants), is used to designate pharmacologically active nitrogen compounds found predominantly in Angiosperms [2]. Their distribution is not uniform in that division. It is estimated that approximately 40% of all plant families have at least one plant species that contains alkaloids. They are easily found in Fabaceaes and Solanaceaes but are rare in Gymnosperms and Pteridophytes such as ferns and monocots [3]. Although most occur in plants, alkaloids have also been isolated from algae, insects, marine and land animals, microorganisms, and fungi [4].

Since the dawn of civilization, there are reports of the use of plant extracts containing alkaloids for various purposes. The death of Socrates in 399 BC, for example, was attributed to the consumption of the extract of *Conium maculatum* containing the alkaloid coniine. There are also reports that during the last century BC, Cleopatra employed extracts of *Hyoscyamus muticus* containing the alkaloid atropine to dilate the pupils to look more attractive. It is known that medieval European women employed extract from *Atropa belladonna* with the same goal. Moreover, 25% of contemporary medicines are plant-derived. For example, morphine is extracted from the poppy. It is a narcotic drug used as analgesic and marketed since 1827 by Merck [2].

© 2015 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Several alkaloids (papaverine, morphine, and cocaine) can cause different stimuli in the nervous system of an animal; a common phenomenon in ecological relationships may constitute, for example, a defense mechanism by plants against herbivores [1].

The importance of alkaloids in the development of medicine is unquestionable since several advances made in the battle against diseases like malaria, leukemia, cancer, and neurodegenerative diseases would not have occurred without the use of these substances [1].

This chapter focuses on the occurrence occurring, chemistry, and biological activity of the quinoline and isoquinoline alkaloids, including the angustureine and congeneric alkaloids.

2. Quinoline and isoquinoline alkaloids

Among the various classes of alkaloid compounds, this section highlights the quinoline and isoquinoline alkaloids. They were originally obtained from natural sources, whose remarkable biological activities and relatively simple structures have attracted great interest in the scientific community, especially researchers involved in the chemistry of natural products. However, these compounds have also attracted the interest of synthetic organic chemists due to the need to obtain increased amounts aimed at additional biological research, as well as in developing efficient synthetic routes for these alkaloids and their derivatives, whose chemical and biological properties could become greatly enhanced by the design of new structures from these modifications.

2.1. Methods of extraction of quinoline and isoquinoline alkaloids

The general method for extracting and isolating alkaloids from plants consists of an acid-base extraction. The dried and pulverized plant is extracted with organic solvents or with acidified water. When the extraction is carried out with organic solvents immiscible with water, the plant sample is alkalized prior to being extracted. For this, the sample is wrapped in filter paper, as a cartridge, humidified with dilute basic solution, e.g., ammonium hydroxide, and extracted under heating using Soxhlet apparatus with organic solvent such as ethyl ether, chloroform, or dichloromethane. After extraction, the solvent volume is reduced by half in rotary evaporator under reduced pressure. The remaining volume of the solvent containing the alkaloid residue is transferred to a separatory funnel and extracted with aqueous phosphoric acid solution (pH 1 to 2) or hydrochloric acid. The wetting of the plant sample with basic solution allows the alkaloids are released from organic acids, thus facilitating its extraction by organic solvents. Meanwhile, when using an acidified solution, alkaloids tend to form salts with these strong acids, facilitating their removal from the two-phase system, water-solvent for the separation of these into the aqueous phase [5].

Another extraction technique uses polar solvent such as ethanol, for example. However, if a plant contains a high amount of lipids, a preextraction with a nonpolar solvent such as hexanes or petroleum ether is necessary for removing these lipids. Next, the organic solvent is concentrated under reduced pressure and the residue solubilized in water [6]. As most alkaloids are

basic or are found in salt form, aqueous solutions of phosphoric acid (pH 1 to 2) or hydrochloric acid are used for their removal. Neutral amide alkaloids, such as colchicine and piperine (Figure 1), remain in the organic phase when treated with an organic solvent (usually ethyl acetate) during the extraction process, while most of the other alkaloids may be extracted using an organic solvent only after neutralization of the aqueous phase with a base [4].

Figure 1. Structure of the neutral amide alkaloids colchicine and piperine.

Distillation methods are rarely employed for isolating alkaloids. This is possible only in cases of low molecular weight alkaloids like coniine and sparteine (Figure 2) [4].

Figure 2. Structure of the alkaloids coniine and sparteine.

Generally, the crude extract is purified by column chromatography (CC), employing silica gel or alumina as the stationary phase and a mixture of appropriate solvents as the mobile phase, following an appropriate choice made via thin layer chromatography (TLC). Often, alkaloids still need additional purification by recrystallization, employing solvent systems such as ethanol/water, methanol/chloroform, or acetone/methanol [4].

While these techniques are successful to extract and to isolate most alkaloids, for those highly water soluble and, therefore, partially or totally insoluble in organic solvents, these methods are not appropriates [6].

Although generally higher solubility in aqueous solutions involves greater bioactivities [6], the employment of more suitable methods for the extraction of highly water-soluble alkaloids is very useful.

In the case of quaternary alkaloids, the residual aqueous solution should be tested with a reagent suitable for the detection of alkaloids (Dragendorff reagent, for example) after completion of conventional partition methods. If it is positive, one should employ methods such as direct crystallization, precipitation as insoluble salts, extraction with polar

solvent water-immiscible, or formation of pseudobases to extract the alkaloids from the aqueous phase [6].

Alkaloids *N*-oxides can be isolated by indirect methods or chemical derivatization, through a reaction of reduction to the corresponding tertiary basis and extracted using the conventional method, and subsequently regenerated by an oxidation reaction, using oxidizing agents such as peroxide hydrogen or *m*-chloroperbenzoic acid. In some cases, the *N*-oxides containing higher aliphatic chains can be almost completely extracted by increasing the basicity of the aqueous phase, but for those who have high levels of hydroxylation, it is infeasible [6].

The methods employed to extract most common polyhydroxylated alkaloids are the direct extraction and ion exchange chromatography [6].

After extraction of alkaloids, a quantitative analysis that may be performed by traditional methods such as simple weighing or base titration after dilution under acidic conditions is recommended. Other widely used techniques for the quantification of alkaloids are by employing the HPLC apparatus with UV detection and by testing the refractive index depending on the structure of the isolated compound(s) [5].

2.2. Occurrence in nature of quinoline and isoquinoline alkaloids

The quinoline and isoquinoline alkaloids were initially extracted from coal tar in 1834 and 1835, respectively. Quinoline, which has high boiling temperature, is commonly employed in organic synthesis as a solvent. Isoquinoline has a low melting temperature and both have moderate basicity (pK_a = 4.9 and 5.1, respectively) [7].

Although the vast majority of quinoline and isoquinoline alkaloids derived from flowered plants, they can also be isolated from animals and microorganisms, some representative examples are given in Table 1 [7].

Structure	Usual name	Source	Biological activity
	Argemonine	*Argemone* sp.	-
	Berberine	Family of *Berberidaceae*	Antiprotozoal Antimalarial Antibacterial Antidiarrheal
	Berlambine	*Thalictrum foliolosum*	-
	Camptothecine	*Camptotheca acuminata*	Topoisomerase inhibitor (anticancer agent)

http://dx.doi.org/10.5772/59819

Structure	Usual name	Source	Biological activity
H_3CO, OCH_3, N, O, O	Canadine	Family of *Berberidaceae*	-
H, HO, N, H, N	Cinchonine	*Cinchona* (tropical species belonging to the family Rubiaceae)	Antimalarial
H_3CO, O, H, NCH_3, HO	Codeine	*Opium*	Analgesic Antiexpectorant
OCH_3, N, O	Dictamnine	*Dictaminus albus*	hepatotoxicity (cytotoxicity to HepG2 cells)
H_3CO, H_3CO, H, N, H, CH_3, OCH_3, HN, OCH_3	Emetine	Family of Rubiaceae	Emetic Antiamebic
H_3CO, H_3CO, N, O, H_3CO	Erysotramidine	*Erythrina genus*	Antifeedant
H_3CO, H_3CO, N, H_3CO	Erythraline	*Erythrina genus*	Antifeedant
H_3CO, H_3CO, H, N, CH_3, H_3CO, OCH_3	Glaucine	*Glaucium genus*	Antiexpectorant
OCH_3, OH, O, O, H, N	Haemanthamine	*Narcisus confusus*	-
O, O, H, N, CH_3, H, O, O, H_3CO, OCH_3	Hydrastine	*Hydrastis canadensis*	Uterine hemostatic Antiseptic
H, CH_3, H, HO, N, H_3CO, N	Hydroquinine	*Cinchona* (tropical species belonging to the family Rubiaceae)	Depigmenting

Structure	Usual name	Source	Biological activity
	Jamtine	*Cocculus hirsutus*	Anti-hyperglycemic
	Lavendamycin	*Streptomyces levendulae*	Antitumor antibiotic
	Lephocereine	*Lephocereus schotti*	-
	Lycorine	*Lycoris radiata*	hypotensive
	Maritidine	-	-
	Morphine	*Opium*	Analgesic
	Nandinine	*Nandina domestica*	-
	Nantenine	*Nandina domestica*	α1 Adrenergic and serotonin antagonist 5-HT_{2A}
	Nitidine	*Zanthoxylum nitidum*	Topoisomerase inhibitor (anticancer agent)
	N-norlaudanosine	-	-
	Palmitine	*Jateorhiza palmate*	-

Structure	Usual name	Source	Biological activity
	Papaverine	*Opium*	Vasodilator
	Pellotine	*Lophophora williamsii*	-
	Protoemetine	*Alangium lamarckii*	-
	Protoemetinol	*Alangium lamarckii*	Emetic Antiamebic Anticancer
	Psychotrine	*Uragoga ipecacuanha*	Inhibitor of HIV-1 transcriptase
	Quinidine	*Cinchona* (tropical species belonging to the family Rubiaceae)	Antiarrhythmic Antimalarial
	Quinine	*Cinchona* (tropical species belonging to the family Rubiaceae)	Antimalarial Muscle relaxant
	d-Quinotoxine	-	-
	Reticuline	*Anona reticulate* *Opium*	-
	Sandramycin	*Norcardioides* sp. (microorganism)	Antitumor antibiotic

Structure	Usual name	Source	Biological activity
	Skimmianine	*Skimmia japoinica*	Sedative Anticonvulsant
	Sanguinarine	*Sanguinaria*	Antibacterial
	Stepholidine	*Stephania intermedia*	D_1 receptor agonist D_2 receptor antagonist
	Streptonigrin	*Streptomyces flocculus*	Antitumor antibiotic
	Tetrahydropa-lmitine	*Corydalis family*	Analgesic
	Thalictricavine	*Corydalis tuberosa*	-
	Tubocurarin	*Chododendron tomentosum*	Neuromuscular blocking agent

Table 1. Quinoline and isoquinoline alkaloids: natural sources and biological activities.

2.3. Biosynthetic origin of quinoline and isoquinoline alkaloids

The most One of the systems used for the classification of alkaloids is based on the kind of nitrogen heterocycle in the structure and its botanical origin (plant family which is extracted); thus, the name given to an alkaloid derived from the genus or species of organism produced, including the term "ine." Another commonly used system takes into account its precursor amino acid. Thus, alkaloid derivatives of tryptophan and tyrosine are biosynthetically classified as quinoline and isoquinoline, respectively (Figure 3) [3].

Thus, quinoline alkaloids are derived from 3-hydroxyanthranilic acid, a metabolite formed from tryptophan through a sequence of enzymatic reactions (Figure 4). The condensation between 3-hydroxyanthranilic acid and malonyl-SCoA, followed by cyclization, produces quinoline alkaloid (Figure 4) [8-10].

http://dx.doi.org/10.5772/59819

Figure 3. Structure of quinoline and isoquinoline alkaloids.

Figure 4. Biosynthesis of quinoline alkaloids.

In the case of isoquinoline systems, the biosynthesis proceeds from the cyclization of the Schiff base formed between the dopamine and an aliphatic aldehyde [8]. Dopamine is obtained from the hydroxylation and decarboxylation of tyrosine. A second intermediate, *p*-hydroxyphenyl acetaldehyde, can also be formed by transamination, decarboxylation, and hydroxylation of tyrosine. The condensation of these intermediates followed by a sequence of steps (cyclization, hydroxylation, and methylation) produces the (*S*)-reticuline, a biosynthetic intermediate of all isoquinoline alkaloids (Figure 5) [11].

2.4. Biological activities of quinoline and isoquinoline alkaloids

In addition to the biological activities shown in Table 1, many quinoline alkaloids and their analogues represent the most important drugs currently used to combat malaria, with chloroquine, amodiaquine, piperaquine, primaquine, quinine, and mefloquine (Figure 6)

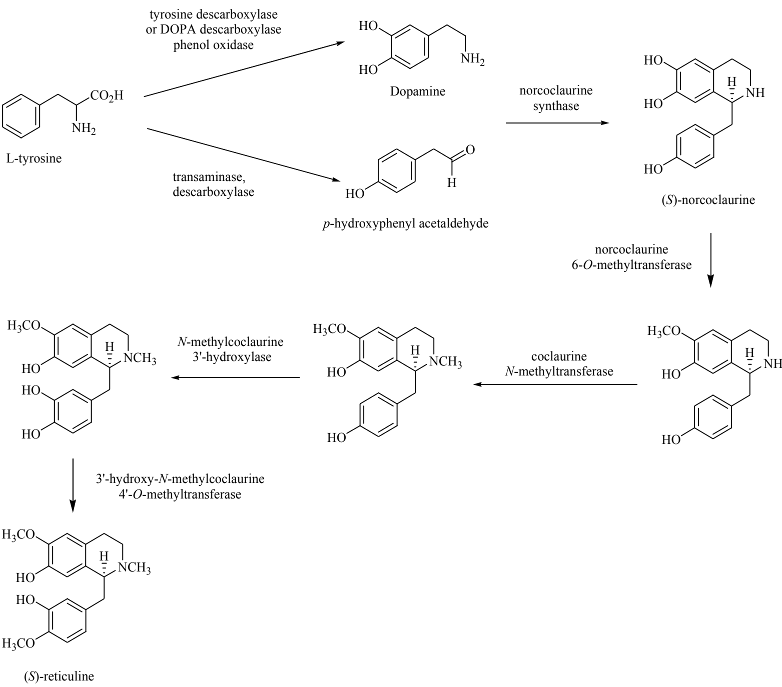


Figure 5. Biosynthesis of isoquinoline alkaloids.

being the most representative drugs of this group [12]. Even today, many studies are still being conducted to increase the efficiency of quinoline derivatives such as AZT-chloro-quinoline, quinine-dihydroartemisinin, and MEFAS, a salt derived from mefloquine and artesunate [13] (Figure 7).

Figure 6. Structure of some quinoline alkaloids and their analogues.

http://dx.doi.org/10.5772/59819

AZT-chloroquinoline

quinine-dihydroartemisinin

MEFAS

Figure 7. Structure of other quinoline derivatives.

On the other hand, the quinoline nucleus has also demonstrated a critical role in the development of new anticancer drugs. Some of its derivatives showed excellent results on various types of cancer cells, through different mechanisms of action. Recently, three protein kinase inhibitors (Bosutinib, Lenvatinib, and Cabozantinib) and an inhibitor of farnesyltransferase (Tipifarnib) (Figure 8), considered as potential anticancer agents, entered into phase of clinical trials [14].

bosutinib

lenvatinib

cabozantinib

tipifarnib

Figure 8. Structure of bosutinib, lenvatinib, cabozantinib, and tipifarnib quinoline nucleus.

Knowing that functionalized quinolines have also shown high anti-carcinogenic potential, in addition to acting as anti-angiogenic agents, and inhibitors of telomerase in various human tumor cells, Muñoz and coworkers [15] selected the DM8 and DM12 tetrahydroquinolines (Figure 9) for testing cytotoxic activity. They observed that individually these compounds significantly inhibited cell growth of breast cancer, and when tested concomitantly with other two anticancer drugs, they work synergistically to increase their cytotoxic activities. The results obtained for the DM12 quinoline made this compound a promising candidate as a new adjunctive anticancer agent.

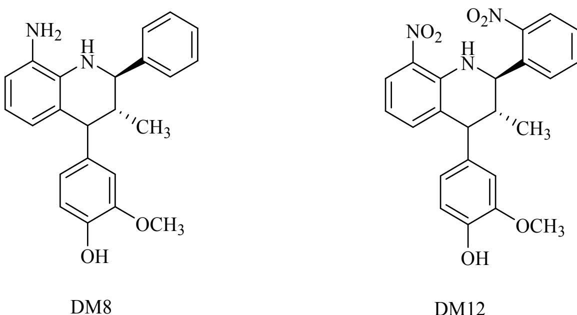


Figure 9. Structure of the DM8 and DM12 tetrahydroquinoline alkaloids.

Furthermore, Villemagne and Okamura [16] obtained promising results with other quinoline derivatives used as selective ligands for Tau proteins, associated with brain damage, which represent a risk factor for diseases such as Alzheimer's, which allowed the investigation of the causes, diagnosis, and treatment of neurodegenerative diseases, encephalopathy, and traumatic brain injury.

Biological activities of hundreds of other substituted quinolines have been reported, many of which are promising in terms of their potential as pharmaceutical agents. 2-Methyl-5-hydroxy-1,2,3,4-tetrahydroquinoline exhibits analgesic activity with a potency one-eighth that of morphine [17]. 1,2,3,4-Tetrahydroquinoline-4-carboxylic acid is used in tissue-irrigating solutions [18]. A wide array of other biological activities have been reported, including *inter alia* inhibition of (H^+/K^+)-ATPase [19], blood serum monoamine oxidase [20], angiotensin I converting enzyme [21], lipoxygenase [22], lipid peroxidation [23], bone resorption [24], leukotriene synthesis [25, 26], and bacterial dihydrofolate reductase [27]. Relevance to many other indications has also been noted [28-54].

In addition to the numerous utilities in the pharmacological area, studies are being performed in order to investigate the potential activities of these alkaloids as agrochemical agents. Sanguinarine and chelerythrine (Figure 10), for example, have shown high activity against fungi and phytopathogenic bacteria *in vitro*, with sanguinarine being the most effective against *Rhizoctonia solani* fungus, which has a wide range of hosts, which causes disease in most plants grown in the world [55].

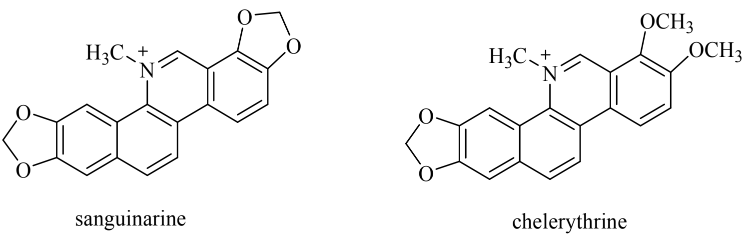


Figure 10. Structure of the sanguinarine and chelerythrine agrochemical agents.

http://dx.doi.org/10.5772/59819

3. Tetrahydroquinoline alkaloids angustureine, galipeine, galipinine, and cuspareine

Among the simplest natural alkaloids that contain the 1,2,3,4-tetrahydroquinoline core are angustureine and its congeners, galipeine, cuspareine, and galipinine (Figure 11). The structures are more narrowly described as 2-alkyl-1-methyl-1,2,3,4-tetrahydroquinolines. They are chiral by virtue of the stereogenic center at position 2. There is an excellent review of the chemistry and synthesis of 1,2,3,4-tetrahydroquinolines by Diaz and Dudley [56].

Figure 11. Structure of the 1,2,3,4-tetrahydroquinolin alkaloids angustureine, galipeine, cuspareine, and galipinine.

In 1999, Jacquemond-Collet and coworkers [57] isolated and characterized these four tetrahydroquinoline alkaloids (Figure 11) from the extract of the bark of *Galipea officinalis*, commonly known as "angostura" [58]. The genus *Galipea* comprises about 20 species that are found predominantly in the Northern of South America. *G. officinalis* is a shrub known by rural communities in the Venezuelan mountains for their pharmacological activities, and its extract is used in the control of dyspepsia, dysentery, and chronic diarrhea, in addition to presenting antimalarial, cytotoxic [59], molluscicidal activities, and antimicrobial properties, inhibiting the growth of *Mycobacterium tuberculosis*, the etiological agent of tuberculosis [60].

In later trials, galipinine and galipeine exhibited promising biological activities *in vitro* such as antimalarial (IC_{50}: 0.24-6.12 and 0.33-13.78 mM, respectively) for the protozoa species *Plasmodium falciparum*, one of the species causing malaria that is already resistant to chloroquine, one of the main drugs used to combat the disease [61].

Due to their structural simplicities associated with the promising pharmacological activities, these alkaloids, especially angustureine, have attracted the attention of the synthetic organic community. For this alkaloid alone, more than 24 different syntheses have already been described [62-85]. Some recent examples of the syntheses of these alkaloids are described below.

Diaz *et al*. [83] described the enantioselective synthesis of both alkaloids (*R*)-(-)- and (*S*)-(+)-angustureine with overall yields of 80% and 44%, respectively, and excellent enantiomeric excesses (95% and 96%, respectively), starting from the (*S*)-β-amino ester and (*R*)-sodium carboxylate, prepared following enzymatic resolution of the β-amino ester racemate [86, 87].

Foubelo *et al*. [85] described a second-generation synthesis of (-)-angustureine by way of 2-allyl-tetrahydroquinoline. A further sequence of three steps from this intermediate provided angustureine in 36% overall yield. However, 2-allyl-tetrahydroquinolines can serve as common precursors to various 2-substituted tetrahydroquinoline alkaloids by making use of the cross-metathesis reaction with the Hoveyda-Grubbs reagent (second-generation ruthenium catalyst) to achieve the appropriate side-chain homologation events.

In turn, Lam *et al*. [88] synthesized analogues of tetrahydroquinoline alkaloids employing asymmetric hydrogenation catalyzed by iridium. These compounds were tested *in vitro* for antitumor activities against lung cancer, breast cancer, hepatocellular carcinoma, and sarcoma cancer and have achieved remarkable results, as well as *in vivo*, using mice as animal models receiving hepatocellular tumor grafts, being that 1,2,3,4-tetrahydroquin-8-ol (Figure 12) revealed the highest toxicity to human cancer cells.

N
H
OH
1,2,3,4-tetrahydroquin-8-ol

Figure 12. Structure of 1,2,3,4-tetrahydroquin-8-ol anticancer agent.

4. Concluding remarks

This chapter is an illustration of the fascinating world of chemistry of quinoline and isoquinoline alkaloids. The range of pharmacological and agrochemical activities, among others, associated with their biosynthetic routes and various natural sources demonstrates the creative universe of the nature of these alkaloids. This universe of compounds arouses interest to organic chemists in general, stimulated by the simplicity and its varied forms of exploitation throughout constant search for the discovery of novel pharmacological agents targeting the treatment of several diseases that affect society.

5. Future directions

In this chapter, we can verify the importance of quinoline and isoquinoline alkaloids due to their potent biological activities demonstrated by *in vitro* and *in vivo* assays, as well as

http://dx.doi.org/10.5772/59819

agrochemical agents reported throughout this chapter. Based on these facts, this family of compounds is presented as an inexhaustible source for research groups working with natural and synthetic products for the pharmaceutical area. In this context, our group intends to continue the study of these alkaloids with the synthesis of novel quinoline and isoquinoline derivatives, aimed at the discovery of new pharmacological agents.

Acknowledgements

The authors are grateful to CAPES and FAPEMIG for their financial support.

Author details

Gaspar Diaz[1*], Izabel Luzia Miranda[2] and Marisa Alves Nogueira Diaz[3]

*Address all correspondence to: gaspardm@qui.ufmg.br

1 Departament of Chemistry, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

2 Departament of Chemistry, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil

3 Departament of Biochemistry and Molecular Biology, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil

References

[1] Aniszewski T. Alkaloids—Secrets of Life. Amsterdam: Elsevier Academic Press; 2007.

[2] Kutchan TM. Alkaloid biosynthesis—the basis for metabolic engineering of medicinal plants. Plant Cell 1995;7(7) 1059-1070.

[3] Ruiz PG. Productos Naturales. Pamplona: Universidad Pública de Navarra; 2002.

[4] Mann J, Davidson RS, Hobbs JB, Banthorpe DV, Harborne JB. Natural Products: Their Chemistry and Biological Significance. London: Longman Scientific & Technical; 1996.

[5] Simões CMO. Farmacognosia—Da planta ao medicamento. Editoras UFRGS e UFSC; 2010.

[6] Colegate SM, Molyneus RJ. Bioactive Natural Products: Detection, Isolation and Structural Determination. Boca Raton, Florida: CRC Press; 1993.

[7] Majumdar KC, Chattopadhyay SK. Heterocycles in Natural Product Synthesis. Singapura: Wiley-VCH; 2011.

[8] Mann J. Secondary Metabolism. Oxford: Clarendon Press; 2005.

[9] Souza MVN, Almeida MV, Hyaric M, Cardoso SH, Amarante WG. Métodos de preparação e atividade biológica do ácido quinolínico e derivados. Química Nova 2003;26(5) 694-698.

[10] Sultana N. Medicinal properties and biosynthetic studies on indigenous medicinal plant *Skimmia laureola*. Critical Review in Pharmaceutical Sciences 2013;2(2) 13-42.

[11] Civjan N. Natural Products in Chemical Biology. Hobokey, New Jersey: John Wiley & Sons; 2012.

[12] Kaur K, Jain M, Reddy RP, Jain R. Quinolines and structurally related heterocycles as antimalarials. European Journal of Medicinal Chemistry 2010;45(8) 3245-3264.

[13] Boechat N, Ferreira MLG, Pinheiro LCS, Jesus AML, Leite MMM, Junior CCS, Aguiar ACC, Andrade IM, Krettli AU. New Compounds Hybrids 1*H*-1,2,3-Triazole-Quinoline Against *Plasmodium falciparum*. Chemical Biology and Drug Design 2014;84(3) 325-332.

[14] Afzal O, Kumar S, Haider MR, Ali MR, Kumar R, Jaggi M, Bawa S. A review on anticancer potential of bioactive heterocycle quinoline. European Journal of Medicinal Chemistry 2014. DOI: 10.1016/j.ejmech.2014.07.044. *In press.*

[15] Muñoz A, Sojo F, Arenas DRM, Kouznetsov VV, Arvelo F. Cytotoxic effects of new trans-2,4-diaryl-r-3-methyl-1,2,3,4-tetrahydroquinolines and their interaction with antitumoral drugs gemcitabine and paclitaxel on cellular lines of human breast cancer. Chemico-Biological Interactions 2011;189(3) 215-221.

[16] Villemagne VL, Okamura N. *In vivo* tau imaging: obstacles and progress. Alzheimer's and Dementia 2014;10(3) 254-264.

[17] Ferranti A, Garuti L, Giovanninetti G, Gaggi R, Roncada P, Nardi P. Preparation and analgesic activity of tetrahydroquinolines and tetrahydroisoquinolines. Farmaco Edizione Scientifica 1987;42(4) 237-249.

[18] LeClerc G, Ruhland B, Andermann G, De Burlet G, Dietz M. For controlling tissue stability during eye surgery: antiswelling agent 1990; US Patent US 4,952,573, 1990.

[19] Uchida M, Chihiro M, Morita S, Kanbe T, Yamashita H, Yamasaki K, Yabuuchi, Y, Nakagawa K. Studies on proton pump inhibitors. II. Synthesis and antiulcer activity of 8-[(2-benzimidazolyl)sulfinylmethyl]-1,2,3,4-tetrahydroquinolines and related compounds. Chemical and Pharmaceutical Bulletin 1989;37(8) 2109-2116.

[20] Gracheva IN, Gridneva LI, Tochilkin AI, Gorkin VZ. Hydrazides and sulfonyl hydrazides from the series of quinoline are human serum amine oxidase inhibitors. Khimiko-Farmatsevticheskii Zhurnal 1988;22(11) 1336-1339.

[21] Miller KE, Huang CT, Portlock DE, Wright GC. Angiotensin I converting enzyme inhibitors containing unnatural alpha-amino acid analogues of phenylalanine. Life Sciences 1987;40(1) 63-70.

[22] Stevens RW, Ikeda T, Wakahayashi H, Nakane M. Antiinflammatory hydroxamic acids and *N*-hydroxyureas. US Patent 1993; US 5,256,789.

[23] Kihara N, Tomino I, Tan H, Ishihara T. Novel nitrogen-containing compound. European Patent 1988; EP 0289.36.5.

[24] Kojima E, Saito K. Cyclic anthranilic acid derivatives as therapeutic agents for treatment of metabolic bone disease. European Patent 1990; EP 402859.

[25] Youssefyeh RD, Magnien E, Lee TDY, Chan WK, Lin CJ, Galemmo JrRA, Johnson JrWH, Tan J, Campbell HF, Huang FC, Nuss GW, Carnathan GW, Sutherland CA, Van Inwegen RG. Development of a novel series of (2-quinolinylmethoxy)phenyl-containing compounds as high-affinity leukotriene receptor antagonists. 1. Initial structure-activity relationships. Journal of Medicinal Chemistry 1990;33(4) 1186-1194.

[26] Paris D, Cottin M, Demonchaux P, Augert G, Dupassieux P, Lenoir P, Peck MJ, Jasserand D. synthesis, structure-activity relationships, and pharmacological evaluation of pyrrolo[3,2,1-ij]quinoline derivatives: potent histamine and platelet activating factor antagonism and 5-lipoxygenase inhibitory properties. potential therapeutic application in asthma. Journal of Medicinal Chemistry 1995;38(4) 669-685.

[27] Rauckman BS, Tidwell MY, Johnson JV, Roth B. 2,4-Diamino-5-benzylpyrimidines and analogs as antibacterial agents. 10. 2,4-Diamino-5-(6-quinolylmethyl)- and -[(tetrahydro-6-quinolyl)methyl]pyrimidine derivatives. Further specificity studies. Journal of Medicinal Chemistry 1989;32(8) 1927-1935.

[28] Ogawa H, Miyamoto H, Kondo K, Yamashita H, Nakaya K, Komatsu H, Tanaka M, Takara S, Tominaga M, Yabuchi Y. Preparation of *N*-benzoyl benzo-fused heterocyclic compounds as vasopressin antagonists. Kokai Tokkyo Koho 1992; JP 04321669.

[29] Bigg D, Mangane M. Tetrahydroquinoline derivatives, their preparation and their therapeutic application 1986; France Patent FR 2,576,308.

[30] Nagasaka T, Kosugi Y, Kawahara T, Kakimoto M, Tamuru K, Hirata A. Preparation of 1,4-dihydro-4-phenyl-3,5-pyridinedicarboxylic acids as calcium antagonists 1993. Japanese Patent 05,339,263.

[31] Moon MW, Morris JK, Heier RF, Chidester CG, Hoffmann WE, Piercey MF, Althaus JS, VonVoigtlander PF, Evans DL, Figur LM, Lahti RA. Dopaminergic and serotonergic activities of imidazoquinolinones and related compounds. Journal of Medicinal Chemistry 1992;35(6) 1076-1092.

[32] Moon MW, His RSP. Synthesis of (*R*)-5-(Di[2,3-$^3H^2$]propylamino)5,6-dihydro-4*H*-imidazo[4,5,1-*ij*]quinolin-2(1*H*)-one ([^{3}H]U-86170) and (*R*)-5-([2,3-$^3H^2$]Propylamino)-5,6-dihydro-4*H*-imidazo[4,5,1-*ij*]quinolin-2(1*H*)-one ([^{3}H]U-91356). Journal of Labelled Compounds and Radiopharmaceuticals 1992;31(11), 933-943.

[33] Buzas A, Ollivier R, El Ahmad Y, Laurent E. 1,4-Dialkylpiperazine derivatives, method for obtaining them, and pharmaceutical compositions containing them. PCT International Applications 1993; WO 9316057.

[34] Agarwal SK, Saxena AK, Jain PC, Sur RN, Srimal RC, Dhawan BN, Anand N. Synthesis and structure activity relationship in 1-aryloxy-3-[*N*′-N4-arylpiperazinyl)] propanes. Indian Journal of Chemistry, Section B 1987; 26B, 642-646.

[35] Uchida M, Chihiro M, Morita S, Yamashita H, Yamasaki K, Kunbe T, Yabuuchi Y, Nakagawa K. Synthesis and antiulcer activity of 4-substituted 8-[(2-benzimidazol)sulfinylmethyl]-1,2,3,4-tetra-hydroquinolines and related compounds. Chemical and Pharmaceutical Bulletin 1990;38(6) 1575-1586.

[36] Uchida M, Morita S, Chihiro M. Preparation of 8-[(2-benzimidazolylthio)alkyl]hydroquinolines and their sulfoxides as antiulcer agents. European Patent 1987; EP 239129.

[37] Atwal K. Indanyl- and quinolylureas and related compounds as cardiovascular agents. European Patent 1992; EP 488616.

[38] Santangelo F, Casagrande C, Miragoli G, Vecchietti V. Synthesis and positive inotropic effect of 1-alkyl- and 1-acyl-6,7-dimethoxy-3-dimethylamino-1,2,3,4-tetrahydroquinolines. European Journal of Medicinal Chemistry 1994;29(11), 877-882.

[39] Galtier D, Lassalle G. Preparation of 1,2,3,4-tetrahydroquinolin-8-sulfonic acid and its chlorides as intermediates for antithrombotics. European Patent 1995; EP 643046.

[40] Lassalle G, Galtier D, Galli F. Preparation of 1-[2-amino-5-[1-(triphenylmethyl)-1H-imidazol-4-yl]-1-oxopentyl]piperidines and their use as intermediates for antithrombotics. European Patent 1995; EP 643047.

[41] Baumgarth M, Lues I, Minck KO, Beier N. Preparation of (benzocyclylethyl)arylpiperidines and -piperazines for treating arrhythmia and tachycardia. German Patent 1995; DE 4321366.

[42] Biller SA, Misra RN. Inhibitors of leukotriene biosynthesis in macrophage cells. US Patent US 4,843,082, 1989.

[43] Lukevics E, Lapina T, Segals I, Augustane I, Verovskii VN. synthesis and antiblastic activity of organosilicon derivatives of quinoline, isoquinoline, and *n*-methylpiperazine. Khimiko-Farmatsevticheskii Zhurnal 1988;22(8) 619-623.

[44] Kohno Y, Kojima E. Preparation of 1,2,3,4-tetrahydroquinoline-4,8-dicarboxylates as drugs. European Patent 1990; EP 403980.

[45] Kohno Y, Awano K, Ishizaki T, Kojima E, Kudoh S, Sekoe Y, Saito K. Preparation of tetrahydroquinoline acetic acid derivatives as immunosuppressants. PCT International Applications 1992; WO 9218482.

[46] Kohno Y, Kojima E, Saito K, Kudoh S, Sekoe Y. Preparation of tetrahydroquinolines as immunosuppressants and inflammation inhibitors. Kokai Tokkyo Koho 1994; JP 06 56788.

[47] Carling RW, Leeson PD, Moseley AM, Smith JD, Saywell K, Triclebank MD, Kemp JA, Marshall GR, Foster AC, Grimwood S. Anticonvulsant activity of glycine-site NMDA antagonists. 2. *trans* 2-carboxy-4-substituted tetrahydroquinolines. Bioorganic and Medicinal Chemistry Letters 1993;3(1) 65-70.

[48] Sangwan NK, Malik MS, Dhindsa KS. Synthesis of 3-benzyl-1,2,3,4-tetrahydro-7-methoxy-4-[4-(2-pyrolidinoethxy)phenyl]-1-tosylquinolines as potential antifertility agentes. Chimica Acta Turcica 1985;13(1) 129-134.

[49] Stevenson GL, Leeson PD, Rowley M, Sanderson I, Stansfield I. Synthesis of *cis* and *trans* 4-amido 2-carboxytetrahydroquinolines, high affinity ligands at the glycine site of the NMDA receptor. Bioorganic and Medicinal Chemistry Letters 1992;2(5) 371-374.

[50] Regnier G, Guillonneau C, Lepagnol J, Lestage P. Morpholine derivatives, process for their preparation and pharmaceutical compositions containing them for treatment of cerebral conditions. European Patent 1991; EP 427605.

[51] Boursier-Neyret C, Baune A, Klippert P, Castagne I, Sauveur C. Determination of *S* 12024 enantiomers in human plasma by liquid chromatography after chiral pre-column derivatization. Journal of Pharmaceutical and Biomedical Analysis 1993;11(11-12) 1161-1166.

[52] Ventura C, Miller R, Wolf HP, Beier N, Jonas R, Klockow M, Lues I, Hano O, Spurgeon HA, Lakatta EG, Capogrossi MC. Novel diazinone derivatives separate myofilament $Ca2^+$ sensitization and phosphodiesterase III inhibitory effects in guinea pig myocardium. Circulation Research 1992;70(6) 1081-1090.

[53] Regnier G, Guillonneau C, Lepagnol J. Preparation of (morpholinylmethoxy) tetrahydroquinoline derivatives as drugs for treatment of cerebral ischemia. European Patent 1988; EP 286495.

[54] Kohno Y, Saito K, Sekoe Y, Kojima E. Preparation of 6-aryl-cyclic anthranilic acids and their use as therapeutics for treatment of metabolic bone disorder. Japanese Patent 1992; JP 04316557.

[55] Cantrell CL, Dayan FE, Duke SO. Natural products as sources for new pesticides. Journal of Natural Products 2012;75(6) 1231-1242.

[56] Diaz MG, Dudley BG. Synthesis of 1,2,3,4-tetrahydroquinolines including angustureine and congeneric alkaloids. Organic Preparations and Procedures International, 2014. In press.

[57] Jacquemond-Collet I, Hannedouche S, Fabre N, Fourasté I, Moulis C. Two tetrahydroquinoline alkaloids from *Galipea officinalis*. Phytochemistry 1999;51(8) 1167-1169.

[58] Taylor LL, Goldberg FW, Hii KK. Asymmetric synthesis of 2-alkyl-substituted tetrahydroquinolines by an enantioselective aza-Michael reaction. Organic Biomolecular Chemistry 2012;10(22) 4424-4432.

[59] Jacquemond-Collet I, Benoit-Vical F, Mustofa Valentin A, Stanislas E, Mallié M, Fourasté I. Antiplasmodial and cytotoxic activity of galipinine and other tetrahydroquinolines from *Galipea officinalis.* Planta Medica 2002;68(1) 68-69.

[60] Houghton PJ, Watabe Y, Woldemariam TZ, Yates M. Activity of alkaloids from Angostura bark against *Mycobacterium tuberculosis*. Journal of Pharmacy and Pharmacology, 1998;50(S9) 230.

[61] Osório EJ, Robledo SM, Bastida J. The Alkaloids: Chemistry and Biology. Amsterdam: Elsevier Academic Press; 2008.

[62] Wang WB, Lu SM, Yang PY, Han XW, Zhou YG. Highly enantioselective iridium-catalyzed hydrogenation of heteroaromatic compounds, quinolines. Journal of American Chemical Society 2003;125(35) 10536-10537.

[63] Avemaria F, Vanderheiden S, Braese S. The aza-xylylene Diels-Alder approach for the synthesis of naturally occurring 2-alkyl tetrahydroquinolines. Tetrahedron 2003;59(35) 6785-6796.

[64] Lin XF, Li Y, Ma DW. Total synthesis of tetrahydroquinoline alkaloid (+)-angustureine. Chinese Journal of Chemistry 2004;22(9) 932-934.

[65] Theeraladanon C, Arisawa M, Nakagawa M, Nishida A. Total synthesis of (+)-(S)- angustureine and the determination of the absolute configuration of the natural product angustureine. Tetrahedron: Asymmetry 2005;16(4) 827-831.

[66] Lu SM, Wang YQ, Han XW, Zhou YG. Asymmetric hydrogenation of quinolines and isoquinolines activated by chloroformates. Angewandte Chemie International Edition 2006;45(14) 2260-2263.

[67] Rueping M, Antonchick AP, Theissmann T. A highly enantioselective Bronsted acid catalyzed cascade reaction: organocatalytic transfer hydrogenation of quinolones and their application in the synthesis of alkaloids. Angewandte Chemie International Edition 2006;45(22) 3683-3686.

[68] Ryu JS. Hydroarylation for the facile synthesis of 2-substituted tetrahydroquinoline: a concise synthesis of (+)-(*S*)-angustureine. Bulletin of the Korean Chemical Society 2006;27(5) 631-632.

[69] Patil NT, Wu H, Yamamoto Y. A route to 2-substituted tetrahydroquinolines via palladium-catalized intramolecular hydroamination of aniline-alkynes. Journal of Organic Chemistry 2007;72(17) 6577-6579.

[70] O'Byrne A, Evans P. Rapid synthesis of the tetrahydroquinoline alkaloids: angustureine, cuspareine and galipinine. Tetrahedron 2008;64(35) 8067-8072.

[71] Shahane S, Louafi F, Moreau J, Hurvois JP, Renaud JL, Van de Weghe P, Roisnel, T. Synthesis of alkaloids of Galipea officinalis by alkylation of an α-amino nitrile. European Journal of Organic Chemistry 2008;2008(27) 4622-4631.

[72] Fustero S, Moscardó J, Jiménez D, Pérez-Carrión MD, Sánchez-Roselló M, del Pozo C. Organocatalytic approach to benzofused nitrogen-containing heterocycles: Enantioselective total synthesis of (+)-angustureine. Chemistry a European Journal 2008;14(32) 9868-9872.

[73] Wang ZJ, Zhou HF, Wang TL, He YM, Fan QH. Highly enantioselective hydrogenation of quinolones under solvent-free or highly concentrated conditions. Green Chemistry 2009;11(6) 767-769.

[74] Kothandaraman P, Foo SJ, Chan PW. H. Gold-catalyzed intramolecular allylic amination of 2-tosylaminophenylprop-1-en-3-ols. A concise synthesis of (±)-angustureine. Journal of Organic Chemistry 2009;74(16) 5947-5952.

[75] Chen BL, Wang B, Lin GQ. Highly diastereoselective addition of alkynylmagnesium chlorides to N-tert-butanesulfinyl aldimines: a practical and general access to chiral α-branched amines. Journal of Organic Chemistry 2010;75(3) 941-944.

[76] Cruz-Lopez O, Nunez MC, Conejo-Garcia A, Kimatrai M, Campos JM. Syntheses of 2,3-dihydro-1,4-benzodioxins and bioisosteres as structural motifs for biologically active compounds. Current Organic Chemistry 2011;15(6) 869-887.

[77] Bentley SA, Davies SG, Lee JA, Roberts PM, Thomson JE. Conjugate addition of lithium N-phenyl-N-(α-methylbenzyl)amide: application to the asymmetric synthesis of (R)-(-)-angustureine. Organic Letters 2011;13(10) 2544-2547.

[78] Wang T, Zhuo LG, Li Z, Chen F, Ding Z, He Y, Fan QH, Xiang J, Yu ZX, Chan ASC. Highly enantioselective hydrogenation of quinolones using phosphine-free chiral cationic ruthenium catalysts: scope, mechanism, and origin of enanioselectivity. Journal of the American Chemical Society 2011;133(25) 9878-9891.

[79] Satyanarayana G, Pflästerer D, Helmchen G. Enantioselective synthesis of tetrahydroquinolines based on iridium-catalyzed allylic substitutions: total synthesis of (+)-angustureine and (-)-cuspareine. European Journal of Organic Chemistry 2011;2011(34) 6877-6886.

[80] Ye KY, He H, Liu WB, Dai LX, Helmchen G, You SL. Iridium-catalyzed allylic vinylation and asymmetric allylic amination reactions with o-aminostyrenes. Journal of the American Chemical Society 2011;133(46) 19006-19014.

[81] Taylor LL, Goldberg FW, Hii KK. Asymmetric synthesis of 2-alkyl-substituted tetrahydroquinolines by an enantioselective aza-Michael reaction. Organic and Biomolecular Chemistry 2012;10(22) 4424-4432.

[82] Tummatorn J, Diaz MG, Dudley GB. Synthesis of (-)-(R)-aungustureine by formal alkynylation of a chiral *β*-amino ester. Tetrahedron Letters 2013;54(10) 1312-1314.

[83] Diaz G, Diaz MAN, Reis MA. Enantioselective synthesis of both (-)-(*R*)- and (+)-(*S*)-angustureine controlled by enzymatic resolution. Journal of the Brazilian Chemical Society 2013;24(9) 1497-1503.

[84] Sirvent JA, Foubelo F, Yus M. Enantioselective synthesis of tetrahydroquinoline alkaloids (-)-angustureine and (-)-cuspareine from chiral *tert*-butanesulfinyl imines. Heterocycles 2014;88(2) 1163-1174.

[85] Sirvent JA, Foubelo F, Yus M. Stereoselective synthesis of indoline, tetrahydroquinoline, and tetrahydrobenzazepine derivatives from o-bromophenyl *N-tert*-butylsulfinyl aldiminas. Journal of Organic Chemistry 2014;79(3) 1356-1367.

[86] Katayama S, Ae N, Nagata R. Enzymatic resolution of 2-substituted tetrahydroquinolines. Convenient approaches to tricyclic quinoxalinediones as potent NMDA-glycine antagonists. Tetrahedron: Asymmetry 1998;9(24) 4295-4299.

[87] Nagata R, Tanno N, Kodo T, Ae N, Yamaguchi H, Nishimura T, Antoku F, Tatsuno T, Kato T, Yanaka Y, Nakamura M. Tricyclic quinoxalinediones: 5,6-dihydro-1H-pyrrolo[1,2,3-de]quinoxaline-2,3-diones and 6,7-Dihydro-1H,5H-pyrido[1,2,3-de]quinoxaline-2,3-diones as potent antagonists for the glycine binding site of the NMDA receptor. Journal of Medicinal Chemistry 1994;37(23) 3956-3968.

[88] Lam K, Lee KK, Gambari R, Wong RS, Cheng GY, Tong S, Chan K, Lau F, Lai PB, Wong W, Chan AS, Kok SH, Tang JC, Chui C. Preparation of *Galipea officinalis* Hancock type tetrahydroquinoline alkaloid analogues as anti-tumor agents. Phytomedicine 2013;20(2) 166-171.

Chapter 7

Aspidosperma Terpenoid Alkaloids — Biosynthetic Origin, Chemical Synthesis and Importance

Pedro Gregório Vieira Aquino, Thiago Mendonça de Aquino,
Magna Suzana Alexandre-Moreira, Bárbara Viviana de Oliveira Santos,
Antônio Euzébio Goulart Santana and João Xavier de Araújo-Júnior

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/59758

1. Introduction

Since a long time ago health sciences and natural products have been linked by the use of remedies and poisons, and nowadays there is little doubt that humans used natural drugs long before the emergence of written history [1,2]. The Ebers Papyrus dating from about 1600 BC, is one of the oldest medical treatise, which documents natural product-derived drugs used by the Sumerians and Akkadians in the 3rd century BC. Today there is information on medicinal plants dating back over about 500 years, as documented in herbaria. Laboratory studies of medicinal natural products started only about 200 years ago, with the isolation of of morphine, an alkaloid, from opium (*Papaver* spp.) [2,3].

Aspisoperma genus (Apocynaceae) species are trees of a great diversity of sizes that grow in different habitats and are distributed mainly among the Americas; In Brazil about 50 species of this genus have been catalogued [4–6]. There are several reports in the literature concerning the folk utilization of plants of this genus, as in treatment of malaria, dysentery, appendicitis, wounds, fever, dyspnea, asthma, scabies, stomachache, cough, constipation, boils, rheumatism, leishmaniasis, toothache, urinary tract inflammation and dermatitis. However several studies show that some plants of the genus are not recommended for pregnant women because of their potential abortifacient and teratogenic effects [7–28].

Given the diversity of popular uses of plants of the genus *Aspidosperma* as well as the predominance of terpenoid-alkaloids production in this genus and the importance of these substances for organic synthesis, medicinal chemistry and for knowledge of the biosynthetic

© 2015 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and eproduction in any medium, provided the original work is properly cited.

pathways used by plants to produce them, we propose to review the literature concerning the aspidosperma-type terpenoid-alkaloids chemical synthesis and their biological potential.

2. Biosynthesis of Aspidosperma terpenoid alkaloids

The isolation of alkaloids from species of *Aspidosperma* trees and their structural elucidation give rise to theories that attempt to explain their biosynthetic origin. In the field of indole-type alkaloids, one of the earliest theories to explain its biosynthesis arose in 1933, proposing that this type of alkaloid has origin in the reaction between tryptophan, phenylalanine and glicine (although at the time the proposed structures do not represent exactly the known reality today) [29]. Revisions of this theory lead to a new biosynthetic route, proposing that the indole-type alkaloids are derived from shikimic and prephenic acids and their interactions with *seco*-prephenate-formadehyde units and aromatic aminoacids as tryptamine and tryptophan (figure 1) [30,31]. As Aspidosperma type alkaloids were isolated theories that tried to explain their biosynthetic origin began to emerge, which, at this moment, were based on the chemical synthesis of such alkaloids, as done by several research groups [32].

Shikimic acid Prephenic acid *seco*-prephenate-formadehyde Tryptamine Tryptophan

Figure 1. Early propositions for indole alkaloid precursors.

Early work on proof of terpenoid alkaloid biosynthesis were based on administration of deuterium-labelled precursors of alkaloids to the plants tested and analysis of the metabolites produced to confirm a proposed biosynthetic way. The earliest proposition for the biosynthesis of Aspidosperma terpenoid alkaloids was the synthesis *via* mevalonate pathway, demonstrated in 1966 by the administration of 1-[$^2H_2$]-geraniol and 2-^{14}C-geraniol to *Vinca rosea* and mass spectrometry detection of labeled vindoline, what allowed the proposition of the biosynthetic route showed in figure 2 [33,34]. This biosynthetic way was refined two years later with the demonstration that administration of ^{14}C-labelled loganin (produced by the administration of 2-^{14}C-geraniol to *Meyanthes trifoliata*) to *V. rosea* and *Rawfolia serpentina* allowed the isolation of ^{14}C-labeled catharantine, serpentine, ajmalicine, vindoline and perivine [35], whose biosynthetic mechanism was detailed elsewhere [36–47].

Geraniol
Loganin
Vindoline

Figure 2. Proposal to Aspidosperma terpenoid alkaloid biosynthesis (adapted from [1-2]).

3. Chemical synthesis of Aspidosperma terpenoid alkaloids

Since the structure elucidation of the first isolated Aspidosperma alkaloids, various alternatives and techniques have emerged, due mainly the great structural complexity of this family of alkaloids. One of the earliest syntheses of an Aspidosperma alkaloid was published by a group from Havard University in 1959, which obtained the recently-isolated alkaloid ellipticine from condensation of indole with 3-acetylpyridine followed by reduction and pyrolisis, as shown in figure 3 [48].

ellipticine

Figure 3. First synthesis of ellipticine.

Many years later, a new synthesis of aspidosperma-type skeleton was published, in a very simple way using four steps (figure 4) [49]. Another example is the synthesis of quebrachamine, one way published in 1966, and another three ways, one of them based on alkylation of cyclic enamines, other starting with 1,3-propanediol and another based on the cleavage of a thioketal group (figure 5) [50–53].

Alkyl-Br
$LiAlH_4$
3-Methylaspidospermidine

Figure 4. Synthesis of 3-Methylaspidospermidine (adapted from [49]).

Figure 5. Total synthesis of quebrachamine.

Despite the many synthetic routes described for obtaining quebrachamine, none was obtained with enantiomeric purity until the problem was addressed in 1980, with the development of a synthetic route to (+)-quebrachamine using L-glutamic acid as a chiral template (figure 6) [54].

Figure 6. Enantioselective total synthesis of quebrachamine

http://dx.doi.org/10.5772/59758

Enamines were also utilized in the synthesis of aspidospermine, as showed in 1971 by a group from Rice University (figure 7) and other groups [55,56].

Figure 7. Synthesis of aspidospermine.

In 1978 was published a work that introduced a conceptually new approach to the synthesis of Aspidosperma-type alkaloids, the photocyclization-rearrangement or heteroatom directed photoarylation of anilinocyclohexanones, exemplified by the synthesis of the indolines A and B shown in figure 8 [57], this concept being expanded many years later, with the demonstration of different techniques of photo-induced reactions [58–60].

Figure 8. Photoarylation in the synthesis of Aspidosperma-type substructures.

Given the biosynthetic route proposed by Wenkert [30], a group from Yale University developed a synthetic route for obtaining the alkaloid minovine in a biogenetically modeled way (figure 9), refined many years later by the same group [61,62].

Figure 9. Synthesis of minovine.

Based on the fact that the Aspidosperma alkaloids share common structural features, a group from the Chinese Academy of Synthesis developed a strategy to aspidophytine enantioselective and stereo-controlled synthesis that could be applied to the synthesis of several other alkaloids of this family by simply varying the initial aniline (figure 10) [63].

Figure 10. Aspidophytine synthesis (adapted from [63]).

Another powerful technique for the Aspidosperma alkaloids skeleton is the utilization of aza-Cope rearrangements, utilized for the first time in 1981 for the stereoselective synthesis of 9a-arylhydrolilolidines precursors of vindoline (figure 11) and later expanded to other alkaloids [64–66].

Figure 11. Application of aza-Cope rearrangement (adapted from [64]).

Based on the premise that Heck reaction is a powerful method for the construction of quaternary carbon centers, researchers from Kyoto University decided to apply this methodology to the entantioselective synthesis of (-)-epieburnamonine (figure 12) [67].

http://dx.doi.org/10.5772/59758

HO OH O O I OH OBn N O O Heck Reaction N Cl O N Boc H N N O (-)-epieburnamonine

Figure 12. Utilization of Heck reaction on the construction of intermediates in terpenoid alkaloids synthesis (adapted from [67]).

Exploiting the possibilities of C-H bond functionalization on the pyrrole ring, a group from Cambridge University recently proposed the total synthesis of rhanizilam-type alkaloids as precursors to Aspidosperma-type alkaloids, as shown in figure 13 [68].

O Boc I NO_2 HN OTSE OH O_2N $ESTO_2C$ N H Aspidospermidine

Figure 13. Metal-catalyzed C-H bond functionalization on terpenoid alkaloid synthesis (adapted from [68]).

3.1. Aspidosperma alkaloid precursors

Another field of great interest is in synthesis of precursors which can serve to the achieve greater structural diversity from common structures. Various approaches have been utilized in this field, such as ketone annelation of endocyclic enamines [69] (figure 14) and the utilization of photochemistry with the one pot synthesis of a 9-membered ring system that could be

applied not only in the synthesis of Aspidosperma-type alkaloids, but also Strychnos, Schizozygane and Eburnamine-types, as shown in figure 15 [70].

Figure 14. Ketone annelation of endocyclic enamines on the synthesis of alkaloids.

Figure 15. Photochemistry on the synthesis of alkaloids.

Another approach relied on the conversion of *para*-substituted anisoles into 4,4-dissubstitued cyclohexenones via cyclohexadiene-$Fe(CO)_3$ complexes, to obtain the tetrasubstituted carbon of Aspidosperma-type alkaloids, as demonstrated by the synthesis shown in figure 16 [71]. The iron complexes were also utilized in the synthesis of limaspermine derivatives, as shown in figure 17 [72]. Iron [73] and others metals were also utilized in Aspidosperma alkaloids synthesis, such as rhodium [74–77], copper [75,78], ruthenium and molybdenum [79], titanium [80] and palladium [81,82].

Figure 16. Synthesis of alkaloids with functionalised C(20) substituents via diene-$Fe(CO)_3$ complex (adapted from [71]).

Another precursor of Aspidosperma type alkaloids was synthesized in 1978, from azocetones or iminomalonates via acid-catalysed and Birch reduction reactions (figure 18) [83].

http://dx.doi.org/10.5772/59758

Figure 17. Utilization of organoiron chemistry in limaspermine synthesis.

Figure 18. Synthesis of synthons for Aspidosperma alkaloids synthesis (adapted from [83]).

3.2. Novel strategies

One of the main concerns of chemists worldwide is the development of more efficient and "green" procedures in organic synthesis procedures. Among the procedures developed we can cite the so-called domino synthesis, where several bonds are formed in sequence, without isolation of intermediates, addition of reagents or changes in reaction conditions, so that the subsequent reaction result as a consequence of the functionality formed in the previous step [84]. One example of domino synthesis application to Aspidosperma alkaloids synthesis was recently published, where the alkaloids (-)-aspidospermidine, (-)-tabersonine and (-)-vinca-difformine were synthesized in an asymmetric domino Michael/Mannich/N-alkylation sequence, as shown in figure 19 [85].

The majority of synthetic strategies employed to obtain natural products are based on the construction of a single target skeleton, in contrast with the strategy utilized by plants, where

Figure 19. Domino Michael/Mannich/N-alkylation sequence to Aspidosperma alkaloids synthesis (adapted from [85]).

divergent molecular cyclizations of a polyunsaturated common intermediate produce different scaffolds, as recently demonstrated in two different papers, by the synthesis of different Aspidosperma alkaloids[81] and diverse indole alkaloids skeletons [86] from a common intermediate in a biogenetically-inspired way, as shown in figure 20 [86].

Figure 20. Synthesis of indole alkaloids in a biogenetically-inspired way (adapted from [86]).

4. Biological importance of Aspidosperma terpenoid alkaloids

One of the research interests of our group is the isolation of alkaloids from *Aspidosperma* species with pharmacological potential. From a chemotaxonomic point of view, alkaloids are substances of great potential in malaria treatment [87,88]. In this perspective, we decided to study the alkaloids produced by *A. pyrifolium*, resulting in the isolation of the alkaloids 15-deme-

thoxypyrifoline, aspidofractinine and *N*-formylaspidofractinine [89]. We have identified in *A. pyrifolium* insecticidal [90], antibacterial [91] and hypotensive activities [92]. Another plant studied by our group was *A. tomentosum*, which showed great anti-hipertensive [93,94], antinociceptive, anti-inflammatory and analgesic [95–98] and *A. macrocarpum*, which showed anti-hypertensive activity in spontaneously hypertensive mice [99].

Some species have been the subject of research in order to identify its pharmacological properties and other biological activities. In vitro assay with aqueous extracts of the aerial parts and roots of A. *pachypterum* against *Staphylococcus aureus* and the Human Immunodeficiency Virus (HIV), respectively, showed that this species exhibited a moderate activity [100,101]. The methanolic extract of the aerial parts of *A. ramiflorum* was active in vitro against gram-negative bacterium *Escherichia coli* [102] and against the fungus *Cryptococcus neoformans* (causing opportunistic infections in humans) [103] while the methanol extract of the stem bark of the same species was found to be moderately active against gram-positive bacteria and inactive against gram-negatives ones [104]. Studying tailings from the processing of hardwoods in Paraná (Brazil), it was found that the methanol extract of the wood of the plant identified as Peroba pink (*Aspidosperma* sp.) had a composition rich in phenols and alkaloids as well as strong activity against gram-negative bacteria *Proteus mirabilis* [105]. In two trials conducted with various plant species, among them five from *Aspidosperma* genre, it was observed that the ethanol extract of the stem bark of *A. excelsum*, *A. megalocarpon*, *A. oblongum* and *A. marcgravianum* were active against gram-positive bacteria *Bacillus subtilis* and that the same extracts and also the ethanol extract of the stem bark of *A. album* were active against gram-positive *S. aureus* [106,107]. In a study of Peruvian plants, it was reported that the extract of the bark of *A. rigidum* showed antibacterial activity against *B. subtilis* [108].

Another reported activity for species was the anti-Leishmania, where in vitro assay for *Leishmania amazonensis* promastigotes ahead and *L. braziliensis*, the fraction rich in alkaloids obtained from the stem bark proved to be active, with the highest activity observed against the first species [109]. Yet in order to find alternatives for the treatment of neglected diseases, the methanol extract of the bark of *A. megalocarpon* was tested against the D2 and F32 *Plasmodium falciparum* strains, being active [110]. The dichloromethane extract of the roots of *A. tomentosum* was active front *P. falciparum* (strain FcB1/Colombia) with a selectivity index of 67.5 compared with the activity front NIH-3T3 cells. In relation to substances with antifungal properties, it was seen that the ethanol extract of the stem of *A. polyneuron* was capable of inhibiting *Cladosporium herbarum* (pathogen of plants) [111].

In order to find alternatives for the treatment of cancer, the dichloromethane extract of the aerial parts of *A. tomentosum* was capable of inhibiting the proliferation of cell lines MCF-7 (breast cancer), UACC62 (melanoma), NCIADR (breast cancer phenotype with resistance to multiple drugs and NCl460 (lung cancer), and we observed that the activity was concentrated in fractions rich in terpenes and species of high polarity [112].

In vivo assay of the ethanol extract of the stem of *A. nitidum* showed significant anti-inflammatory activity when evaluated in the trial of edema induced by carrageenan in mice. Prospecting for sources of antioxidant compounds, the hot aqueous extract of *A. quebracho-*

blanco was tested for oxidation power / ferric reduction, showing a low activity and is therefore not considered as potential producer of antioxidant compounds [113].

It was observed that administration of a fraction rich in alkaloids obtained from the root bark of *A. ulei* exerted pro-erectile effect in rats and suggested a mechanism of action via blocking presynaptic α2-adrenergic receptors, the activation of the dopaminergic system and release of nitric oxide [114]. When the same fraction was tested in corpus cavernosum penis obtained from rabbit, its ability to cause relaxation was observed and the proposed mechanism blocking the influx of calcium into the cells [115]. In assay using α-adrenergic receptors isolated from human penis, it was shown that the crude extract and four fractions obtained from the bark of *A. quebracho-blanco* were able to block them, and the magnitude of interaction directly proportional to the content of the alkaloid yohimbine [116].

Despite reports of low toxicity associated with the use of plants of the genus *Aspidosperma* [109,110,117–119], some studies show a contrary position regarding the species *A. pyrifolium* [89,120]. In a study of the species *A. pyrifolium* cases of abortion in goats were reported due to ingestion of parts of the plants and when the ethanol extract of the leaves was administered to pregnant rats reduced fetal weight and maternal toxicity was observed, as well as hemolysis and toxicity test the front microcrustacean *Artemia salina* [120]. In a toxicity study with the microcrustacean *A. franciscana* with several species found in the Brazilian Amazon, among them seven species of *Aspidosperma*, it was reported that the bark extracts of *A. marcgravianum*, *A. vargasii*, *A. nitidum* and *A. sprucenaum* led to mortality of 100, 94, 70 and 65% of the crustaceans, whilst extracts from the bark of *A. desmanthum*, *A. sandwithianum* and *A. shultesii* led to a mortality rate of 6, 0 and 0% crustaceans, showing the potential toxicity of some species gender [121]. In another test with brine shrimp, both the dichloromethane extract and the methanol extract of the bark of *A. excelsum* showed toxicity [14].

5. Conclusion

The present literature review shows the importance of the study of Aspidosperma type alkaloids due to the widespread usage of plants that produce these substances in folk medicine and the great array of potential biomedical applications that these substances exhibit. Beyond this it is clear the importance of developments in synthetic organic chemistry to obtain these substances without the necessity of extraction from natural sources.

Acknowledgements

We would like to thank the financial support given by CNPq, CAPES, FINEP and FAPEAL agencies.

http://dx.doi.org/10.5772/59758

Author details

Pedro Gregório Vieira Aquino[1], Thiago Mendonça de Aquino[1],
Magna Suzana Alexandre-Moreira[2], Bárbara Viviana de Oliveira Santos[3],
Antônio Euzébio Goulart Santana[1] and João Xavier de Araújo-Júnior[1*]

*Address all correspondence to: jotaaraujo2004@gmail.com

1 Institute of Chemistry and Biotechnology, Federal University of Alagoas, Maceió, Brazil

2 Institute of Biological and Health Sciences, Federal University of Alagoas, Maceió, Brazil

3 Pharmaceutical Sciences Department, Federal University of Paraíba, João Pessoa, Brazil

References

[1] Newman DJ, Cragg GM, Snader KM. The influence of natural products upon drug discovery. Natural Products Report 2000;17:215–34.

[2] Kinghorn AD. Drug discovery form natural products. In: Lemke TL, Williams DA, Roche VF, Zito SW, editors. Foye's Principles of Medicinal Chemistry 6th ed., Philadelphia: Lippincott Williams & Wilkins; 2008, p. 12–25.

[3] De Pasquale A. Pharmacognosy: the oldest modern science. Journal of Ethnopharmacology 1984;11:1–16.

[4] Amorim IL De, Sampaio EVSB, Araújo E de L. Flora e estrutura da vegetação arbustivo-arbórea de uma área de caatinga do Seridó, RN, Brasil. Acta Botânica Brasileira 2005;19:615–23.

[5] Corrêa MP. Diccionário das Plantas Úteis do Brasil e das Exóticas Cultivadas. Volumes I a VI. Rio de Janeiro: Imprensa Nacional; 1984.

[6] Oliveira VB, Freitas MSM, Mathias L, Braz-Filho R, Vieira IJC. Atividade biológica e alcalóides indólicos do gênero Aspidosperma (Apocynaceae): uma revisão. Revista Brasileira de Plantas Medicinais Botucatu 2009;11:92–9.

[7] Agra MF, Baracho GS, Nurit K, Basílio IJLD, Coelho VPM. Medicinal and poisonous diversity of the flora of "Cariri Paraibano", Brazil. Journal Ethnopharmacology 2007;111:383–95.

[8] Botsaris AS. Plants used traditionally to treat malaria in Brazil: the archives of Flora Medicinal. Journal of Ethnobiology and Ethnomedicine 2007;3:1.

[9] Bourdy G, DeWalt SJ, Chávez de Michel LR, Roca a, Deharo E, Muñoz V, et al. Medicinal plants uses of the Tacana, an Amazonian Bolivian ethnic group. Journal Ethnopharmacology 2000;70:87–109.

[10] Bourdy G, Chāvez de Michel LR, Roca-Coulthard a. Pharmacopoeia in a shamanistic society: the Izoceño-Guaraní (Bolivian Chaco). Journal of Ethnopharmacology 2004;91:189–208.

[11] Brandão MGL, Grandi TSM, Rocha EMM, Sawyer DR, Krettli AU. Survey of medicinal plants used as antimalarials in the Amazon. Journal of Ethnopharmacology 1992;36:175–82.

[12] De Almeida CDFCBR, Ramos MA, de Amorim ELC, de Albuquerque UP. A comparison of knowledge about medicinal plants for three rural communities in the semiarid region of northeast of Brazil. Journal of Ethnopharmacology 2010;127:674–84.

[13] Dele Vitto LA, Petenatti EM, Petenatti ME. Recursos herbolarios de San Luis (República Argentina) primera parte: plantas nativas. Multequina 1997;6:49–66.

[14] Desmarchelier C, Mongelli E, Coussio J, Ciccia G. Studies on the cytotoxicity, antimicrobial and DNA-binding activities of plants used by the Ese'ejas. Journal of Ethnopharmacology 1996;50:91–6.

[15] Filipov A. Medicinal plants of the Pilagá of central Chaco. Journal of Ethnopharmacology 1994;44:181–93.

[16] Goleniowski ME, Bongiovanni G a, Palacio L, Nuñez CO, Cantero JJ. Medicinal plants from the "Sierra de Comechingones", Argentina. Journal of Ethnopharmacology 2006;107:324–41.

[17] Hajdu Z, Hohmann J. An ethnopharmacological survey of the traditional medicine utilized in the community of Porvenir, Bajo Paraguá Indian Reservation, Bolivia. Journal of Ethnopharmacology 2012;139:838–57.

[18] Kvist LP, Christensen SB, Rasmussen HB, Mejia K, Gonzalez a. Identification and evaluation of Peruvian plants used to treat malaria and leishmaniasis. Journal of Ethnopharmacology 2006;106:390–402.

[19] Martínez GJ, Barboza GE. Natural pharmacopoeia used in traditional Toba medicine for the treatment of parasitosis and skin disorders (Central Chaco, Argentina). Journal of Ethnopharmacology 2010;132:86–100.

[20] Monteles R, Pinheiro CUB. Plantas medicinais em um quilombo maranhense: uma perspectiva etnobotânica. Revista Biologia e Ciências da Terra 2007;7:38–48.

[21] Muñoz V, Sauvain M, Bourdy G, Arrázola S, Callapa J, Ruiz G, et al. A search for natural bioactive compounds in Bolivia through a multidisciplinary approach Part III. Evaluation of the antimalarial activity of plants used by Alteños Indians. Journal of Ethnopharmacology 2000;71:123–31.

http://dx.doi.org/10.5772/59758

[22] Rodrigues E. Plants of restricted use indicated by three cultures in Brazil (Caboclo-river dweller, Indian and Quilombola). Journal of Ethnopharmacology 2007;111:295–302.

[23] Ruiz L, Ruiz L, Maco M, Cobos M, Gutierrez-Choquevilca A-L, Roumy V. Plants used by native Amazonian groups from the Nanay River (Peru) for the treatment of malaria. Journal of Ethnopharmacology 2011;133:917–21.

[24] Sanz-Biset J, Cañigueral S. Plant use in the medicinal practices known as "strict diets" in Chazuta valley (Peruvian Amazon). Journal of Ethnopharmacology 2011;137:271–88.

[25] Sanz-Biset J, Campos-de-la-Cruz J, Epiquién-Rivera M a, Cañigueral S. A first survey on the medicinal plants of the Chazuta valley (Peruvian Amazon). Journal of Ethnopharmacology 2009;122:333–62.

[26] Scarpa GF. Medicinal plants used by the Criollos of Northwestern Argentine Chaco. Journal of Ethnopharmacology 2004;91:115–35.

[27] Schmeda-Hirschmann G. Magic and medicinal plants of the Ayoreos of the Chaco Boreal (Paraguay). Journal of Ethnopharmacology 1993;39:105–11.

[28] Alves M, Silva B, Melo LVL, Ribeiro R V. Levantamento etnobotânico de plantas utilizadas como anti-hiperlipidêmicas e anorexígenas pela população de Nova Xavantina-MT, Brasil. Revista Brasileira de Farmacognosia 2010;20:549–62.

[29] Barger G, Scholz C. Über Yohimbin. Helvetica Chimica Acta 1933;16:1343–54.

[30] Wenkert E. Biosynthesis of Indole Alkaloids. The Aspidosperma and Iboga Bases. Journal of the American Chemical Society 1962;84:98–102.

[31] Wenkert E, Bringi N V. A Stereochemical Interpretation of the Biosynthesis of Indole Alkaloids 1. Journal of the American Chemical Society 1959;81:1474–81.

[32] Kutney JP, Piers E. The Chemistry of Cleavamine: A Novel Transannular Cyclization Relating to Biosynthesis of Aspidosperma Alkaloids. Journal of the American Chemical Society 1964;86:953–5.

[33] Hall ES, McCapra F, Money T, Fukumoto K, Hanson JR, Mootoo BS, et al. Concerning the terpenoid origin of indole alkaloids: biosynthetic mapping by direct mass spectrometry. Chemical Communications 1966:348–50.

[34] Battersby AR, Brown RT, Kapil RS, Martin JA, Plunkett AO. Role of loganin in the biosynthesis of indole alkaloids. Chemical Communications 1966;22:812–3.

[35] Battersby AR, Kapil RS, Martin JA, Mo L. Loganin as Precursor of the Indole Alkaloids. Chemical Communications 1968:133–4.

[36] Battersby AR, Byrne JC, Kapil RS, Martin JA, Payne TG, Arigoni D, et al. The Mechanism of Indole Alkaloid Biosynthesis. Chemical Communications 1968:951–3.

[37] Battersby AR, Burnett AR, Parsons PG. Preparation of secologanin: its conversion into ipecoside and its role in indole alkaloid biosynthesis. Chemical Communications 1968:1280–1.

[38] Qureshi AA, Scott AI. Interconversion of Coryanthe, Aspidosperma, and Iboga alkaloids. A model for indole alkaloid biosynthesis. Chemical Communications 1968:945–6.

[39] Kutney JP, Cretney WJ, Hadfield JR, Hall ES, Nelson VR, Wigfield DC. Indole alkaloid biosynthesis. Journal of the American Chemical Society 1968;90:3566–7.

[40] Scott AI, Qureshi AA. Biogenesis of Strychnos, Aspidosperma, and Iboga alkaloids. Structure and reactions of preakuammicine. Journal of the American Chemical Society 1969;91:5874–6.

[41] Qureshi AA, Scott AI. Biosynthesis of indole alkaloids: sequential precursor formation and biological conversion in Vinca rosea. Chemical Communications 1968:948.

[42] Battersby AR, Burnett AR, Parsons PG. Alkaloid biosynthesis. Part XV. Partial synthesis and isolation of vincoside and isovincoside: Biosynthesis of the three major classes of indole alkaloids from vincoside. Journal of Chemical Society 1969:1193–200.

[43] Battersby AR, Burnett AR, Parsons PG. Alkaloid biosynthesis. Part XIV. Secologanin: Its cnversion into ipecoside and its role as biological precursor of the indole alkaloids. Journal of Chemical Society 1969:1187–92.

[44] Kutney JP, Ehret C, Nelson VR, Wigfield DC. Studies on indole alkaloid biosynthesis. II. Journal of the American Chemical Society 1968;90:5929–30.

[45] Kutney JP, Nelson VR, Wigfield DC. Indole alkaloid biosynthesis. III. Journal of the American Chemical Society 1969;91:4278–9.

[46] Kutney JP, Nelson VR, Wigfield DC. Indole alkaloid biosynthesis. IV. Journal of the American Chemical Society 1969;91:4279–80.

[47] Kutney JP, Beck JF, Eggers NJ, Hanssen HW, Sood RS, Westcott ND. Indole alkaloid biosynthesis. VII. Later stages of Aspidosperma alkaloid biosynthesis. Journal of the American Chemical Society 1971;93:7322–4.

[48] Woodward RB, Iacobucci GA, Hochstein IA. The synthesis of ellipticine. Journal of the American Chemical Society 1959;81:4434–5.

[49] Barton JED, Harley-Mason J. Total synthesis of Hunteria and Aspidosperma alkaloids from a common intermediate. Chemical Communications 1965:298–9.

[50] Kutney JP, Abdurahman N, Le Quesne P, Piers E, Vlattas I. A New Total Synthesis of dl-Quebrachamine and dl-Aspidospermidine. A General Entry into the Aspidosperma Alkaloids. Journal of the American Chemical Society 1966;88:3656–7.

[51] Ziegler FE, Zoretic PA. The alkylation of cyclic enamines: A synthesis of the quebrachamine skeleton. Tetrahedron Letters 1968:2639–41.

[52] Kutney JP, Abdurahman N, Gletsos C, Le Quesne P, Piers E, Vlattas I. Total synthesis of indole and dihydroindole alkaloids. V. Total synthesis of dl-quebrachamine and dl-aspidospermidine. General entry into the aspidosperma alkaloids. Journal of the American Chemical Society 1970;92:1727–35.

[53] Takano S, Hatakeyama S, Ogasawara K. Synthesis of the non-tryptamine moiety of the aspidosperma-type indole alkaloids via cleavage of cyclic.alpha.-diketone monothioketal. An efficient synthesis of (dl)-quebrachamine and a formal synthesis of (dl)-tabersonine. Journal of the American Chemical Society 1976;98:3022–3.

[54] Takano S, Chiba K, Yonaga M, Ogasawara K. Enantioselective Synthesis of (+)-Quebrachamine using L-glutamic acid as chiral template. Chemical Communications 1980:616–7.

[55] Michael J, Zimmerman L. General methods of alkaloid synthesis. A new approach to functionalized Hydrolulolidone aspidosperma alkaloid precursors. A formal synthesis of (+-)-aspidospermine. Chemical Communications 1971:857–8.

[56] Martin SF, Desai SR, Philips GW, Miller AC. General methods for alkaloid synthesis via intramolecular [4+2] cycloaddition reactions of enamides. A new approach to the synthesis of Aspidosperma alkaloids. Journal of the American Chemical Society 1980;102:3294–6.

[57] Schultz AG, Chiu I-C. Heteroatom directed photoarylation; an approach to the synthesis of Aspidosperma alkaloids. Journal of Chemical Society Chemical Communications 1978:29.

[58] Ban Y, Yoshida K, Goto J, Oishi T. Novel photoisomerization of 1-acylindoles to 3-acylindolenines. General entry to the total synthesis of Strychnos and Aspidosperma alkaloids. Journal of the American Chemical Society 1981;103:6990–2.

[59] Ibrahim-Ouali M, Sinibaldi M-E, Troin Y, Guillaume D, Gramain J-C. Diastereoselective photochemical synthesis of 3,3′-dissubstituted indolines. Tetrahedron 1997;53:16083–96.

[60] Ibrahim-Ouali M, Sinibaldi M-E, Troin Y, Gramain J-C. Photocyclization of enaminoesters: Access to 2,3-dihydroindoles spiroimides. Tetrahedron Letters 1996;37:37–8.

[61] Ziegler FE, Spitzner EB. The biogenetically modeled total synthesis of (+-)-Minovine. Journal of the American Chemical Society 1970;92:3492–4.

[62] Ziegler FE, Spitzner EB. Biogenetically modeled synthesis via an indole acrylic ester. Total synthesis of (+-)-minovine. Journal of the American Chemical Society 1973;95:7146–9.

[63] Yang R, Qiu FG. General entry to aspidosperma alkaloids: enantioselective total synthesis of (-)-aspidophytine. Angewandte Chemie International Edition English 2013;52:6015–8.

[64] Overman LE, Sworin M, Bass LS, Clardy J. Synthesis applications of aza-cope rearrangements. Tetrahedron 1981;37:4041–5.

[65] Overman LE, Sworin M, Burk RM. Synthesis applications of aza-Cope rearrangements. Part 10. A new approach for the total synthesis of pentacyclic Aspidosperma alkaloids. Total synthesis of dl-16-methoxytabersonine. Journal of Organic Chemistry 1983;48:2685–90.

[66] Overman LE, Robertson GM, Robichaud AJ. Use of aza-Cope rearrangement-Mannich cyclization reactions to achieve a general entry to Melodinus and Aspidosperma alkaloids. Stereocontrolled total syntheses of (.+-.)-deoxoapodine, (.+-.)-meloscine, and (.+-.)-epimeloscine and a formal synthesis of (. Journal of the American Chemical Society 1991;113:2598–610.

[67] Yasui Y, Takeda H, Takemoto Y. Toward general access to the aspidosperma-type terpenoid indole alkaloids: synthesis of the key 3,3-disubstituted piperidones through enantioselective intramolecular heck-type reaction of chloroformamides. Chemical and Pharmaceutical Bulletin (Tokyo) 2008;56:1567–74.

[68] McMurray L, Beck EM, Gaunt MJ. Chemical synthesis of Aspidosperma alkaloids inspired by the reverse of the biosynthesis of the rhazinilam family of natural products. Angewandte Chemie International Edition English 2012;51:9288–91.

[69] Stevens R V, Mehra RK, Zinmerman RL. Synthesis of Aspidosperma Alkaloid Precursors. A New Application of the Methyl Vinyl Ketone Annelation of Endocyclic Enamines in Alkaloid Synthesis. Chemical Communications 1969:877–8.

[70] Ban Y, Toshida K, Goto J, Oishi T, Takeda E. A synthetic road to the forest of Strychnos, Aspidosperma, Schizozygane and Eburnamine alkaloids by way of the novel photoisomerization. Tetrahedron 1983;39:3657–68.

[71] Pearson AJ, Ress DC. New synthetic approaches to Aspidosperma alkaloids with functionalised C(20) substituents: Some key intermediates via diene-Fe(CO)3 complexes. Tetrahedron Letters 1980;21:3937–40.

[72] Pearson AJ, Rees DC. Total synthesis of (.+-.)-limaspermine derivatives using organoiron chemistry. Journal of the American Chemical Society 1982;104:1118–9.

[73] Kuehne ME, Bandarage UK, Hammach A, Li Y, Wang T. Application of Ferrocenylalkyl Chiral Auxiliaries to Syntheses of Indolenine Alkaloids: Enantioselective Syntheses of Vincadifformine, ψ-and 20-epi-ψ-Vincadifformines, Tabersonine, Ibophyllidine, and Mossambine. Journal of Organic Chemistry 1998;63:2172–83.

[74] Padwa A, Price AT. Synthesis of the Pentacyclic Skeleton of the Aspidosperma Alkaloids Using Rhodium Carbenoids as Reactive Intermediates. Journal of Organic Chemistry 1998;63:556–65.

[75] Edwankar R V, Edwankar CR, Namjoshi O a, Deschamps JR, Cook JM. Brønsted acid mediated cyclization of enaminones. Rapid and efficient access to the tetracyclic framework of the Strychnos alkaloids. Journal of Natural Products 2012;75:181–8.

[76] Mejía-Oneto JM, Padwa A. Application of the Rh(II) cyclization/cycloaddition cascade for the total synthesis of (+/-)-aspidophytine. Organic Letters 2006;8:3275–8.

[77] Hong X, Mejía-oneto JM, France S, Padwa A. Preparation of 2-diazo-2-oxopiperidin-3-yl-3-oxopropanoates. Useful reagents for Rh (II)-catalyzed cyclization-cycloaddition chemistry. Arkivoc 2007:125–38.

[78] Temme O, Taj S-A, Andersson PG. Highly Enantioselective Intermolecular Cu(I)-Catalyzed Cyclopropanation of Cyclic Enol Ethers. Asymmetric Total Synthesis of (+)-Quebrachamine. Journal of Organic Chemistry 1998;63:6007–15.

[79] Sattely ES, Meek SJ, Malcolmson SJ, Schrock RR, Hoveyda AH. Design and stereoselective preparation of a new class of chiral olefin metathesis catalysts and application to enantioselective synthesis of quebrachamine: catalyst development inspired by natural product synthesis. Journal of the American Chemical Society 2009;131:943–53.

[80] Cheng X, Duhaime CM, Waters SP. Total synthesis of the Aspidosperma alkaloid (±)-subincanadine F via a titanium-mediated intramolecular nucleophilic acyl substitution strategy. Journal of Organic Chemistry 2010;75:7026–8.

[81] Shen X, Zhao R, Mo M, Peng F, Zhang H, Shao Z. Catalytic enantioselective and divergent total synthesis of (+)-10-oxocylindrocarpidine, (+)-cylindrocarpidine, (-)-N-acetylcylindrocarpinol, and (+)-aspidospermine. Journal of Organic Chemistry 2014;79:2473–80.

[82] Jiao L, Herdtweck E, Bach T. Pd(II)-catalyzed regioselective 2-alkylation of indoles via a norbornene-mediated C-H activation: mechanism and applications. Journal of the American Chemical Society 2012;134:14563–72.

[83] Takano S, Shishido K, Sato M, Yuta K, Ogasawara K. New synthetic routes to synthons for the synthesis of functionalized aspidosperma alkaloids. Journal of Chemical Society Chemical Communications 1978:943.

[84] Tietze LF. Domino Reactions in Organic Synthesis. Chemical Reviews 1996;96:115–36.

[85] Zhao S, Andrade RB. Domino Michael/Mannich/N-alkylation route to the tetrahydrocarbazole framework of aspidosperma alkaloids: concise total syntheses of (-)-aspidospermidine, (-)-tabersonine, and (-)-vincadifformine. Journal of the American Chemical Society 2013;135:13334–7.

[86] Mizoguchi H, Oikawa H, Oguri H. Biogenetically inspired synthesis and skeletal diversification of indole alkaloids. Nature Chemistry 2014;6:57–64.

[87] Wright CW. Plant Derived Antimalarial Agents: New Leads and Challenges. Phytochemistry Reviews 2005;4:55–61.

[88] Foley M, Tilley L. Quinoline antimalarials: mechanisms of action and resistance. International Journal of Parasitology 1997;27:231–40.

[89] Araújo-Jr. JX, Antheaume C, Trindade RCP, Schmitt M, Bourguignon J-J, Sant'Ana AEG. Isolation and characterisation of the monoterpenoid indole alkaloids of Aspidosperma pyrifolium. Phytochemistry Reviews 2007;6:183–8.

[90] Trindade RCP, da Silva PP, Araújo-Júnior JX, de Lima IS, de Paula JE, Sant'Ana AEG. Mortality of Plutella xylostella larvae treated with Aspidosperma pyrifolium ethanol extracts. Pesquisa Agropecuária Brasileira 2008;43:1813–6.

[91] Pessini GL, Aquino PG V, Bernardo VB, Costa MA, Nakamura C V, Ribeiro EAN, et al. Evaluation of antimicrobial activity of three Aspidosperma species. PharmacologyOnLine 2012;1:112–9.

[92] Araújo-Júnior JX, Oliveira DA, Oliveira MP, Aquino PG V, Sant'Ana AEG, Ribeiro EAN. Evaluation of cardiovascular response induced by ethanolic extract of Aspidosperma pyrifolium (Apocynaceae) wood on arterial pressure in spontaneously hypertensive rats. XX Congr. Italo-Latinoamericano Etnomedicina, Fortaleza: 2011.

[93] Furtado FF, Herculano EA, Araújo-Júnior JX, Sat'Ana AEG, Ribeiro EAN, Medeiros IA, et al. Vasorelaxant effect induced by ethanolic extract of Aspidosperma tomentosum in the isolated rat mesenteric artery. XVII Congr. Italo-Latinoamericano di Etnomedicina, Palermo: 2008.

[94] Furtado FF, Tenorio EP, Sant'Ana AEG, Araújo-Júnior JX, Aquino PG V, Ribeiro EAN, et al. Antihypertensive and vasorelaxant activities of Aspidosperma tomentosum are mainly through calcium channel blockade. XIX Italo-Latinamerican Congr. Etnomedicine, Villassimius: 2010.

[95] Aquino AB De, Cavalcante-Silva LHA, Matta CBB Da, Epifânio WADN, Aquino PGV, Santana AEG, et al. The antinociceptive and anti-inflammatory activities of Aspidosperma tomentosum (Apocynaceae). ScientificWorldJournal 2013;2013:218627.

[96] Aquino AB, Queiroz AC, Oliveira MP, Epifanio WAN, Junior WB, Sant'Ana AEG, et al. Avaliação da atividade antinociceptiva e antiinflamatória da espécie Aspidosperma tomentosum (Apocynacea). 40º Congr. Bras. Farmacol. e Ter. Exp., Águas de Lindóia: 2008.

[97] Epifanio WAN, Oliveira MP, Melo GMA, Silva SAS, Moreira MSA, Sant'Ana AEG, et al. Isolamento de flavonóide das cascas do caule de Aspidosperma tomentosum e atividade analgésica preliminar. 30ª Reun. Anu. da Soc. Bras. Química, Águas de Lindóia: 2007.

[98] Oliveira MP, Silva DJC, Aquino AB, Epifanio WAN, Araújo-Júnior JX, Sant'Ana AEG, et al. Evaluation of the anti-nociceptive and antiinflammatory activities of Aspidosperma tomentosum (Apocynaceae). XVI Congr. Italo-latinoamericano Etnomedicina, La Plata: 2007.

[99] Araújo-Júnior JX, Oliveira MP, Paulino JVS, Aquino PG V, Sant'Ana AEG, Ribeiro EAN. Anti-hypertensive activity of Aspidosperma macrocarpum on spontaneously hypertensive rats. XX Congr. Italo-Latinoamericano Etnomedicina, Fortaleza: 2011.

[100] Suffredini IB, Varella D, de Oliviera a a, Younes RN. In vitro anti-HIV and antitumor evaluation of Amazonian plants belonging to the Apocynaceae family. Phytomedicine 2002;9:175.

[101] Suffredini IB, Bacchi EM, Mary T, Sakuda K, Ohara MT, Younes RN, et al. Antibacterial activity of Apocynaceae extracts and MIC of Tabernaemontana angulata stem organic extract. Revista Brasileira de Ciências Farmacêuticas 2002;38:89–94.

[102] Agripino DG, Leite EML, da Silva RM, Meda CI, Bolzani V da S, Cordeiro I, et al. Screening of brazilian plants for antimicrobial and DNA-damaging activities. I. Atlantic rain forest-Ecological station Juréia-Itatins. Biota Neotropica 2004;4:1–15.

[103] De Souza ACM, Hasimoto e Souza LK, Silva MRR, Oliveira CMA, Kato L, da Silva CC, et al. Propriedades antifúngicas dos alcalóides de Aspidosperma ramiflorum. 29ª Reun. Anu. da Soc. Bras. Química, vol. 46, Águas de Lindóia: 2006.

[104] Tanaka JCA, da Silva CC, de Oliveira AJB, Nakamura C V, Dias Filho BP. Antibacterial activity of indole alkaloids from Aspidosperma ramiflorum. Brazilian Journal of Medicine and Biological Research 2006;39:387–91.

[105] Granato D, Nunes DS, Mattos PP De, Moura E De, Samples W. Chemical and Biological Evaluation of Rejects from the Wood Industry Microbiological activity of the extracts. Brazilian Archives of Biology and Thecnology 2005;48:237–41.

[106] Verpoorte R, Tsoi ATA, Doorne HVAN, Svendsen AB. Medicinal plants of Suriname. I. Antimicrobial activity of some medicinal plants. Journal of Ethnopharmacology 1982;5:221–6.

[107] Verpoorte R, Van Beek TA, Thomassen PHAM, Aandewiel J, Svendsen AB. Screening of antimicrobial activity of some plants belonging to the Apocynaceae and Loganiaceae. Journal of Ethnopharmacology 1983;8:287–302.

[108] Rojas R, Bustamante B, Bauer J, Fernández I, Albán J, Lock O. Antimicrobial activity of selected Peruvian medicinal plants. Journal of Ethnopharmacology 2003;88:199–204.

[109] Ferreira ICP, Lonardoni MVC, Machado GMC, Leon LL, Gobbi Filho L, Pinto LHB, et al. Anti-leishmanial activity of alkaloidal extract from Aspidosperma ramiflorum. Memórias do Instituto Oswaldo Cruz 2004;99:325–7.

[110] Weniger B, Robledo S, Arango GJ, Deharo E, Aragón R, Muñoz V, et al. Antiprotozoal activities of Colombian plants. Journal of Ethnopharmacology 2001;78:193–200.

[111] Ferreira DT, da Silva Jr. J V, Soeira LS, Zanolli LA, Ishikawa NK, Barbosa AM, et al. Avaliação da atividade antifúngica dos extratos etanólicos de raiz, caule e folha de Aspidosperma polyneuron. An. do XI Encontro Química da Região Sul, 2003, p. QO83.

[112] Kohn LK, Pizão PE, Foglio MA, Antônio MA, Amaral MCE, Bittric V, et al. Antiproliferative activity of crude extract and fractions obtained from Aspidosperma tomentosum Mart. Revista Brasileira de Plantas Medicinais Botucatu 2006;8:110–5.

[113] Borneo R, León a. E, Aguirre a., Ribotta P, Cantero JJ. Antioxidant capacity of medicinal plants from the Province of Córdoba (Argentina) and their in vitro testing in a model food system. Food Chemistry 2009;112:664–70.

[114] Campos AR, Lima RCP, Uchoa DE a, Silveira ER, Santos F a, Rao VSN. Pro-erectile effects of an alkaloidal rich fraction from Aspidosperma ulei root bark in mice. Journal of Ethnopharmacology 2006;104:240–4.

[115] Campos a R, Cunha KM a, Santos F a, Silveira ER, Uchoa DE a, Nascimento NRF, et al. Relaxant effects of an alkaloid-rich fraction from Aspidosperma ulei root bark on isolated rabbit corpus cavernosum. International Journal of Impotency Research 2008;20:255–63.

[116] Sperling H, Lorenz A, Krege S, Arndt R, Michel MC. An extract from the bark of Aspidosperma quebracho blanco binds to human penile alpha-adrenoceptors. Journal fo Urology 2002;168:160–3.

[117] Goloni R, Alves NM, Garrote CFD, Paula JR, Valadares MC, Bara MTF, et al. Estudo da toxicidade aguda do Aspidosperma subincanum Martius. Revista Eletrônica de Farmácia 2005;2:89–91.

[118] Pereira MDM, Jácome RLRP, Alcântara AFC, Alves RB, Raslan DS. Alcaloides indólicos isolados de espécies do gênero Aspidosperma (APOCYNACEAE). Quimica Nova 2007;30:970–83.

[119] Santos SR, Rangel ET, Lima JCS, Silva RM, Lopes L, Noldin VF, et al. Toxicological and phytochemical studies of Aspidosperma subincanum Mart. stem bark (Guatambu). Pharmazie 2009;64:836–9.

[120] De Souza Lima MCJ, Soto-Blanco B. Poisoning in goats by Aspidosperma pyrifolium Mart.: biological and cytotoxic effects. Toxicon 2010;55:320–4.

[121] Quignard ELJ, Pohlit AM, Nunomura SM, da Silva Pinto AC, dos Santos EVM, de Morais SKR, et al. Screening of plants found in amazonas state for lethality towards brine shrimp. Acta Amazonica 2003;33:93–104.

Section 3

Extraction and Identification of Phytochemicals

Chapter 8

Bud Extracts as New Phytochemical Source for Herbal Preparations — Quality Control and Standardization by Analytical Fingerprint

D. Donno, G.L. Beccaro, A.K. Cerutti,
M.G. Mellano and G. Bounous

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/59759

1. Introduction

1.1. General, economic, and social notes

1.1.1. Historical background of the gemmotherapy method

The use of buds for therapeutic purposes dates back many centuries. Ancient Indian medicine (Ayurveda) used, and still uses, plant buds for therapeutic purposes. In Western Europe, Galen (second century A.D.) prepared Acopon, one of the most renowned vulnerary balsams of that time, by soaking poplar buds in olive oil for three months. Nicolas De Myrepse, a medieval Greek physician, revived this idea and formulated the famous "Unguentum Populeum."

Buds and sprouts were identified with the persistence of the life cycle, which renewed itself each spring [1, 2]. Paracelsus realized that the different parts of a single plant had distinct properties [3].

In ancient times, physicians, botanists, and naturalists were interested in defining plants and their derivative properties. According to Theophrastus, herb quality was dependent on a series of factors, including plant age, collection method, used plant part, and geographical origin, as well as preparation methods and storage conditions of the final extracts. In the first century AD, Plinio il Vecchio warned his readers about the herbal preparation quality in relation to possible substitutes, pollutants, deterioration due to age, pests, or inadequate storage. Two centuries later, Galen emphasized the importance of distinguishing good quality products on the basis of sensory tests, drug potency, and geographical origin. During the Renaissance,

© 2015 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

scientists and scholars also performed medicinal plant quality control through studies and experiments on fresh plants. Safety, quality, and efficacy, therefore, have always been the key factors distinguishing a therapeutic plant-based product, and over time they have become increasingly complex and important criteria.

Pol Henry was the first researcher who systematically studied bud-preparations. He is therefore considered to be the true "founding father" of gemmotherapy in its current form. He was the first to have the idea of using extracts derived from meristematic tissues rather than adult plant parts for human therapy and proposed a therapeutic method based on analogical protocols. He conducted a series of clinical trials on humans and animals to establish the psychopharmacological effects of, initially, some twenty or so gemmotherapy remedies. The results of his initial works were published in the "Archives Homeopathiques de Normandie" in 1959. Since then, many scientific publications have confirmed the validity of this therapeutic method [8, 9].

Gemmotherapy is a therapeutic method belonging to the field of Biotherapy, which, based on the analogical–biochemical principles of biological drainage, uses hydroglyceroalcoholic solutions of macerated fresh plant extracts in the first decimal dilution for therapeutic purposes [1, 6]. These extracts consist of meristematic tissues, such as buds, sprouts, young stems, rootlets, catkins, the inner root cortex, the young branch cortex, sap, seeds, and other meristematic plant tissues in the growth phase [10]. To express the concept at a taxonomic level, it would be more correct to describe the method as "meristemotherapy," since the used plant tissues are of meristematic origin.

Gemmotherapy/meristemotherapy is therefore a medical method that uses extracts of fresh plant tissues in the growth phase. Sprouts, buds, and other meristematic plant tissues retain all the anabolic faculties of the primitive plant cell, which is capable of developing all the potential no longer found in the adult plant. This therapeutic plant medicine belongs to "renewed phytotherapy," a branch of the biotherapeutic medicines classed between allopathic medicine and homeopathy. It was relatively unknown until a few years ago, but it is now growing considerably, in Italy and elsewhere [13]. The reason for this popularity lies in the fact that bud-preparations are easy to administer: they are sold ready-for-use and simply need to be diluted in water.

1.1.2. New interest and current diffusion of phytotherapy

Phytotherapy has thus developed from knowledge that was handed down over the course of millennia and has been confirmed, modified, or disproved through many pharmacological studies and clinical trials (Figure 1). It has therefore become a fully fledged medical discipline, since the knowledge gleaned from folk medicine has since been subjected to methodical scientific assessment in order to provide evidence of its efficacy [1, 15].

Progressively deteriorating environmental quality, pollution, excessive reliance on technology, continuous innovation of products and synthetic drugs perceived as alien to the organism, the indiscriminate use of many chemicals, and the change in the concept of health, which is no longer seen merely as the absence of disease but as a general sense of well-being, have

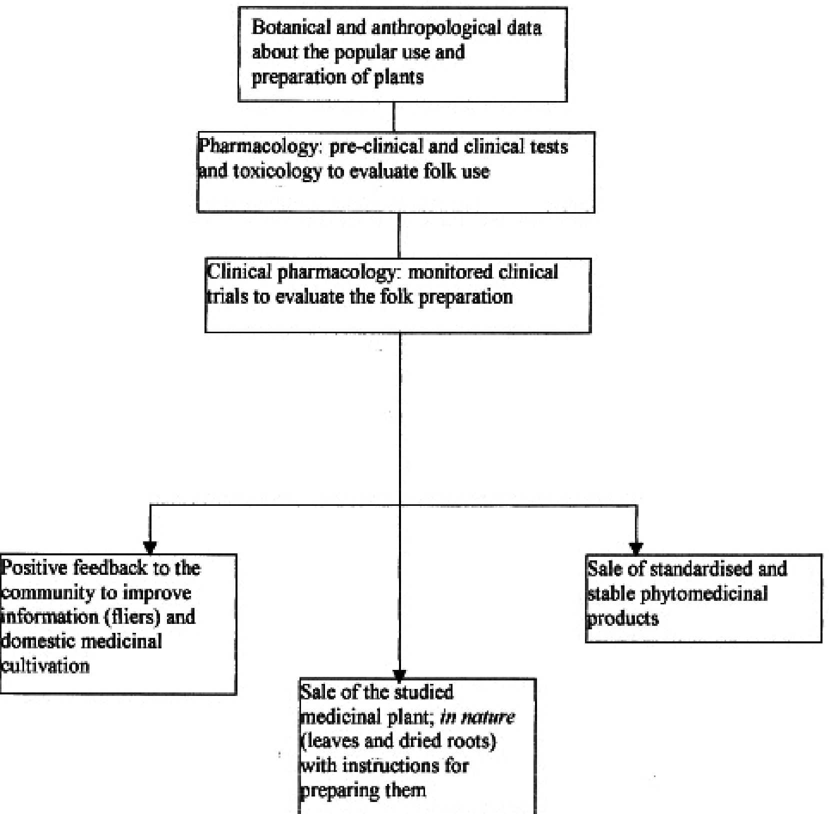


Figure 1. Methods for pharmacological validation of the popular use of medicinal plants [12].

driven "modern people" to "return to nature." The use of phytotherapy has considerably increased in recent years, thanks to important studies confirming the medicinal properties of plant extracts and the continuous growth in scientific knowledge of their positive effects on humans and animals.

However, the use of plants for therapeutic purposes should be assessed by both the treating physician as well as the patient; the bioactive substances found in plants and their derivatives can also cause unwanted side effects, allergic reactions, or interactions with other plants or medicines [17]. For some diseases, moreover, no "miracle plants" are currently available, and so official medicine is the only option. They can, however, replace or complement synthetic drugs for several functional and somatic disorders. For example, many laxatives, painkillers, and tranquilizers can be replaced by a milder treatment based on medicinal plants [18].

The recent ISTAT multipurpose survey "Health conditions and the use of health services," conducted in 2005, has a section on the use of nonconventional therapies, which are also referred to as alternative, complementary, or traditional (at times ambiguously). The survey was carried out on a sample of approximately 60,000 households and showed that 13.9% of

the Italian population (7.9 million) used nonconventional medicine during the three years preceding the survey, particularly homeopathy (7%), manual therapies (6.4%), and phytotherapy (3.7%). There was also a clear prevalence in the north of the country (North-east Italy 21.9%, North-west Italy 17.9%, Central Italy 13.6%, Southern Italy 5.4% and the Italian islands 7%). The users were mostly women (15.8% compared to 11.2% of men) between thirty-five and forty-four years of age.

The inclination to use nonconventional treatment methods increases with the level of education: 18.7% of people with a degree or diploma, 13.5% of people with a middle-school diploma, and 9.2% of those with primary school certificates use these methods. Executives, entrepreneurs, professionals, and office workers, in particular, most often used alternative therapies during the period covered by the survey [19]. According to the ISTAT survey, 71.3% of the patients stated that they chose nonconventional medicine because they considered it less toxic than conventional medical treatments.

General public opinion regarding UMs remained divided. However, 48.8% considered them to be effective and 51.2% regarded them as useless. Men were found to be more critical than women in regard to the usefulness of these methods. Most (73.5%) of the people interviewed in 2005 combined both homeopathy and phytotherapy with standard medicines. Specifically, 44.2% of them tried homeopathy and phytotherapy but mainly relied on conventional medical therapies, whereas 29.3% stated that they had combined conventional medical treatments with alternative therapies, relying mainly on the latter; 17% of the questioned people used homeopathy and phytotherapy exclusively, without combining them with other conventional medical treatments.

More recently, Eurispes indicated that 14.5% of Italians used nonconventional medicines in 2011, a decrease of 4% from 2010. Homeopathy remains the preferred therapy (70.6%), followed by phytotherapy (39.2%), osteopathy (21.5%), acupuncture (21%), and chiropractic (17.2%). There is a greater tendency toward regular use of alternative therapies in other European countries: 49% and 46% of the population in France and Germany, respectively, used it regularly, along with 35%, 31%, and 25% of the population in the United Kingdom, Belgium, and the countries of Northern Europe, respectively [20].

There are many reasons for the increasing interest in and availability of nonconventional medicines. Many patients try them because they have not benefited from traditional medical remedies or because they caused side effects. Moreover, the basic UM doctrines are holistic in nature, i.e., they consider the patient in his or her psychophysical entirety. A particular doctor–patient relationship is thereby established, based on the premise that a specific disorder may originate from a multiplicity of factors [3, 21]. The approach to the problem is therefore not confined to the treatment of a particular symptom, but extends to an analysis of the patient's personal and family background.

The growing popularity of these alternative medicine practices is also reflected in the efforts over the last twenty years by the World Health Organization (WHO), and, more recently, by the National Center for Complementary and Alternative Medicine (NCCAM) of the National

http://dx.doi.org/10.5772/59759

Institute of Health (USA) to establish guidelines for methods to research and evaluate the effectiveness of nonconventional medicine [1, 22].

1.2. Bud-preparations

1.2.1. Buds and others meristematic tissues

Research has shown that, in addition to cytoplasmic substances, meristematic tissue cells also contain plant hormones (auxins, gibberellins, cytokinins, abscisic acid, and ethylene), enzymes, aminoacids, trace elements, vitamins, flavonoids [23, 24], and nucleic acids (DNA and RNA), many bioactive compounds are often found only in trace amounts in adult plants [9, 25]. The effectiveness of bud-preparations is due to the combination of all these substances (called "botanicals"), which constitute the bud-phytocomplex.

Plants preserve meristematic cells in their meristematic tissue. The meristem biochemical profile is not comparable to the adult plant organ profile, because plant growth imposes a specialization aimed at specific biological roles [11, 26]. Meristematic tissues are composed of groupings of meristematic cells: the specialized adult tissue cells are subsequently differentiated from these cells. Buds are particular organs produced by the plant to protect these tissues during the cold season: new plant parts will emerge at the end of this unfavorable period from these tissues [25].

The following parts are used in gemmotherapy: buds, sprouts, rootlets, root cortex, young branch cortex, and seeds. Buds and sprouts are the most common tissues used for the bud-preparation production [8, 27].

The buds spend the winter in a sleeping state, which begins with a period marked by decreasing temperatures or a progressive reduction of the photoperiod. The end of dormancy is determined by the exposure of the buds to relatively low temperatures for a certain number of hours (chilling requirement) and by a decrease in the level of abscisic acid, with an increase in cytokinin and gibberellin content. Indeed, in preparation for winter, abscisic acid is produced in terminal buds: this slows plant growth and directs leaf primordia to develop scales to protect the dormant buds during the cold season. It also inhibits the division of cells in the vascular cambium, adjusting to cold conditions in the winter by suspending primary and secondary growth. Gibberellins and cytokinins are also involved in the natural process of breaking dormancy and cell differentiation [25].

The breaking of dormancy is also associated with an increase in organic acid content and respiration in buds. Organic acid content was inversely related to carbohydrate content in developing buds [28].

Moreover, in the early stages of development, the plant tissues contain specific bioactive compounds: later, these molecules are subjected to metabolic transformations, as the removal/adding of hydroxyl groups, diversifying, for example, the polyphenolic patrimony; in this case, recent histochemical tests revealed the presence of three types of polyphenolic compounds in buds, differing in what their intracellular localization is concerned: granular polyphenols, vacuolar polyphenols, and droplike polyphenols. Finally, the sugar moieties are diversified in

mature organs: in buds, the preferred sugar for glycosylation is galactose, while, later, the glycosylation preferentially occurs with rhamnose [27].

1.2.2. Preparation protocols

The official procedure for bud-preparation production is detailed in the monograph "Homeopathic preparations" published in the 8th edition of the *French Pharmacopoeia* and subsequent editions [29].

This book describes the different stages of the extraction and preparation process:

Collection. Meristematic tissues should be collected in their balsamic period, which generally coincides with late winter–early spring, as the greatest concentration of bioactive compounds occurs at this time.

Cleaning. The newly harvested fresh parts are cleaned thoroughly to remove any foreign parts or soil residues.

Measurement of humidity level and dry weight. Samples are placed in a stove at 105°C to dehydrate for few days in order to reach a changeless weight, defined as the dehydrated weight. This operation allows the quantity of ethanol and glycerol to be calculated for the subsequent maceration step.

Grinding. The clean portion of fresh plant material is manually fragmented, using a mortar, for example, in the case of large buds. This operation facilitates extraction by the solvent.

Maceration. The fresh plant material is left to macerate for 21 days in a mixture of 95% ethanol and glycerol in a 1:1 ratio: the solvent quantities are calculated in order to obtain a 1:20 glycerine macerate, so that the final product is 20 times the weight of the raw material in the dry state. The maceration must be performed immediately after harvesting, to prevent the fresh tissue undergoing enzymatic and oxidative degradation.

Decanting, filtration, and pressing. Once the maceration is finished, the mixture is decanted and then filtered. The filter is pressed using a manual press. The product obtained from the pressing is added to the filtrate, which is then left to settle for another 48 hours. Finally, it is filtered once more, obtaining the mother preparation.

Dilution. The macerate is diluted to the first Hahnemann decimal (DH 1): one part of the mother preparation is diluted with nine parts of a mixture containing 50 parts (by weight) of glycerol, 30 parts of ethanol, and 20 parts of water. The mixture is then stirred for optimal mixing of the preparation. The alcohol content normally attained is 38°.

Checks. The final product is checked to assess and determine the smell and taste (by sensory analysis), color (by spectrophotometry), and alcohol content (e.g., by gas chromatography). A further test is useful to detect the presence of any contaminants, such as molds, yeasts, methanol, or 2-propanol.

http://dx.doi.org/10.5772/59759

1.2.3. Relationship between drugs/bud-preparations and phytochemicals

The principle of pure and pharmacologically active compounds was and remains the goal of pharmaceutical-chemical research in this century. Plants have been considered a very important source of active molecules for direct extraction or as a basis for the synthesis of molecules with specific pharmacological properties. Chemists have identified some of these many compounds and extracted them from plants, and physicians have studied their action mechanisms and therapeutic effects. This has allowed the identification not only of bioactive compound classes, such as flavonoids, saponins, terpenes, but also of some single molecules (caffeic acid, ferulic acid, chlorogenic acid, quercetin, and many more). Pharmaceutical chemistry has thus begun to reproduce their fundamental structures, sometimes modifying them to obtain products with therapeutic effects of equal, if not greater, effect.

Conventional drugs contain a single active compound (represented by molecules by synthesis or by plant extraction), which has the purpose of interacting with a specific tissue-target in order to produce a fast and effective result. They are therefore suitable for acute diseases or in deteriorating chronic conditions [21, 33]. Phytotherapy treatments, like synthetic drugs, act on the organism by chemical substances, which have a pharmacological effect [6]. Due to the large quantity of bioactive compounds found in phytotherapeutic products, many of which act synergistically, there is a preference to attribute the pharmacological effect to the "phytocomplex," rather than to any single active compound, as in the case of standard medicine.

The phytocomplex consists of a combination of different substances, both active principles and other plant components, which contribute to the overall therapeutic effect. The combination of individual components contained in the phytocomplex is able to release a synergistic action as a whole [21] and to reduce the risk of addiction and toxicity, producing a more complete and less drastic pharmacological effect [9] than that of one or a few of its components taken separately.

The main compounds with phytotherapeutic action are the plant secondary metabolites, i.e., compounds without a direct function in regard to growth and development. Unlike the primary metabolites, such as chlorophyll, aminoacids, nucleotides, or simple carbohydrates, they do not have roles in the processes of assimilation, respiration, transport, and differentiation. Although they are widely distributed throughout the plant kingdom, secondary metabolites are normally found in a specific plant species or in a group of related species and are only localized in specific tissues and organs or in particular stages of development [6, 34].

1.3. Main critical points

1.3.1. Final product and supply chain

The current literature on bud-preparations shows a discrepancy regarding the preparation protocols. The *French Pharmacopoeia* requires the bioactive compounds to be extracted in a mixture consisting of ethanol and glycerol in equal parts. It also requires the "mother solution" to be diluted with a mixture of water, alcohol, and glycerol in a 1:10 ratio (one part of base macerated in nine parts of this mixture).

According to some experts, however, if the base preparation is diluted in a ratio of 1.5:10 (a more concentrated product), the bud-preparation will provide a higher number of clinical benefits [8]. Other herbal companies propose yet another maceration method, divided into two steps. During the first step, which lasts for a week, the plant material is macerated in ethanol alone. In the second step, after seven days, a solution of water (revitalized through a particular process) and glycerol in equal parts is added. In other cases, the bud-preparation is prepared by macerating the plant material (100 g) in a 1 L mixture with different extraction solvents, in a 1:2 ratio, while stirring vigorously. After a month, the macerate is filtered and the solvent removed with a rotary evaporator [24].

Another critical point concerns the botanical nomenclature showed in the bud-preparation package. It is not uncommon to find inaccuracies regarding the species names, as in lemon and chestnut herbal products. The latter, for example, is named as *Castanea vesca*, and this scientific name was replaced many years ago by *Castanea sativa*. In the European market, most of the plant material (80%) for herbal preparations is harvested in the wild, but also when harvested from specialized cultivations herbal products are only generally labeled with the species name without any regard to genotype (Figure 2).

Figure 2. No cultivar information is reported on the bud-preparation packages.

No plants are specifically grown for the phytotherapeutic market, and there is a lack of basic knowledge, i.e., researches on the potential qualitative and quantitative differences between different genotypes of a species.

Finally, the bud-preparation regulatory classifications are ambiguous (Figure 3): they have been associated with homeopathic products by the *French Pharmacopoeia*, based on their dilution method, but they can also now be associated with the food supplement category.

Figure 3. Bud-preparation regulatory classification is still contradictory and ambiguous.

1.3.2. Analytical techniques

Compared to other phytotherapic products, bud-preparations are currently affected by critical aspects, due to the total lack of analytical studies and comparative references to other research experiences at national and international levels [38].

An important issue concerns the analysis methods. Technological innovation in the analytical instruments has allowed increasingly sophisticated qualitative and quantitative assessments of the plant extract chemical composition. Chromatographic techniques are mainly used to analyze herbal preparations with some analytical problems related to the complexity of the initial matrix and the nature of the extraction solvents, because glycerol does not allow for the application of certain commonly used analytical techniques. Thin Layer Chromatography (TLC), for example, which is normally used to analyze mother tinctures, cannot be applied to

bud-preparations, because the glycerol does not allow the analyte migration to the chromatographic plate. With paper chromatography and TLC, very small samples could be analyzed, and the resolution and reproducibility could be improved. However, quantitation is still inadequate and the resolution of similar compounds is difficult.

Chromatography includes a range of different instrumental techniques, from the simplest to the most sophisticated (Table 1).

Chromatographic Method	Separation Mechanism
Liquid–solid chromatography	Adsorption
Paper	(Adsorption), partition
Gas liquid chromatography (GLC)	Adsorption, partition
Thin-layer chromatography (TLC)	Adsorption, partition
High-performance liquid chromatography (HPLC)	Adsorption, partition
Ultra-performance liquid chromatography (UPLC)	Adsorption, partition
Supercritical fluid chromatography (SFC)	Adsorption, partition
Liquid–liquid chromatography (LLC)	Partition
Countercurrent chromatography (CCC)	Partition
Ion-exchange chromatography (IEC)	Ion exchange
Capillary electrophoresis	Charge
Ion-pair chromatography	Ion pair formation, ion interaction
Hydrophobic interaction chromatography (HIC)	(Adsorption), partition
Size exclusion chromatography (SEC)	Size of analyte
Affinity chromatography	Biological affinity

Table 1. The major chromatographic methods used in phytochemistry [40].

Their common feature is a separation achieved through the flowing of a mobile phase, the eluent, containing the analytes to be separated, over a fixed phase, the adsorbent, which exerts a selective action on the compounds. Chromatography offers very powerful separation ability, such that the complex chemical components in herbal extracts can be separated into many relatively simple subfractions. Furthermore, the recent approaches of applying hyphenated chromatography and spectrometry such as high-performance liquid chromatography–diode array detection (HPLC–DAD), gas chromatography–mass spectroscopy (GC–MS), capillary electrophoresis–diode array detection (CE-DAD), HPLC–MS, and HPLC–NMR, can provide additional spectral information. This is very helpful for the qualitative analysis and for the on-line structural elucidation.

More specifically, High-Performance Liquid Chromatography is currently the most widely used analytical method, combined with different detectors (diode arrays, mass spectrometry,

and fluorescence). All the compounds in herbal preparations can be analyzed by HPLC because this popular analytical method is easy and not limited by compound volatility and stability.

It is a chemical–physical separation method: different molecules distribute themselves, according to determined relationships, between two separate and distinct phases, one fixed into a column and the other mobile. In this case, there is a real molecules separation, while in other analytical methods, as spectrophotometry, the single compounds remain united and the separation consists in highlighting the different analyte interaction with the radiation. In the analysis of herbal products, reversed-phase (RP) HPLC columns are the most used stationary phases for compound separation. Different factors are involved in chromatographic separation as mobile phase chemical composition, pH of solvents, pump pressure, flow rate, and selected wavelengths [44].

Finally, the implementation of fast and reliable methodologies for analysis based on GC–MS, HPLC–UV–MS, etc., made possible the identification and quantification of known compounds in plants and other organisms [16].

2. Phytochemical composition and quality of gemmotherapy products

Gemmotherapy can provide effective therapy only through the availability of suitable plant extracts that are as uniform as possible. A great deal of information is necessary to ensure the herbal preparation's safety and quality (Table 2).

Botanical source	1. Identity: family, genus, and species of source plant (with authority) and, if relevant, variety and chemotype; common names
	2. Part(s) of plant used
	3. Geographic origin
Growing conditions	1. Wild or cultivated
	2. Use of good agricultural practice
	3. Site and time of harvest stage of growth at harvest
	4. Postharvest treatment (drying, fermentation, etc.)
	5. Storage conditions
	6. Phytosanitary measures pre- and postharvest (including use of and limits for pesticides)
Raw material (e.g., dried plant material)	1. Specifications according to standard reference (e.g., herbal pharmacopoeias)
	2. Identity tests (macroscopic, microscopic, FT-IR, TLC, GC, HPLC, etc.)
	3. Quantitative tests (especially constituents relevant *for* efficacy and/or toxicity–not necessarily the same)

Process applied to starting material	1. Steps in preparation (e.g., separation, extraction processes, solvents)
	2. Methods used
	3. Specific precautions addressing, e.g., light/temperature sensitivity, oxidation
Botanical preparation	1. Standardization criteria (markers: active constituents, other relevant components; plant extract ratio)
	2. Specifications: levels and range for markers
	3. Physicochemical properties of relevant constituents; stability
	4. Purity criteria by chain control or analysis; microbiological, mycotoxins, pesticides, environmental contaminants
	5. Nature and level of excipients; formulation methodology
	6. Storage conditions
End product (food or supplement	1. Fate in food or formulated product
containing the botanical preparation)	2. Fate on industrial processing and/or domestic preparation for consumption

Table 2. Information relating to herbal product identification, characterization, and standardization [50].

2.1. Genotype and bud phenological stage

The bud-preparation quality begins in the field through the species identification before bud collecting. Species identification should be done by botanists or agronomists, as the diagnostic features typically used in botanical identification (leaves, flowers, etc.) are not usually present in the bud-harvesting season. However, some dichotomous keys allow trees to be identified in winter, through bud color and shape analysis [8]. Identification is an important step in the wild plant collection, even if it is often overlooked by the herbal companies: in the European market only 20% of the plants for herbal products are collected in germplasm repositories. Moreover, an individual plant species may have different cultivars, with different genetic characteristics, chemical compositions, and health effects on final product efficacy and consumer's health [52].

The buds are picked up in the early spring, when they emerge from winter sleeping. This time is known as the "balsamic period," a specific phenological stage in which the meristems have high concentrations of bioactive compounds [8]. The balsamic period, specific to each plant part, depends on the plant vegetative cycle; it can also vary because of different sampling sites and years. Tree species development is also influenced by natural factors (endogenous or exogenous), as well as by human factors. These factors can influence the plant organ chemical composition (and the respective herbal products).

2.2. Pedoclimatic conditions and agrotechnique

The herbal preparation quality is not only dependent on intrinsic characteristics (genetic factors), but is also closely linked to the collecting-area pedoclimatic conditions. Indeed, plants

growing in more suitable areas have greater productive potential in terms of product quantity and quality [56]. In the case of plant material for bud-preparation production, knowledge of environmental characteristics, such as the best pedoclimatic conditions for the plant development, can help choose the best raw material to be used for bud-preparations [3, 12].

Each species requires its own best soil and climate, in terms of altitude, latitude, mean temperature, average annual rainfall, light availability, and physico-chemical soil properties. Climatic conditions show a direct effect on plant physiological processes and phenology, such as growth, flowering, and fruit ripening, thus they can also affect the availability of essential metabolites for the active compound biosynthesis [56, 57]. A plant can almost completely lose the ability to synthesize specific bioactive molecules outside of its own habitat [25]. The use of advanced agrotechniques together with a dedicated genetic improvement allow tree species to fit better with the cultivation site and can also improve the herbal product final quality [57].

2.3. Extraction methods

However, the herbal preparation quality is also determined by the following processing and storage procedures. The extraction technique is an important processing step that requires specific chemical and analytical knowledge. The extraction method and the used solvents show a high influence on the extract releasing and bioactivity. Each plant organ, due to the different chemical composition, has its own specific extraction method. The extraction method and its effectiveness depend on the solvent nature, which is linked to the different chemical compound polarities [24, 35], and to the extraction time and temperature [58].

Due to bud-preparation variability and complexity, it is very difficult to control their product quality: the key factors in achieving this objective are determination of the chemical composition and standardization. The definition of an analytic fingerprint allows for qualitative and quantitative phytocomplex component evaluation: for this reason, botanical fingerprints become an effective tool for quality control [44]. This quantity should not be below a minimum level; otherwise the product will not have the proper therapeutic effect.

3. Analysis of phytochemicals in herbal products

3.1. Analytical methods

Identification and quality control of the herbal material can be performed through macroscopic and microscopic techniques and the use of marker compounds [41]: a marker compound is a chemical constituent of a botanical raw material, drug substance, or drug product that is used for identification and/or quality control purposes, especially when the active constituents are not known or identified. The active compounds are responsible for the intended pharmacological activity or synergistic therapeutic effects [35]. It is important to assess the phytochemical composition of herbal medicines in order to study pharmacological and clinical properties (bioactivity and possible side effects) of these products and their bioactive constituents. [44].

Sample preparation is the most important step in the development of analytical methods for the analysis of botanicals and herbal preparations. The basic operation included steps, as prewashing, drying of plant materials or freeze drying, grinding to obtain a homogeneous sample and often improving the kinetics of analyte extraction: the official extraction method, detailed in the *French Pharmacopoeia*, should be chosen in order to produce commercial preparations.

After bioactive compound extraction, in order to evaluate the entire pattern, chromatography combined with a suitable detection technique offers a powerful tool for separating the individual compounds and developing a characteristic profile of the sample, called a fingerprint. In particular, High-Performance Liquid Chromatography (HPLC) is the most popular analytical technique for analyzing herbal products: HPLC is an easy to operate, fully automatable technique with high resolution, selectivity, and sensitivity. One of the main advantages of HPLC is the possibility to couple the technique to different detectors: combining a chromatographic separation system on-line with a spectroscopic detector has become the most important approach for the identification and/or confirmation of the identity of target and unknown chemical compounds in herbal preparations. For most (trace-level) analytical problems in the research field of herbal medicines, the combination of column liquid chromatography with a UV–vis or a mass spectrometer (HPLC–DAD, LC–MS, respectively) becomes the preferred approach for the analysis of herbal medicines. HPLC coupled with UV photodiode array detection (DAD) allow the running of a chromatographic separation with simultaneous detection at different wavelengths. The UV spectra of bud-products give useful information on the type of constituents: indeed, new instruments allow the recording of UV spectra of compounds, which can be performed automatically when screening for known constituents [40]. The data obtained from hyphenated instruments are the so-called two-way data (one way for chromatogram and the other way for spectrum), which provide much more information than the classic one-way chromatography in order to obtain a complete chemical fingerprint. If hyphenated chromatography is further combined with chemometric approaches, clear pictures might be developed for chromatographic fingerprints obtained.

3.2. Statistical data analysis

After selecting an appropriate technique to analyze the bud-preparation samples, an adequate method should be developed, optimized, and validated with a multivariate statistical approach, as the Design of Experiments (DoE) or experimental design. In the multivariate approaches, a predefined number of experiments are performed according to a well-designed experimental setup in order to examine several factors simultaneously. The factors having the most effect on the outcome of a chromatographic analytical method are determined from screening designs: these factors included, amongst others, mobile phase composition, buffer concentration and pH, detection wavelength, column temperature, injection volume, sample concentration, and column type.

Due to the complexity of herbal medicines, and in particular bud-preparations, it is necessary to consider many factors for their fingerprint evaluation: some minor differences between strongly related genotypes may not be observed, but can largely affect the health of the patient.

Classification of, and discrimination between herbal species is an important strategy in the quality control of herbal products [71]: the quality of bud-preparations is closely related to the chemical constituents and their concentration, and might vary slightly according to differences in climate, cultivation conditions, harvest time, drying, and storage [42]. To analyze the large number of generated data, a variety of analytical methods prove to be useful and versatile tools for the extraction, visualization, and interpretation of the information. Pattern recognition methods as Principal Component Analysis (PCA) allow better visualizing of the information that is included in the fingerprints. Exploratory data analysis is easier if represented as a multivariate data table as a low-dimensional plane. The original variables are transformed into latent variables summarizing the systemic patterns of variation between the samples. For this reason, PCA is often applied to chemical data expressed by bud-preparation fingerprinting.

4. Product quality

4.1. Safety

Although botanicals have played a role in the marketing of health products to reduce age effects, there is an increased interest today due to their perceived health benefits. Not only do consumers increasingly take charge of their health, but the scientific information and understanding of the beneficial health effects of bioactive substances in food, functional foods, and food supplements have improved. Increasing use of these products has also led to concerns about their actual safety.

The safety assessment of botanicals and botanical preparations in food and food supplement is complicated by, amongst others, the variability of composition and it should at least involve:

- The origin and characterization of the raw material and its quality control
- The intended use and consequent dosage
- History of use and exposure
- Product comparison(s)
- Toxicological information gathering
- Risk characterization/safety assessment

An increasing number of botanical products are used as food ingredients or supplements and these are a commercially important part of the health and food market. Botanical products may have different formulations, from whole foods to pharmaceutical-like preparations in unit dose form, as tablets, capsules, or drops [75].

The regulatory position with regard to food supplements is unclear (food or medicine?), and the increasing number of products in the food market raises concern about the safety assurance of these products, as many cases of intoxication with the use of botanical products are reported [50]. In some cases, these have resulted from contamination with other plant

species. In addition, different parts of the plant source may have widely different concentrations of toxic constituents, e.g., pyrrolizidine alkaloids [76], and climatic and agronomic differences may lead to great variability in composition [53]. Since compositional variability of bud-products may occur, it is important that the safety-evaluated botanical material is representative of the commercial material and has adequate specifications of identity and purity. Also, identification of the plant material, details of all the applied processes and compositional standards for the finished product are pivotal. Data should be based on a sufficient number of batches to permit the assessment of the natural variability under different agronomic conditions and establishment of appropriate specifications, and sampling plans should take the variability into account [33].

For some botanical products, good epidemiological and clinical human studies addressing both efficacy and safety may be available. However, if a herbal extract was to be used as a food supplement, the safety evaluation may be focused on the effects of the fractionation process, i.e., nature and concentrations of bioactive compounds relative to the traditional source and likely effects on bioavailability and intake [20].

Hazard identification (adverse effects and adverse effect nature) and hazard characterization (human-relevant dose levels) are necessary to determine the nature and level of risk associated with a particular level of herbal preparation exposure (intake) [75]. The risk assessment/safety evaluation relates to the material as consumed, thus data applicable to the finished products are of major importance. Therefore, detailed analytical fingerprint and other data are required to give assurance that the tested material provides relevant information, bearing in mind the possibility of matrix effects, e.g., on bioavailability, absorption, and pharmacokinetic behavior [60]. There is need of adequate chemical information related to all the phenological stages and to all the potential biological properties of the botanical material, also evaluating the possible botanical interactions with pharmaceuticals, aspects not normally considered for food additives [50].

4.2. Standardization

Scientists have been proceeding in a systematic way in order to validate the claims of many medicinal plant species. For some symptoms and ailments, this may be fairly easy to prove, but in more complex health conditions, the situation becomes a bit complicated. Nonetheless, medicinal plant extracts are showing a great deal of promise, even in instances of complexity of illnesses.

Millions of dollars are spent each year on herbal products that are marketed as food supplements, but in reality very few know, chemically, what they are purchasing or using. Very often the dosage varies from the different brands of the same herbal product. The chemistry and efficacy of many of these plants are relatively unknown and there is a chance of toxicity or overdose until the secondary compounds are known and understood [74, 77]. There is a tendency in the United States and in Europe to regulate and license this market, and this has led to greater and more effective use of these important medicinal plants. There is also general agreement that chemical standardization is the way forward in order for herbal remedies to be prescribed to patients who seek to be treated with medicinal plants [2].

http://dx.doi.org/10.5772/59759

Standardization is a method for assuring a minimum level of bioactive ingredients in the extracts and is becoming increasingly important as a means of ensuring a consistent supply of high-quality phyto-pharmaceutical products. It can be defined as the establishment of reproducible pharmaceutical quality by comparing a product with established reference substances, and by defining minimum amounts of one or several compounds or groups of compounds [35]. In general, in the field of phyto-medicines, standardization is only applied to extracts and not to diluted products. Standards for bioactive molecules to be used in medicinal products may be found in monographs and/or pharmacopeias. A pharmacopeia is a collection of quality standards for medicines and their components. In order to obtain marketing authorization for a medical product, the ingredients or the medicinal product must generally comply with a pharmacopeial standard. Thus, pharmacopeial standard may provide guidance on acceptable purity criteria for that ingredient.

It is accepted that concentration or dosages are very important because herbal medicines (in common with conventional medicines) contain biologically active substances that may produce nontrivial side effects when taken in excessive doses. Very low doses, on the other hand, may have no therapeutic value. Moreover, plant material is often highly variable, so that a minimum concentration or a concentration range is often used rather than an exact level. An upper limit is necessary with highly active or potentially harmful ingredients, as most plants have a wide therapeutic window (e.g., a toxic compound is considerably higher than the therapeutic dose). In the case of compounds with a narrow therapeutic window, chemical entities are favored, as opposed to extracts. Standardization also allows comparison of the clinical effectiveness, pharmacological effects and side effects of a series of products (e.g., against a placebo) [5]. Standardized products provide more security and increase the level of trust people have in herbal drugs.

Different kinds of extracts should be considered depending on standardization:

- Standardized extracts: are those for which the bioactive constituents (single or groups) are known. They can thus be standardized to be a defined content of the active constituents giving a clearly defined amount of an active natural product.
- Quantified extracts: are those having constituents with known therapeutic or pharmacological activity. Groups of compounds likely to have the desired pharmacological activity are unknown, but are not solely responsible for the clinical efficacy of the extract. The pharmacopiea monograph must define a range of content of the selected constituent(s) some of which are lead compounds.

Standardization by blending different batches of a herbal drug before extraction, or by mixing different lots of herbal drug preparation, is acceptable but adjustment using excipients is not acceptable [22, 77].

4.3. Quality control

Quality control for natural medicine begins in the field and ends with a safe and effective product being delivered to the patient, followed by post-marketing pharmacovigilance: the evaluation of genetic, agronomic, and environmental parameters is the first critical step in all

the quality control supply chain. It is an evolutionary process. The World Health Organization has recognized this, and developed a series of guidelines to assist nations to develop their strategies for the quality control of herbal medicines.

Phytochemistry has an important and pivotal role in the implementation of each of the quality control steps. Each step requires knowing what is in the plant in terms of bioactive compounds, knowing what is happening to those constituents during the processing of the plant, and assuring appropriate analysis methods when materials are being tested biologically, and eventually delivered to the patient in a finished product [33].

There are serious concerns being expressed all over the world with respect to contamination and adulteration of traditional medicines. Contaminants may include various pesticides, herbicides, insecticides, heavy metals (arsenic, cadmium, lead, and mercury), microbial species, radiation, etc., and adulterants may include other (perhaps cheaper) plant materials with similar biological effects or synthetic drugs [5]. Microbial contamination can be controlled. It is a fact that natural materials harbor a large number of spores and other microorganisms. However, on the contrary, the maximum number or microorganisms allowed is regulated in most pharmacopeia [4].

Quality control is also very important to control side effects (plant extract toxicity). Botanical secondary compounds are not benign molecules. Ecologically speaking, these have evolved as chemical defenses that can repel, stun, poison, or kill other species. It would be easy on anybody's behalf to think that every plant extract is necessarily safe for human consumption. Nonetheless, it would be difficult for an inexpert consumer to distinguish between effective medicines from deadly poisons. Over and above the dosage factor, it is adulteration either accidentally or intentionally.

It is not a simple exercise of applying modern technologies to quality control of the products that have been in constant use for centuries. New technologies for botanical authentication, phytochemical analysis, and biological determination are employed for the development of new strategies to improve the quality control of medicinal plants [4]. Microscopy is less important here as opposed to phytochemical methods. In the case of the herbal extracts, Thin-Layer Chromatography is only feasible for some components, as the flavonoids, while the presence of vital minor compounds may be masked and may not be very visible. It is in this particular instance that the complementarity of analytical methods as HPLC are of paramount importance for analyzing both the lead and the minor compounds, as polyphenols and vitamins.

The use of chromatographic fingerprinting demonstrates to be an effective and efficient tool for the bud-extract quality control of the available herbal products. The complex relationship between the chromatographic fingerprints and efficacy of the herbal medicines could also be one of the most important aspects for the herbal medicine quality control. Chemical fingerprints might be linked to biological assays to provide assurance of efficacy and consistency. In general, the research work on this aspect is far from sufficient to meet the needed criteria: the studies on the analytical fingerprint of herbal preparations in relation to their efficacy are an important task for quality control of these products. At the same time, it is urgent to evaluate

http://dx.doi.org/10.5772/59759

the possible contaminations in phytotherapic medicines (e.g., pesticides, microbial agents, heavy metals, and toxins).

4.4. Phytochemical fingerprint

Few data are available on natural product quality (intended as safety and efficacy) because adequate and validated methods and health care policies for herbal medicine evaluation are still lacking. Currently, only one or two biomarkers were considered for the evaluation of herbal product quality and authenticity, and for the assessment of the quantitative chemical composition of a herbal preparations and in assessing the quantitative herbal composition of a herbal product [81, 44]. This kind of determination, however, does not give a complete picture of a herbal product because multiple constituents are usually responsible for its therapeutic effects: it is necessary a chemical fingerprint be obtained by several analytical techniques.

By definition, a chromatographic fingerprint of a herbal medicine is a chromatographic pattern of the extract of the most common pharmacologically active compounds. With the help of obtained chromatographic fingerprints, the authentication and identification of herbal medicines can be accurately conducted even if the amount and/or concentration of the chemically characteristic constituents are not exactly the same for different samples of this preparation: the chromatographic fingerprints could demonstrate both the “sameness” and “differences” between various samples successfully.

A herbal preparation may contain several natural compounds and, for this reason, a combination of different herbs could create interactions with many of these constituents during the extraction phases: therefore, a chromatographic fingerprint may present a complete representation of all the chemical molecules of a natural medicine.

The chromatographic technique allows obtaining a relatively complete profile of bud-preparations, the chromatographic fingerprint, representing the so-called phytocomplex. Obtaining a good chromatographic fingerprint representing the herbal preparation phytocomplex depended on several analytical factors, such as the extracting methods, the measurement instruments and the measurement conditions, according to other studies [66]. In particular, it is necessary to have a good extracting method, in order to obtain an informative fingerprint of herbal products with almost all the bioactive compounds to represent the integrity of the bud-preparations. Moreover, a chromatogram with good separation and a representative concentration profile of the bioactive components detected by a useful detector, as diode array detector, is also required.

A botanical fingerprint with all of its peaks just completely separated should be featured by maximal information content, and further separation cannot provide any more information and becomes unnecessary. It is a multivariate system, since in general it embraces most of the phytochemical constituents of herbal products [62]. Once different analytical fingerprints are obtained from the same herbal product or from different preparations, first of all it is important to evaluate any similarities and/or differences between them, also considering possible variation sources and their effects (e.g., retention time shift) from one chromatographic pattern to another. Moreover, the concentration profiles changes greatly for the herbal medicines

depending on the different producing places and/or harvest seasons and wrong results would have been obtained by simply seeking the optimal correlation coefficient between the chromatograms.

The construction of chromatographic fingerprints aims at evaluating the bud-product quality. Even if one or two markers or bioactive compounds are currently employed for evaluating the quality and authenticity of a herbal medicine, this determination does not give a complete picture of a herbal product. The fundamental reason of herbal medicine quality control is based on the concept of total herbal phytocomplex in order to identify the real herbal medicine and the false one [41]. If some fingerprints from different extraction methods and/or from different herbal medicines are considered, it is possible to see the same phytochemical constituents or to understand their bioactivities and possible side effects: it is very important for the quality assessment of different samples to determine the presence or absence of interested components among the different chromatographic fingerprints.

4.5. Case study: Phytochemicals in Ribes nigrum bud-preparations

Ribes nigrum L. (Family: *Grossulariaceae*) is commonly used as herbal medicine and it is popularized for its alleged tonic effect and possible curative and restorative properties; for this reason, blackcurrant bud-preparations, derived from embryonic fresh plant tissues, are important therapeutic remedies, prescribed in hepatic, respiratory, circulatory, and inflammatory disorders, but data on their chemical composition are lacking as, to date, phytochemical studies have principally been performed on barks, roots and root exudates, leaves, fruit, and seeds. The most important industrial product of *Ribes nigrum* is fruit; however, due to their particular chemical composition and excellent flavor, leaves and buds are also used in some applications as a raw material for the herbal and cosmetic industries: many people use the buds as medicinal preparation for their anti-inflammatory activity and antidermal diseases (eczema and psoriasis) [86].

By nature herbal preparations are complex matrices, comprising a multitude of compounds that are prone to variation due to environmental factors and manufacturing conditions. The current practice of identifying herbal extracts is by measuring the concentration of the main botanicals; their concentrations are used to characterize the herbal preparations and fingerprinting is recommended by the main pharmacopeias as a potential and reliable strategy for the quality control of complex mixtures.

The aim of this research was to perform an analytical study of *Ribes nigrum* bud-preparations, in order to identify and quantify the main bioactive compounds, obtaining a specific chemical fingerprint to evaluate the single class contribution to herbal preparation phytocomplex. The same analyses were performed using a High-Performance Liquid Chromatograph-Diode Array Detector (HPLC-DAD). In March 2013, samples of *Ribes nigrum* L. buds were picked up in a germplasm repository in San Secondo di Pinerolo, Turin Province (Italy): two different varieties (Rozenthal and Tenah) were sampled in different phenological stages (bud sleeping, bud break, first leaves), in order to test the genotype/sampling time effect on the chemical composition of the final product. Buds were used fresh to produce herbal preparations.

The protocol of bud-preparations is detailed in the monograph "Homeopathic preparations," quoted in the *French Pharmacopoeia*, 8th edition, 1965 [29]. Bioactive compounds were extracted through a cold maceration process for 21 days, in a solution of ethanol (95%) and glycerol, followed by a first filtration, a manual pressing and, after two days of decanting, a second filtration. Macerated samples were then stored at N.A., at 4°C and 95% R.H. Different chromatographic methods were used to analyze the macerated samples, two for polyphenols and one for terpenic compounds, organic acids, and vitamins, respectively. Mineral elements were also considered.

The content of total bioactive compounds in the evaluated bud-preparations is reported in Figure 4. Statistically significant differences were observed among the analyzed samples depending on genotypes and bud phenological stages.

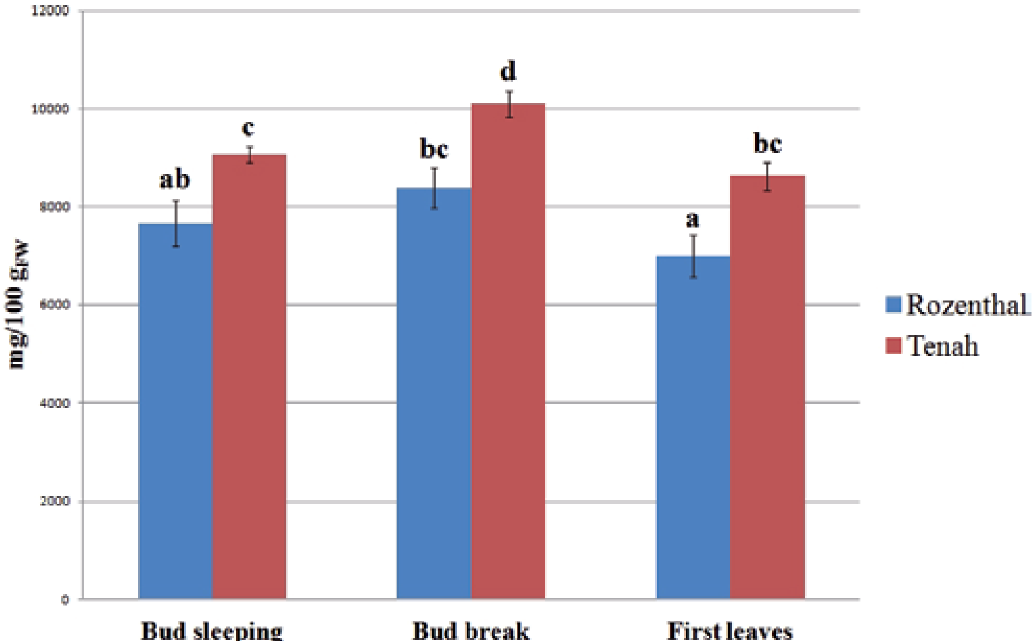


Figure 4. Total bioactive compound content in Ribes nigrum bud-preparations.

Ribes nigrum was identified as a rich source of anti-inflammatory and antioxidant compounds: the observed analytical fingerprint demonstrated that these bud-preparations represent a rich source of organic acids, terpenic and polyphenolic compounds, especially catechins and phenolic acids (Table 3).

Chemical fingerprint showed the prevalence of organic acids and monoterpenes in chemical composition of all the analyzed samples (mean values were considered): the most important class was organic acids (75.57%), followed by monoterpenes (18.45%), polyphenolic compounds (4.94%), vitamins (0.95%), and mineral elements (0.09%) (Figure 5).

	Bioactive Compounds	
	mg/100 g_{FW}	
Benzoic Acids	*gallic acid*	46.018
	ellagic acid	34.270
Cinnamic acids	*caffeic acid*	1.757
	chlorogenic acid	20.354
	coumaric acid	7.645
	ferulic acid	87.175
Catechins	*catechin*	53.026
	epicatechin	63.501
Flavonols	*hyperoside*	n.d.
	isoquercitrin	1.801
	quercetin	68.921
	quercitrin	34.066
	rutin	n.d.
Mineral elements	*calcium*	0.701
	iron	0.165
	magnesium	0.697
	phosphorus	1.626
	potassium	4.694
Monoterpenes	*limonene*	121.583
	phellandrene	163.176
	sabinene	27.607
	γ-terpinene	57.633
	terpinolene	1194.037
Organic acids	*citric acid*	28.063
	malic acid	2189.727
	oxalic acid	57.434
	quinic acid	3259.735
	succinic acid	481.857
	tartaric acid	391.420
Vitamins	*B1*	0.015
	B2	0.002
	C	80.747

Table 3. Information relating to botanical fingerprint of Ribes nigrum bud-preparations.

Analytical fingerprinting could be an important tool to study the assessment of chemical composition and bioactivities of plant-derived products, helping in finding new sources of natural health-promoting compounds: this study allowed to develop an effective tool for quality control through the botanical fingerprinting of bud-preparations.

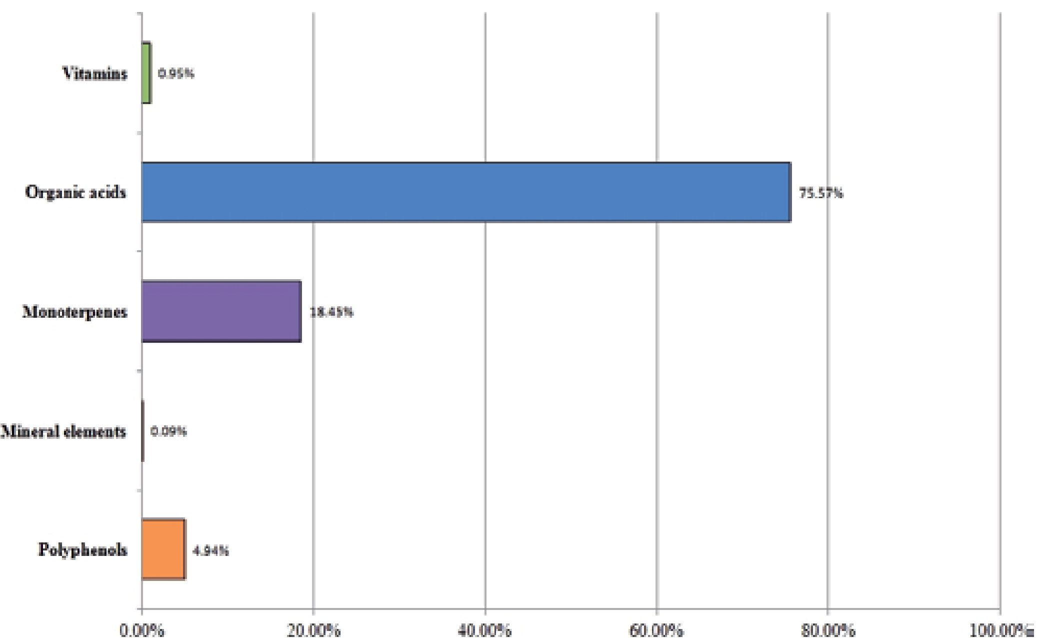


Figure 5. Ribes nigrum bud-preparation phytocomplex.

5. Conclusion

5.1. Herbal preparation supply chain

The creation of specific horticultural models for bud-production could be an interesting opportunity to obtain a high standardized raw material for herbal preparations. If natural products are to be produced for utilization in the pharmaceutical industry, there will be certain criteria that a system of production must meet. First, it clearly must be economical: if the drug costs hundreds to thousands of dollars per dose, there is no viable product [39].

The production system must be sustainable and reliable because the plant part has to be harvested directly from its source (thus endangering the survival of the species), and the natural compound extraction may not be economically viable [8]. A source of bioactive compounds must be available to meet different medical needs, but today's society requires that production of natural products to be environmentally safe and nonenvironmentally impacting. Alternative methods for herbal preparation production include cultivation of the target species, synthesis, and biotech production. These approaches closely follow the original phytochemical study in order to assess the quality and yield of the production and the purity of the final product, but to be economical, these methods must also include fast analytical techniques.

To meet these criteria and establish a viable production system, all the steps of production of a natural product must be systematically evaluated. First, a source of bioactive substances must be identified: cultivars of the same species must be considered in order to choose the highest and the most stable concentration of the natural compounds. Once this source has been identified, an uninterruptible and stable supply of plant material must be secured. This means that an agronomic system for biomass production must be developed in order to allow the full expression of the genetic capability of the cultivar.

Climate and soil should fit with the species requirements. The impact of fertilization and irrigation on the biomass production and its chemical constituents must be understood and, generally, the economic growth and cultivation of the raw material must be explored [53]. In the future, a plant production of secondary metabolites may be controlled with different growth regulators according to Good Agriculture Practices (G.A.P.) used in this agronomic production [90].

The successful production of biomass should include the development of appropriate harvest techniques. Topics to consider are the determination of the best bioactive compound concentration in the plant material during the growing season and the handling of freshly harvested biomass to retain bioactive content: the choice of the best phenological stage during bud-harvesting period is a fundamental step to obtain high values of bioactive compounds in bud-products. Moreover, mechanization of the harvest process must be addressed in order to maintain an economic system of production. Harvested biomass should require an economic processing facility in order to process biomass for the plant material isolation over an entire calendar year, not just immediately after harvesting. Therefore, technology should be developed to stabilize the biomass so that it retains bioactive content during storage before its ultimate processing: this usually involves developing an appropriate drying process. For this reason, analytical fingerprint could be also an important tool to evaluate the bioactive compound stability during product storage.

Finally, once the biomass processing is initiated, the extraction–purification system should be economical and efficient in its recovery of the bioactive compounds from the biomass. Moreover, it should be safe in its operation, and the generation of waste products should be minimized so that there is no deleterious environmental impact from the material processing.

5.2. Directions for further studies

Research into medicinal plants and the search for plant-derived preparations require a multidisciplinary approach with integrated projects, financial and technical support, and a very carefully planned strategy [12]. The research field of herbal medicine quality control is really an interdisciplinary research: it needs a crossover of chemistry, agricultural science, pharmacology, medicine, and even statistics to provide a platform for the quality control of herbal products and further discovery of the novel therapeutics composed of multiple chemical compounds [44]. The aims should consider demands in terms of public health, preservation of Biodiversity and the technical qualification of each laboratory or research group involved ; advances in technology and knowledge of natural products should be viewed not merely from the perspective of drug development, but also as a special tool for the understanding of

http://dx.doi.org/10.5772/59759

biological phenomenon in order to contribute to the well-being of humanity [4]. Chemical analysis of extracts from plant material could play a central role in the development and modernization of natural medicines, botanicals and herbal preparations.

In order to evaluate the entire substances pattern of complex bud-products, chromatography offers a powerful tool for separating the individual compounds and hereby creates a specific fingerprint profile. Later on, when the analyses are performed, data handling methods should be used to extract the useful information residing within the enormous amounts of generated data. The use of an experimental design for the selection of the most influential factors and their optimization is important.

Once the analytical method is established, the herbal fingerprints can be recorded. Although major improvements in the quality control of herbal medicines have been made, proper fingerprint analysis does not only include an adequate choice of the analytical technique [40]. For this reason, more attention has been paid to the optimization of the extraction and separation procedures using appropriate experimental designs, even if an adequate preprocessing of the fingerprints and a critical validation of the results obtained by the data handling techniques are too often neglected [41].

Compounds as the macromolecules (e.g., polysaccharides), a major part of the bioactive compounds in herbal species, are often not considered in herbal fingerprinting, and should be taken into account during future development of quality control criteria. For this, several innovative methods based on the interaction with biological systems and dialysis methods are at the point of breakthrough and could allow scientists to approach quality control issues from other angles, reducing the complexity of the matrix and focusing on the directly related biologically active compounds. Accordingly, the biological activity of herbal samples can be integrated in the fingerprint development, focusing on the relevant biological information.

Just as for pharmaceutical products, a well-validated method can help in monitoring the stability of the botanicals and herbal preparations over a period of time: the fingerprint could be also applied in this direction. It is likely that the combination of chemical standardization with biological assay could provide further knowledge about therapeutic effects of the medicinal plants [37].

Finally, the use of validated methods in the chemical standardization of botanicals and herbal preparations could enhance the quality of the products, assist in pharmacological studies, perform credible clinical trials and propel the move toward evidence-based medicine.

Author details

D. Donno*, G.L. Beccaro, A.K. Cerutti, M.G. Mellano and G. Bounous

*Address all correspondence to: dario.donno@unito.it

Department of Agricultural, Forest and Food Sciences, University of Turin, Grugliasco (TO), Italy

References

[1] Firenzuoli F. *Interazioni tra erbe, alimenti e farmaci*. Milano: Tecniche Nuove; 2009, p. 360.

[2] Khan IA. Issues related to botanicals. *Life Sciences* 2006; 78: 2033-8.

[3] Lockwood GB. Fundamentals of pharmacognosy and phytotherapy. *Phytochemistry* 2005; 66: 1636-7.

[4] Cordell GA. Phytochemistry and traditional medicine – A revolution in process. *Phytochem Lett* 2011; 4: 391-8.

[5] Gurib-Fakim A. Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Mol Aspects Med* 2006; 27: 1-93.

[6] Firenzuoli F. *Fitoterapia. Guida all'uso clinico delle piante medicinali*. III edn. Milano: Masson; 2002, p. 411.

[7] Fowler MW. Plants, medicines and man. *J Sci Food Agri* 2006; 86: 1797-804.

[8] Bertinat C. GM, Pilotti PP. *La filiera del gemmoterapico*. Torino: Simbiosi; 2007.

[9] Brigo B. *L'uomo, la fitoterapia, la gemmoterapia. 211 sindromi cliniche trattate con fitocomplessi e gemmoderivati*. Milano, Italy: Tecniche Nuove; 2009, p.768.

[10] Phillipson JD. Phytochemistry and pharmacognosy. *Phytochemistry* 2007; 68: 2960-72.

[11] Amaral JS, Seabra RM, Andrade PB, Valentao P, Pereira JA and Ferreres F. Phenolic profile in the quality control of walnut (*Juglans regia* L.) leaves. *Food Chem* 2004; 88: 373-9.

[12] Rates SMK. Plants as source of drugs. *Toxicon* 2001; 39: 603-13.

[13] Foroncewicz B, Mucha K, Gryszkiewicz J, et al. Dietary supplements and herbal preparations in renal and liver transplant recipients. *Transplant Proc* 2011; 43: 2935-7.

[14] Nicoletti M. Nutraceuticals and botanicals: Overview and perspectives. *Int J Food Sci Nutri* 2012; 63 Suppl 1: 2-6.

[15] Phillipson JD. Phytochemistry and medicinal plants. *Phytochemistry* 2001; 56: 237-43.

[16] Macías FA, Galindo JLG and Galindo JCG. Evolution and current status of ecological phytochemistry. *Phytochemistry* 2007; 68: 2917-36.

[17] McChesney JD, Venkataraman SK and Henri JT. Plant natural products: Back to the future or into extinction? *Phytochemistry* 2007; 68: 2015-22.

[18] Penso G. *Piante medicinali nella terapia medica: compendio di farmacognosia pratica per medici e farmacisti*. Organizzazione editoriale medico farmaceutica; II edn. Milano: 1987, p. 249.

[19] Silano V, Coppens P, Larranaga-Guetaria A, Minghetti P and Roth-Ehrang R. Regulations applicable to plant food supplements and related products in the European Union. *Food Funct* 2011; 2: 710-19.

[20] Konik EA, Jungling RC and Bauer BA. Herbs and dietary supplements in the European Union: A review of the regulations with special focus on Germany and Poland. *J Diet Suppl* 2011; 8: 43-57.

[21] Ercoli A. *Clinica medica in fitogemmoterapia e omeopatia*. Milano: Tecniche Nuove; 2002, p. 275.

[22] Bent S and Ko R. Commonly used herbal medicines in the United States: A review. *Am J Med* 2004; 116: 478-85.

[23] Campanini E. *Dizionario di fitoterapia e piante medicinali*. Milano: Tecniche Nuove; 2012, p. 952.

[24] Jahan N. Correlation of polyphenolic contents of indigenous medicinal plants with their bioactivities. Department of Chemistry & Biochemistry, Faculty of Sciences Faisalabad: University of Agriculture, 2011, p. 214.

[25] VV.AA. *General Arboriculture*. Bologna: Pàtron Editore; 2012, p. 536.

[26] Suglia L. *La verde età*. FITO·NewS. Milano: Fitomedical, 2003.

[27] Peev CI, Vlase L, Antal DS, Dehelean CA and Szabadai Z. Determination of some polyphenolic compounds in buds of *Alnus* and *Corylus* species by HPLC. *Chem Natur Comp* 2007; 43: 259-62.

[28] Wang SY, Ji ZL and Faust M. Metabolic changes associated with bud break induced by thidiazuron. J Plant Growth Regul 1987; 6: 85-95.

[29] Pharmaciens ONd. Pharmacopée Française, Codex Medicamentarius Gallicus, Codex Français: Monographie, Préparations Homéopathiques. In: Population Mdlspedl (ed.), VIII edn. Paris: 1965.

[30] Espín JC, García-Conesa MT and Tomás-Barberán FA. Nutraceuticals: Facts and fiction. *Phytochemistry* 2007; 68: 2986-3008.

[31] Boni U and Patri G. *Scoprire, riconoscere, usare le Erbe*. Milano: Fabbri Edizioni; 2006, p. 730.

[32] Newman DJ and Cragg GM. Natural products as sources of new drugs over the last 25 years. *J Natur Prod* 2007; 70: 461-77.

[33] Amagase H and Farnsworth NR. A review of botanical characteristics, phytochemistry, clinical relevance in efficacy and safety of *Lycium barbarum* fruit (Goji). *Food Res Int* 2011; 44: 1702-17.

[34] Sabandar CW, Ahmat N, Jaafar FM and Sahidin I. Medicinal property, phytochemistry and pharmacology of several *Jatropha* species (*Euphorbiaceae*): A review. *Phytochemistry* 2013; 85: 7-29.

[35] Ong ES. Extraction methods and chemical standardization of botanicals and herbal preparations. *J Chromatography* B 2004; 812: 23-33.

[36] Qiao X, Yang WZ, Guo DA and Ye M. Extraction, separation, detection, and structural analysis of flavonoids. *Curr Org Chem* 2011; 15: 2541-66.

[37] Chandra A, Rana J and Li Y. Separation, identification, quantification, and method validation of anthocyanins in botanical supplement raw materials by HPLC and HPLC–MS. *J Agric Food Chem* 2001; 49: 3515-21.

[38] Dvaranauskaitė A, Venskutonis PR, Raynaud C, Talou T, Viškelis P and Sasnauskas A. Variations in the essential oil composition in buds of six blackcurrant (Ribes nigrum L.) cultivars at various development phases. *Food Chem* 2009; 114: 671-9.

[39] Barnes S and Prasain J. Current progress in the use of traditional medicines and nutraceuticals. *Curr Opin Plant Biol* 2005; 8: 324-8.

[40] Marston A. Role of advances in chromatographic techniques in phytochemistry. *Phytochemistry* 2007; 68: 2786-98.

[41] Tistaert C, Dejaegher B and Heyden YV. Chromatographic separation techniques and data handling methods for herbal fingerprints: A review. *Anal Chim Acta* 2011; 690: 148-61.

[42] Hibbert DB. Experimental design in chromatography: A tutorial review. *J Chromatography* B 2012; 910: 2-13.

[43] Steinmann D and Ganzera M. Recent advances on HPLC/MS in medicinal plant analysis. *J Pharmaceut Biomed Anal* 2011; 55: 744-57.

[44] Liang Y-Z, Xie P and Chan K. Quality control of herbal medicines. *J Chromatography B* 2004; 812: 53-70.

[45] Peev CI, Dehelean CA, Antal DS, Negrea P and Tamas M. The analysis of the main mineral elements in foliar buds of some medicinal plants. *Rev Chim* 2006; 57: 494-7.

[46] Donno D, Beccaro GL, Mellano MG, Bonvegna L and Bounous G. *Castanea* spp. buds as a phytochemical source for herbal preparations: Botanical fingerprint for nutraceutical identification and functional food standardisation. *J Sci Food Agric* 2014: n/a-n/a.

[47] Donno D, Beccaro GL, Mellano MG, Cerutti AK and Bounous G. Medicinal plants, chemical composition and quality: May blackcurrant buds and blackberry sprouts be a new polyphenol source for herbal preparations? *J Appl Botany Food Qual* 2013; 86: 79-89.

[48] Píry J and Príbela A. Application of high-performance liquid chromatography to the analysis of the complex volatile mixture of blackcurrant buds (*Ribes nigrum* L.). *J Chromatography A* 1994; 665: 105-9.

[49] Ravandeh M, Valizadeh J, Noroozifar M and Khorasani-Motlagh M. Screening of chemical composition of essential oil, mineral elements and antioxidant activity in Pulicaria Undulata (L.) C. A. Mey from Iran. *J Med Plants Res* 2011; 5: 2035-40.

[50] Kroes R and Walker R. Safety issues of botanicals and botanical preparations in functional foods. *Toxicology* 2004; 198: 213-20.

[51] Donno D, Beccaro GL, Mellano GM, Cerutti AK, Canterino S and Bounous G. Effect of agronomic and environmental conditions on chemical composition of tree-species buds used for herbal preparations. *Int J Plant Res* (VEGETOS) 2012; 25: 21-9.

[52] Kerslake MF and Menary RC. Varietal differences of extracts from blackcurrant buds (*Ribes nigrum* L.). *J Sci Food Agric* 1985; 36: 343-51.

[53] Lakusic B, Ristic M, Slavkovska V, Milenkovic M and Lakusic D. Environmental and seasonal impacts on the chemical composition of *Satureja horvatii* SILIC (*Lamiaceae*) essential oils. *Chem Biodivers* 2011; 8: 483-93.

[54] Garland SM, Menary RC and Claye CJ. Variation during dormancy and the effect of freezing and postharvest incubation on the chemical composition of blackcurrant buds (*Ribes nigrum* L.). *J Horticult Sci Biotechnol* 2002; 77: 489-97.

[55] Tibaldi G, Fontana E and Nicola S. Growing conditions and postharvest management can affect the essential oil of *Origanum vulgare* L. ssp *hirtum* (Link) Ietswaart. *Ind Crop Prod* 2011; 34: 1516-22.

[56] Vegvari G, Brunori A, Sandor G, Jocsak I and Rabnecz G. The influence of growing place on the rutin content on *Fagopyrum esculentum* and *Fagopyrum tataricum* varieties seeds. *Cereal Res Commun* 2008; 36: 599-602.

[57] Capasso F, Grandolini G and Izzo AA. *Fitoterapia – Impiego razionale delle droghe vegetali*. III edn. Milano: Springer; 2006, p.1031.

[58] Yang D, Wang Q, Ke L, Jiang J and Ying T. Antioxidant activities of various extracts of lotus (*Nelumbo nuficera* Gaertn) rhizome. *Asia Pacific J Clin Nutri* 2007; 16 Suppl 1: 158-63.

[59] Raskin I, Ribnicky DM, Komarnytsky S, et al. Plants and human health in the twenty-first century. *Trends Biotechnol* 2002; 20: 522-31.

[60] Rietjens IM, Slob W, Galli C and Silano V. Risk assessment of botanicals and botanical preparations intended for use in food and food supplements: Emerging issues. *Toxicol Lett* 2008; 180: 131-6.

[61] Gong F, Liang Y-Z, Xie P-S and Chau F-T. Information theory applied to chromatographic fingerprint of herbal medicine for quality control. *J Chromatography A* 2003; 1002: 25-40.

[62] Gong F, Wang B-T, Liang Y-Z, Chau F-T and Fung Y-S. Variable selection for discriminating herbal medicines with chromatographic fingerprints. *Analy Chim Acta* 2006; 572: 265-71.

[63] Qiao X, Han J, He WN, et al. Chemical fingerprint of commercial *Radix echinopsis* and quantitative analysis of alpha-terthienyl. *J Sep Sci* 2010; 33: 530-8.

[64] Zhao ZY, Dong LL and Lin F. Fingerprint analysis of *Euonymus alatus* (Thuhb) siebold by LC-DAD and LC-ESI-MS. *Chromatographia* 2009; 69: 429-36.

[65] Zhou Y, Jiang SY, Ding LS, et al. Chemical fingerprinting of medicinal plants "Guijiu" by LC-ESI multiple-stage MS. *Chromatographia* 2008; 68: 781-9.

[66] Xu C-J, Liang Y-Z, Chau F-T and Heyden YV. Pretreatments of chromatographic fingerprints for quality control of herbal medicines. *J Chromatography A* 2006; 1134: 253-9.

[67] Liu M, Liu S, Shi X, Zhang YD and Wang Q. Quality evaluation of a herbal prescription through quantification of 40 components by HPLC-ESI-MS/MS. *Phytochem Anal: PCA* 2012; 23: 365-72.

[68] Liang QL, Qu J, Luo GA and Wang YM. Rapid and reliable determination of illegal adulterant in herbal medicines and dietary supplements by LC/MS/MS. *J Pharmaceut Biomed Anal* 2006; 40: 305-11.

[69] Uchiyama N, Kikura-Hanajiri R, Kawahara N and Goda Y. Identification of a cannabimimetic indole as a designer drug in a herbal product. *Forensic Toxicol* 2009; 27: 61-6.

[70] Donno D, Beccaro GL, Mellano MG, Cerutti AK, Marconi V and Bounous G. Botanicals in *Ribes nigrum* bud-preparations: An analytical fingerprinting to evaluate the bioactive contribution to total phytocomplex. *Pharm Biol* 2013; 51: 1282-92.

[71] Xu S, Yang L, Tian R, et al. Species differentiation and quality assessment of *Radix Paeoniae Rubra* (Chi-shao) by means of high-performance liquid chromatographic fingerprint. *J Chromatography A* 2009; 1216: 2163-8.

[72] Ieri F, Martini S, Innocenti M and Mulinacci N. Phenolic distribution in liquid preparations of Vaccinium myrtillus L. and Vaccinium vitis idaea L. *Phytochem Anal: PCA* 2013; 24: 467-75.

[73] Carratu B, Federici E, Gallo FR, et al. Plants and parts of plants used in food supplements: an approach to their safety assessment. *Ann Ist Super Sanita* 2010; 46: 370-88.

[74] Latif A and Rahman SZ. Drug safety in traditional herbal medicine. *J Pharmacol Toxicol Meth* 2007; 56: e47.

[75] Singh D, Gupta R and Saraf SA. Herbs-are they safe enough? An overview. *Crit Rev Food Sci Nutri* 2012; 52: 876-98.

[76] Betz JM, Eppley RM, Taylor WC and Andrzejewski D. Determination of pyrrolizidine alkaloids in commercial comfrey products (*Symphytum* sp.). *J Pharmaceut Sci* 1994; 83: 649-53.

[77] Labadie RP. Problems and possibilities in the use of traditional drugs. *J Ethnopharmacol* 1986; 15: 221-30.

[78] Hardy G. Nutraceuticals and functional foods: Introduction and meaning. *Nutrition* 2000; 16: 688-9.

[79] Dell'Agli M, Di Lorenzo C, Badea M, et al. Plant food supplements with anti-inflammatory properties: A systematic review (I). *Crit Rev Food Sci Nutri* 2013; 53: 403-13.

[80] Ganzera M. Quality control of herbal medicines by capillary electrophoresis: Potential, requirements and applications. *Electrophoresis* 2008; 29: 3489-503.

[81] Liu EH, Qi LW, Li K, Chu C and Li P. Recent advances in quality control of traditional Chinese medicines. *Combinat Chem High Throughput Screen* 2010; 13: 869-84.

[82] Sheridan H, Krenn L, Jiang RW, et al. The potential of metabolic fingerprinting as a tool for the modernisation of TCM preparations. *J Ethnopharmacol* 2012; 140: 482-91.

[83] Fan X-H, Cheng Y-Y, Ye Z-L, Lin R-C and Qian Z-Z. Multiple chromatographic fingerprinting and its application to the quality control of herbal medicines. *Anal Chim Acta* 2006; 555: 217-24.

[84] Tabart J, Franck T, Kevers C, et al. Antioxidant and anti-inflammatory activities of *Ribes nigrum* extracts. *Food Chem* 2012; 131: 1116-22.

[85] Tabart J, Kevers C, Evers D and Dommes J. Ascorbic acid, phenolic acid, flavonoid, and carotenoid profiles of selected extracts from *Ribes nigrum*. *J Agric Food Chem* 2011; 59: 4763-70.

[86] Dvaranauskaite A, Venskutonis PR, Raynaud C, Talou T, Viškelis P and Dambrauskiene E. Characterization of steam volatiles in the essential oil of black currant buds and the antioxidant properties of different bud extracts. *J Agric Food Chem* 2008; 56: 3279-86.

[87] Cerutti AK, Beccaro GL, Bagliani M, Donno D and Bounous G. Multifunctional Ecological Footprint Analysis for assessing eco-efficiency: A case study of fruit production systems in Northern Italy. *J Cleaner Prod* 2013; 40: 108-17.

[88] Cerutti AK, Bruun S, Donno D, Beccaro GL and Bounous G. Environmental sustainability of traditional foods: The case of ancient apple cultivars in Northern Italy assessed by multifunctional LCA. *J Cleaner Prod* 2013; 52: 245-52.

[89] Joubert E, Richards ES, Van der Merwe JD, De Beer D, Manley M and Gelderblom WCA. Effect of species variation and processing on phenolic composition and in vi-

tro antioxidant activity of aqueous extracts of *Cyclopia* spp. (honeybush tea). *J Agric Food Chem* 2008; 56: 954-63.

[90] Donno D, Beccaro GL, Mellano MG, Canterino S, Cerutti AK and Bounous G. Improving the nutritional value of kiwifruit with the application of agroindustry waste extracts. *J Appl Botany Food Qual* 2013; 86: 11-5.

[91] Spinelli R, Schweier J and De Francesco F. Harvesting techniques for non-industrial biomass plantations. *Biosyst Eng* 2012; 113: 319-24.

[92] Amaral S, Mira L, Nogueira JMF, da Silva AP and Florencio MH. Plant extracts with anti-inflammatory properties – A new approach for characterization of their bioactive compounds and establishment of structure-antioxidant activity relationships. Bioorg Med Chem 2009; 17: 1876-83.

Section 4

Effect of Stress on Phytochemical Activation

Chapter 9

Effects of Abiotic Stress (UV-C) Induced Activation of Phytochemicals on the Postharvest Quality of Horticultural Crops

Rohanie Maharaj

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/60050

1. Introduction

Phytochemicals are bioactive non-nutrient plant components that have gained considerable attention as photoprotective agents in providing certain health benefits and are well known for their powerful antioxidant and free radical scavenging potential [1-2, 8]. Phytochemicals found in plant-based foods (fruits, vegetables, legumes, nuts, cereals, and grains) belong to several classes according to their chemical structures and physiological functions and include polyphenols, flavonoids, isoflavonoids, phytoalexins, phenols, anthocyanidins, nitrogen compounds (polyamines), chlorophyll derivatives, beta carotene (pro-vitamin A), and other carotenoids, ascorbic acid (vitamin C), folic acid, and α-tocopherol (vitamin E). Figure 1 illustrates the classification of dietary phytochemicals, which are widely distributed with different structures at the tissue, cellular, and sub-cellular levels. While it has been reported that there are over 4,000 phytochemicals, the most studied are the phenolics (largest category of phytochemicals) and carotenoids [3, 5], whereas some phytochemicals are distributed only among limited taxonomic groups. For example, glucosinolates are only found in the cruciferous vegetables crops, whereas the occurrence of sulfides is restricted to the Liliaceae. Additionally, each fruit and vegetable species has a distinct profile of phytochemicals, which is also within a special phytochemical group [4]. Many phytochemicals, particularly the pigment molecules, are often concentrated in the outer layers of the various plant tissues [5]. Some sources of phytochemicals include lycopene from tomatoes, isoflavones from soy, β-carotene from carrots, and anthocyanins from blueberries and grapes. The phytochemicals lutein and zeaxanthin are carotenoids found in spinach, kale, and turnip greens. Another group of phytochemicals called allyl sulfides are found in garlic and onions. http://www.cancer.org/treatment/treatmentsandsideeffects/complementaryandalternativemedicine/herbsvitaminsandminerals/phytochemicals

© 2015 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

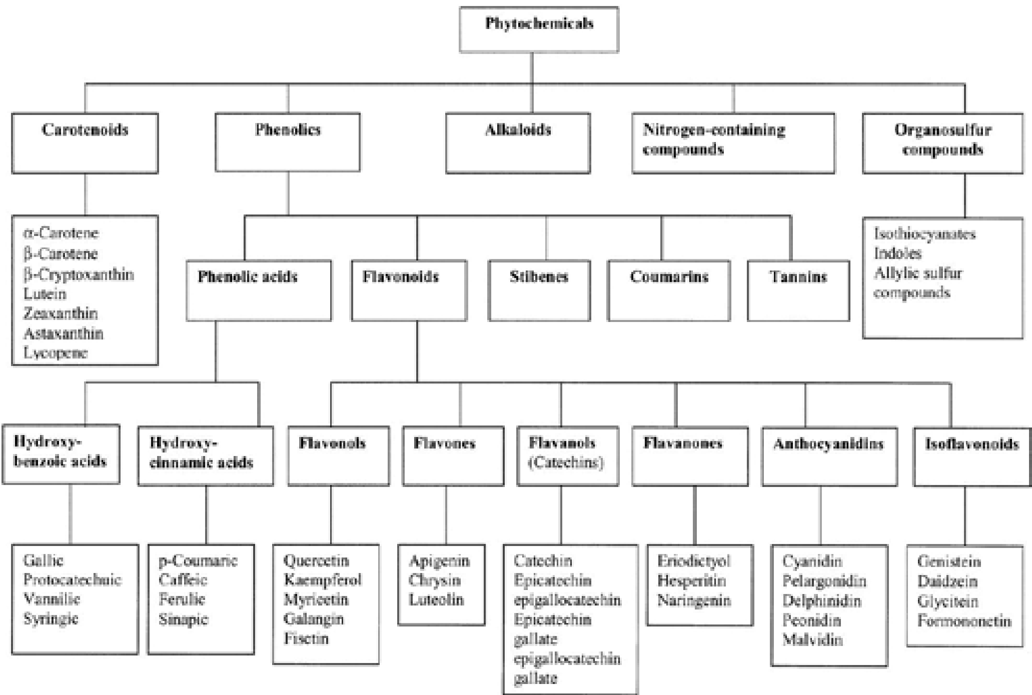


Figure 1. Classification of dietary phytochemicals (Adapted from [3]).

The beneficial effects of phytochemicals are mediated through several mechanisms that include enzyme stimulation, interference with DNA replication, anti-bacterial effect, and physical action. Enhancing the health benefits of fresh produce through physical methods could therefore add value and create new opportunities for growers and processors by tapping into health-oriented markets [12, 57]. Abiotic stresses (negative impact of non-living environmental factors on growth and productivity of crops) are significant determinants of quality and nutritional value of crops along the food value chain. While abiotic stress is essentially unavoidable, simple modifications to postharvest handling systems may result in significant reduction in stress exposure, which will positively impact on phytochemical content and storage and/or shelf-life extension [7, 10]. There is a need to provide technologies that can ensure the delivery of high-quality products with high levels of the desired phytonutrients [6, 11-12, 15]. There is now considerable literature on the use of low dose UV-C radiation to control postharvest pathogens, delay postharvest senescence, and improve shelf-life of many horticultural crops. It also has potential for commercial use as a surface treatment in the food industry, particularly for whole and fresh-cut produce. However, the use of UV-C radiation as an elicitor of phytochemicals which enhances additional health benefits and mechanisms related to improvement in shelf-life or increase in the content of phytonutrients are still not well understood. The objective of this chapter will focus on a review of recent studies of UV-C abiotic stress on the production and activation of phytochemicals and its effect on the quality of crops.

http://dx.doi.org/10.5772/60050

2. UV radiation and its interactions with biological systems

UV radiation is generally divided into three classes: UV-C (200-280 nm), UV-B (280-320 nm), and UV-A (315-400 nm) [21]. Each band can induce significantly different biological effects in plant systems. UV-C wavelengths (highly energetic) result in high levels of damage very quickly [22]; however, such radiations are effectively absorbed by ozone and atmospheric gases and are not present in sunlight at the earth's surface. UV radiation below 320 nm is actinic, which means it causes photochemical reactions [21]. Such reactions occur as a result of the absorption of photons by chromophores (chemical grouping of molecules) at particular wavelengths.

2.1. Direct and indirect effects of UV radiation

The destructive action of UV radiation results from both direct absorption of photons by DNA and indirect mechanisms involving excitation of photosensitizers and the generation of reactive oxygen species (ROS). Nucleic acids, proteins, lipids, indole acetic acids, flavor-proteins, and phytochromes are molecules that contain conjugated double bonds and absorb energy in the UV region. They have key roles in plant cell function and structure and any alterations of these compounds due to UV radiation might be expected to cause physiological alterations in crops. Plants may differ in resistance to ROS depending upon the efficiency of the plant defense systems that involve DNA repair systems and quenching systems involving either a suppression of ROS production or scavenging of ROS, which have already been produced with enzymatic and non-enzymatic scavenging pathways. This can affect the secondary metabolism of fresh produce and increase the synthesis of phytochemicals or reduce the synthesis of undesirable compounds [4, 12, 16]. The biological consequences of free radical reactions leading to cellular dysfunction and cell death are summarized in Figure 2.

3. Postharvest abiotic stress and the production of phytochemicals

Harvested crops can be potentially exposed to various abiotic stresses that impact on quality in the food value chain. These include qualitative and quantitative losses including sensorial, microbial, and nutritional losses [7]. Wide variation in resistance to stress injury exists in higher plants and the stress tolerance threshold are determined by plant species, the developmental stage of the plant, type of stressor, exposure time, as well as other factors such as the plant stress-coping mechanisms. Abiotic stresses play a major role in determining the distribution of plant species across different types of environments. Many breeding programs impose abiotic stresses on the plants in a very quantitative way to develop stress tolerance that will allow crops to adapt to climate change [10]. However, it is not clear whether breeding in the field will also extend stress resistance characteristics in the postharvest phase including shelf-life extension and phytonutrient quality of crops.

Various abiotic stresses (salinity, water stress, drought, heavy metals, wind, air pollution, altered gas composition, light, temperature extremes, and exposure to ultraviolet or gamma

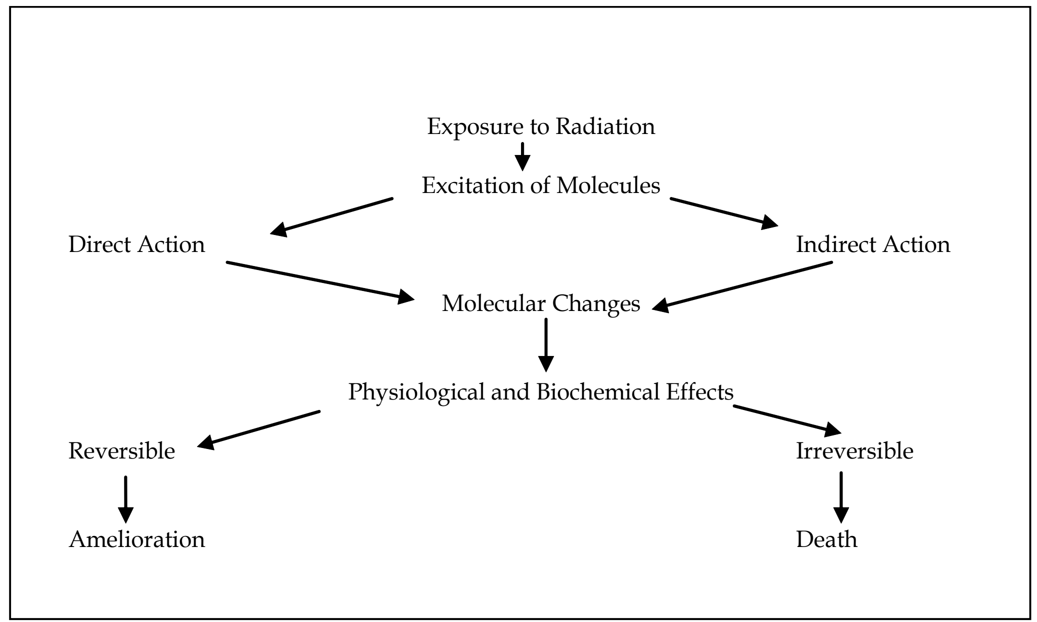


Figure 2. Schematic representation of the photobiological effects of UV-C radiation in plants [26].

radiation) are known to have deleterious effects on plant tissues as a result of the production of ROS, causing progressive oxidative damage, which can damage biomolecules and may affect biochemical pathways, leading to cell death (as shown in Figure 2) and cause substantial crop losses worldwide [13, 16, 18-19]. Postharvest abiotic stressors can lead to numerous quality problems in fruits and vegetables, including scald, core and flesh browning of fruits, sweetening, pitting, water-soaked appearance, abnormal ripening, russeting, tissue softening, and loss of nutrient constituents [10]. Hence an understanding of the effects of field abiotic stresses on postharvest stress susceptibility will become more important since postharvest stresses limit the storage and shelf-life potential of crops. Characterization of mutants and transgenic plants with altered expression of antioxidants is also a potentially powerful approach to understanding the functioning of the antioxidant system and its role in protecting plants against stress [6, 13]. The use of genetically modified crops has its biases as in most cases it is considered as potential biological hazards that create an ecological imbalance [6, 9].

As a secondary response, some postharvest abiotic stress treatments could induce some mechanisms that affect the metabolic activity of the treated produce, such as triggering antioxidants. Studies have shown that plants with higher levels of antioxidants, whether constitutive or induced, showed a greater resistance to different types of environmental stresses [13]. Plants possibly protect themselves against increased UV irradiation by an increased synthesis of pigments in the epidermis [23]. However, the mechanisms of cellular oxidative stress are complex, and other biochemical and physiological mechanisms may act in concert under UV stress. Figure 3 illustrates possible mechanisms for using controlled postharvest abiotic stresses to enhance the phytochemical content of crops [7].

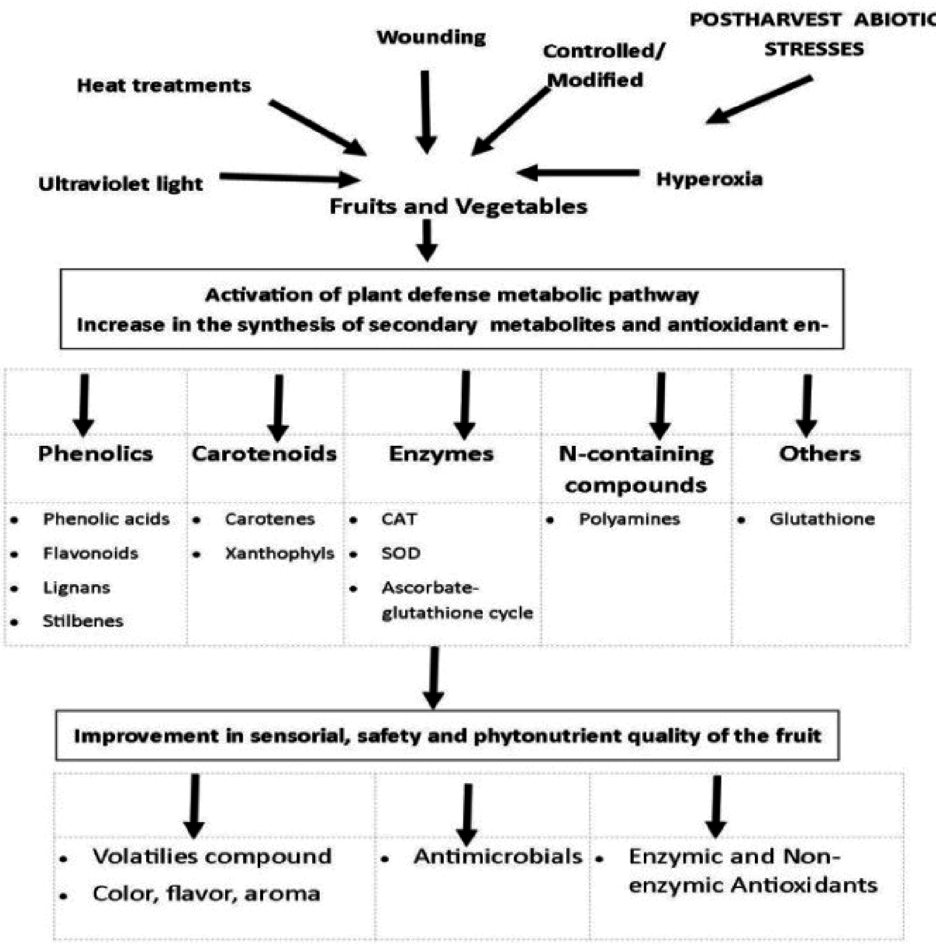


Figure 3. Effect of postharvest abiotic stress on the production of phytochemicals (adapted from [7]).

4. UV-C as a postharvest treatment

Non-ionizing artificial UV-C irradiation is a postharvest treatment that can be adjunct to refrigeration for delaying postharvest ripening, senescence, and decay in different fruit and vegetable species. UV-C radiation is mainly used as a surface treatment because it penetrates only 5-30 microns of the tissue and is beneficial for keeping the integrity and freshness of fruits and vegetables [27, 65]. It has been extensively used for many years in the disinfection of equipment, glassware, and air by food and medical industries. Exposure to abiotic UV-C radiation stress is well known to have deleterious effects on plant tissues however low levels may stimulate beneficial responses of plants, a phenomenon known as Hormesis [14, 28]. The use of UV-C hormesis to improve the postharvest quality of fresh fruits and vegetables has

been the subject of numerous research activities during the last two decades, with some early applications in the eighties on the control of postharvest pathogens. UV-C doses above the optimal level can lead to detrimental color development and poor appearance of fruits and vegetables, thus lowering aesthetic value and lead to poor marketability of such treated produce [15]. Turtoi [27] and Ribeiro [11] conducted recent reviews on the effects of UV-C primarily on decontamination and disease control in fresh fruits and vegetables. UV-C irradiation also holds considerable promise as a non-chemical treatment to delay ripening and senescence and to improve shelf-life of fresh fruits and vegetables and the associated health and therapeutic benefits with their consumption [11, 28]. In a more recent study, UV-C hormesis was induced at the pre-harvest stage, which has commercial implications particularly for crops that are easily damaged during postharvest treatment [20]. UV-C treated crops, with the ability to scavenge and/or control the level of cellular ROS with the consequential activation of phytochemicals may be useful to improve postharvest storage life and enhance nutritional quality and health benefits. However, the reported biochemical and molecular mechanisms of such effects are complex and quite diverse and depend on several factors such as differences in crops sensitivity, maturity levels, UV-C doses, equipment, and environmental conditions as previously noted.

4.1. UV-C technology

UV-C irradiation can be applied using low and medium pressure mercury vapor discharge lamps with a peak emission at 254 nm inside an enclosed chamber that is kept closed during irradiation. The lamps are suspended directly over the sample in the chamber at a fixed distance from the sample and radiation doses are varied using different exposure times. The intensity of the light is affected by the distance the source is from the sample. Manual rotation is generally employed to the treated commodity to ensure irradiation uniformity. The use of devices to rotate the crops to ensure radiation uniformity is noted in [20]. The dose is calculated from the product of exposure time and irradiance, as measured by portable handheld digital radiometers. The irradiance, sometimes called intensity, has the preferred units of $mWcm^{-2}$ while the UV dose has the preferred units of kJm^{-2} [21]. UV dose may be calculated from the following formula:

$D = I\ (T)$

where D = dosage, I = applied intensity, and T = time of exposure.

There are several advantages in the use of UV-C technology such as:

- it leaves no residues after treatment, including moisture residues,
- it does not involve complex expensive equipment,
- it is simpler and more economical to use than ionizing radiation, and
- it generally lacks regulatory restrictions.

The beneficial effects from UV-C radiation result from the use of very low UV doses ranging from 0.125 kJm^{-2} to 9 kJm^{-2} and the time scale for the induction of such effects is generally

measured over hours (h) or even days [11, 15, 17]. The design of UV-C equipment and technology on a commercial scale for fresh fruits and vegetables and fresh-cut food industry poses a challenge with respect to ensuring affordable technology and simultaneously protecting operators from harmful effects of UV-C rays while effectively treating fruit tissue to ensure significant and consistent microbial load reduction and activation of beneficial phytochemicals.

5. UV-C radiation and its effects on phytochemicals

Plants possess a variety of phytochemicals to protect against the adventitious production of ROS caused by specific postharvest elicitor treatments [4, 13]. Such phytochemicals include the enzymes superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reducatase (DHAR), glutathione reductase (GR), polyphenol oxidases (PPO), guaiacol peroxidase (GPX), as well as water and fat-soluble antioxidants (ascorbic acid, thiol containing compounds, tocopherols, carotenes) and general antioxidants such as phenolic compounds. Non-enzymic phytochemicals play a significant role in protecting the cell from oxidative stress that occurs when there is a disturbance between the production of free radicals and antioxidant defenses [13, 18, 24]. A summary of some changes in enzymic and non-enzymic phytochemicals in postharvest UV-C treatment of some crops are illustrated below.

5.1. Enzymic phytochemcials

5.1.1. Superoxide Dismutase (SOD), Glutathione Reductase (GR), Catalase (CAT)

SOD is a ubiquitous defensive enzyme against superoxide damage to aerobic organisms. SOD catalyzes the dismutation of the one-electron reduced form of oxygen ($O_2{\cdot}{-}$) or superoxide radical resulting in the production of the less toxic hydrogen peroxide and oxygen: $2\ O_2{\cdot}{-} + 2H^+ \rightarrow H_2O_2 + O_2$ [18, 25]. The H_2O_2 formed by the action of SOD is itself a strong oxidant and is toxic to cells. It can then be converted to oxygen and water in consecutive reactions with ascorbate and glutathione or with CAT in plant tissues [20]. SOD activity and enzymes of the H_2O_2 scavenging pathway are induced by diverse environmental stresses. High SOD activity has been reported to increase in plants exposed to various stresses [18]. GR is a key enzyme in the glutathione-ascorbate cycle and can regenerate reduced glutathione from its oxidized form. Together with glutathione, it is an important component of the ROS scavenging system in plant cells. GR has its main function as a detoxifier of peroxide from the cells. Peroxide decomposes to form highly reactive free radicals which can damage proteins, DNA and lipids. CAT a high capacity but low affinity enzyme destroys H_2O_2 and promotes the redox reaction:-$2H_2O_2 \rightarrow 2H_2O + O_2$. This enzyme has been reported to show a general decline in activity with increasing illumination, with degradation exceeding the capacity for repair [13]. In one study, activities of SOD, GR, and CAT were more than 2-fold higher during the first 1 h after UV-C treatment of peanut seedling [38]. Overall, the general trend is that UV-C stressed crops show an initial increase in enzyme activity followed by a decrease in the antioxidant enzymes.

Table 1 summarizes the changes in SOD, GR, and CAT activities with postharvest UV-C treatment of some crops.

Crop	UV-C conditions	Results	Source
Banana (cv. Cavendish)	0.02 kJm^{-2}, 0.03 kJm^{-2} and 0.04 kJm^{-2} and storage at 5°C and 25°C for 7 days and 21 days	SOD activity in both control and UVC-treated fruit decreased with storage time, with a slight increase at the end of storage, but UV-C treated fruit maintained higher SOD activity throughout storage. UV-C treatment led to significantly higher activities of SOD, CAT, POD, APX, and GR compared to control fruit during later storage.	[30]
Yellow Bell Pepper	2.2 kJm^{-2}, 4.4 kJm^{-2}, and 6.6 kJm^{-2} and storageat 12 ± 1°C for 15 days	UV-C illumination at 6.6 kJm^{-2} enhanced the activities of enzymes such as CAT, SOD, GPX, and APX in yellow bell pepper during storage when compared to control fruit.	[32]
Pear (cv. Yali)	5 kJm^{-2} and storage at 20°C for 42 days	Activities of SOD, GR, and CAT in Yali pear fruit were significantly enhanced with UV-C dose compared to control fruit even up to the end of the storage period.	[33]
Strawberry	0.43 kJm^{-2}, 2.15 kJm^{-2}, and 4.3 kJm^{-2} and storage at 10°C for 15 days	Total SOD activity decreased in both control and UV-C treated strawberries, but after 15 days of storage all UV-treatments showed higher SOD levels that controls. UV-treated fruits at 2.15 kJm^{-2} dose (5 min) had the highest GR activity. GR activity increased during the first 10 days of storage and then declined after 15 days of storage.	[34]
Strawberry	0.25, 0.5 kJm^{-2} and 0.75 kJm^{-2} and storage at 10°C for 15 days	SOD activity increased at day 5 but then declined during storage. Extracts from all UV-C treated fruits had higher SOD activities compared to control fruits during storage. UV-C doses at 0.5 kJm−2 and 0.75 kJm^{-2} enhanced SOD and CAT activities in fruit after 5 days but levels declined after.	[35]
Tomato (cv. Capello)	3.7 kJm^{-2} and 24.4 kJm^{-2} and storage at 16°C for 28 days	SOD levels were lowest at day 0 and increased during the pre-climacteric phase attaining a maximum level by day 14 for control and day 21 for UV-C treated fruits (both doses) prior to declining in the post-climacteric phase. SOD levels were generally lower for UV-C treated fruit than controls.	[36]
Turnera diffusa Willd (*Damiana* medicinal plant)	0.38 $mWcm^{-2}$; 5, 10, and 20 min day-1 and storage for 10 days	No significant differences were found in SOD activity of *damiana* leaves between UV-C treatments and control plants.	[37]
Fresh-cut watermelon	1.6, kJm^{-2}, 2.8 kJm^{-2}, 4.8 kJm^{-2}, and 7.2 kJm^{-2} and storage at 5°C for 11 days	The general trend in CAT activity was a decline from initial values however the 1.6 kJm^{-2} and 4.8 kJm^{-2} treatments kept the initial activity of this enzyme throughout shelf-life.	[39]

Table 1. Summary of changes in SOD, GR, and CAT activities with postharvest UV-C treatment of crops.

5.2. Non-enzymic phytochemicals

5.2.1. Phytoalexins

Resistance to infection by pathogen or abiotic stress is correlated with the induction of plant defense mechanisms and this is manifested through the stimulation of anti-microbial compounds such as phytoalexins. Allixin was the first compound isolated from garlic as a phytoalexin and a possible compound for cancer prevention. Trans-resveratrol produced by a large number of plants has a wide range of beneficial biological properties including being attributed with a relatively low incidence of cardiovascular disease and associated with reduced cancer risk [8]. A more detailed review of the stilbene resveratrol in grape and grape products irradiated with UV-C can be found in [8] and in peanuts [38, 43]. Resveratrol was present in substantial amounts (1.2-2.6 μg/g FW) in leaves, roots, and shells, but very little (0.05-0.06 μg/g FW) was found in developing seeds and seed coats of field-grown peanuts. Accumulation of resveratrol in leaves increased over 200-fold in response to UV light, over 20-fold in response to paraquat, and between 2- and 9-fold in response to wounding, H_2O_2, salicylic acid (SA), jasmonic acid, and ethephon, 24 h after treatment. Changes in resveratrol content were correlated with levels of resveratrol synthase (RS) mRNA, indicating a transcriptional control of resveratrol synthase activity [40]. These gene changes underline the biochemical and physiological changes induced by UV-C such as increased defense ability, delayed softening, better maintenance of nutritional and sensory qualities and extension of shelf-life [30-31]. Table 2 summarizes the changes in phytoalexin content with postharvest UV-C treatment of some crops.

Crop	UV-C conditions	Results	Source
Tomato (cv. Zhenfen 202)	4.0 kJm^{-2} and storage at 14°C for 24 h	A number of PR genes, like PR5-like protein, β-1,3-glucanase and chitinase were highly up-regulated in response to UV-C treatment.	[31]
Peanut Seedlings	300 μWcm^{-2} at 15 cm for 1 h and storage at 25°C for 60 h	Resveratrol concentrations increased immediately after UV-C radiation and peaked at 12 h, followed by a decline nearly to control concentrations by 24 h, indicating that resveratrol synthesis could be induced by UV-C irradiation.	[38]
Star Ruby grapefruit	0.5 kJm^{-2}, 1.5 kJm^{-2}, and 3.0 kJm^{-2} and storage at 7°C for 28 days	Scoparone and scopoletin increased in flavedo tissue with UV treatment. Both phytoalexins showed similar accumulation patterns, although the concentrations of scoparone were much lower than those of scopoletin and when compared to non-irradiated fruit that exhibited no detectable levels of scoparone and scopoletin.	[41]
Tomato	3.7 kJm^{-2} and storage at 7°C for 35 days	UV-C dose induced synthesis and accumulation of rishitin. The capacity to accumulate rishitin declined with ripening in both control and UV-treated fruit.	[42]

Table 2. Summary of changes in phytoalexin content with postharvest UV-C treatment of crops.

5.2.2. Phenolics and flavonoids

The largest category of phytochemicals and most widely distributed secondary products in plants are the phenolics. They exist in higher plants in many different forms including hydroxybenzoic derivatives, cinnamates, flavonoids (flavonols, flavones, flavanols, flavanones, isoflavones, proanthocyanidins), lignans, and stilbenes, and affect quality characteristics of plants such as appearance, flavor, and health-promoting properties. Besides their role as antioxidants, phenolic compounds also possess antimicrobial properties and are involved in disease resistance by contributing to the healing of wounds by lignification of cell walls around wounded sites [44]. The accumulation of phenolic and flavonoid compounds may act as a protective filter against excessive UV radiation [28, 45-48]. Phenolics also serve as substrates for browning reactions. The enzymes phenylalanine ammonia-lyase (PAL; EC 4.3.1.5), polyphenol oxidases (PPO; EC 1.14.18.1) and peroxidises (POD; EC 1.11.1.7) are the main enzymes responsible for phenolic degradation that often leads to quality loss in crops [44]. Skin discoloration or external browning is one effect of UV-C treatment and is dose dependent with the effect being more pronounced as the dose is increased and has been reported in several crops notably tomato, strawberries, peaches, and Star Ruby grapefruit [28]. Table 3 summarizes the changes in Phenolics content with postharvest UV-C treatment of some crops. Time course measurements of the effects of UV-C have shown that the strongest responses of fruit to UV-C treatment occurred instantly after the illumination and the effects diminished with time.

Crop	**UV-C conditions**	**Results**	**Source**
Spilanthes acmella (toothache plant)	1.5 kJm^{-2} and storage in field conditions after 3 and 5 days	Anthocyanin and flavonoid concentration increased with UV-C treatment.	[29]
Banana (cv. Cavendish)	0.02 kJm^{-2}, 0.03 kJm^{-2}, and 0.04 kJm^{-2} and storage at 5 and 25°C for 7 and 21 days	UV-C doses reduced both the incidence of CI and its severity compared with controls in banana fruit peel. UV-C treatment activated PAL and resulted in higher levels of total phenolic compounds in comparison with untreated controls.	[30]
Yellow Bell Pepper	2.2 kJm^{-2}, 4.4 kJm^{-2}, and 6.6 kJm^{-2} and storage at 12 ± 1°Cfor 15 days	UV-C illumination at 6.6 kJ/m^2 enhanced total flavonoid content.	[32]
Pear (cv. Yali)	5 kJm^{-2} and storage at 20°C for 42 days	Enzyme activities of PAL and β-1,3-glucanase were induced to high levels by UV-C treated pears and thought to be responsible for the reduction in postharvest decay.	[33]
Tomato (cv. Capello)	3.7 kJm^{-2} and 24.4 kJm^{-2} and storage at 16°C for 28 days	A 4.5- and 4.9-fold increase in total phenols with UV doses of 3.7 kJm^{-2} and 24.4 kJm^{-2}, respectively, when compared to controls on day 14 was observed. Levels of total phenols were higher with UV-treated fruits than controls at the end of the storage.	[36]

Crop	UV-C conditions	Results	Source
Turnera diffusa Willd (*Damiana* medicinal plant)	0.38 $mWcm^{-2}$; 5, 10 min day^{-1} and 20 min day-1 exposure and storage for 10 days	Levels of phenols increased immediately upon exposure to UV-C dose (5 min day^{-1}) and in all UV-C doses (5, 10 and 20 min day^{-1}) by day 7.	[37]
Tomato (Light Red and Mature Green)	1.0 kJm^{-2}, 3.0 kJm^{-2}, and 12.2 kJm^{-2} and storage atRT for 2 days	UV-C doses (3.0 kJm^{-2} and12.2 kJm^{-2}) caused a 1.2-fold increase in total phenolics of both maturities but the same effect was not observed in the individually analyzed phenolic compounds.	[45-46]
Fresh-cut pineapple (cv. Honey), banana (cv. Pisang mas) and Thai seedless guava	10 min day^{-1}, 20 min day^{-1}, and 30 min day^{-1} exposure and storage at RT	In bananas and guava, UV-C radiation resulted in an increase in total phenols and flavonoids. In pineapples however, there was a significant increase in flavonoids but UV-C irradiation did not have any significant increase in total phenol content.	[47]
Fresh-cut Mango (cv. Tommy Atkins)	1 min day^{-1}, 3 min day^{-1}, 5 min day^{-1}, and 10 min day^{-1} exposure and storage at 5°C for 15 days	An increase in the total phenols and total flavonoids were observed for all doses, with the longer irradiation exposure time proportional to the increases in levels of total phenols and flavonoids and lowest antioxidant capacity with controls.	[48]
Blueberries	0.43 kJm^{-2}, 2.15 kJm^{-2}, 4.30 kJm^{-2}, and 6.45 kJm^{-2} and held forvarious times at 20°C	UV-C doses increased total phenols and anthocyanins levels versus controls. Levels of flavonoids in blueberries increased with UV-C doses. Significantly higher antioxidant capacity in blueberries was detected in fruit treated with doses of 2.15 kJm^{-2} and 4.30 kJm^{-2} compared to the control fruit.	[49]
Button mushrooms	0.225 kJm^{-2}, 0.45 kJm^{-2}, and 0.90 kJm^{-2} and storage at 4°C for 21 days	Although there was a general increase in total phenolics during storage, UV doses showed lower total phenolics content during the first week of storage beyond which there was no significant difference among treatments.	[50]
Korla Pears	3 kJm^{-2} and 6 kJm^{-2} and storage at 20°C for 15 days	UV-C 3 kJm^{-2} dose had higher levels of total phenolics compared to the dose of 6 kJm^{-2} and control. Similar results were noted for flavonoids where at the end of storage, residual flavonoid content in control was 82% of initial values compared with 108% in 3 kJm^{-2} dose, and 98% in 6 kJm^{-2} dose of initial values.	[51]
Broccoli heads (cv. de Cicco)	4 kJm^{-2}, 7 kJm^{-2}, 10 kJm^{-2}, or 14 kJm^{-2} and storage at 20°C for 6 days	Total phenols and flavonoids increased in both control and UV-treated broccoli. Lower levels of total phenols and flavonoids were found in UV-treated florets after 4 and 6 days in storage compared to controls.	[53]

Table 3. Summary of changes in phenolics and flavonoids content with postharvest UV-C treatment of crops.

5.2.3. Photosynthetic pigments, antioxidants carotenoids, lycopene and vitamins α-tocopherol and ascorbic acid

5.2.3.1. Chlorophyll

During chlorophyll degradation, chlorophyll *a* is transformed to chlorophyllide *a* through the action of the chlorophyllase enzyme [52]. Chlorophyllide *a* is then acted by the enzyme Mg-dechelatase removing Mg^{2+} from the molecule and forming pheophorbide *a*, consequently the green color is lost. In general, the chloroplast is the first organelle to show injury response with UV radiation and reduction in the chlorophyll contents may be due to inhibition of biosynthesis or due to degradation of chlorophyll and their precursors [29]. Table 4 summarizes the changes in chlorophyll content with postharvest UV-C treatment of some crops. Photosynthetic pigments such as chlorophyll *a* and *b* as well as total chlorophyll contents were considerably reduced in UV-C treated crops [29, 37]. In these studies, chlorophyll *a* and chlorophyll *b* were more sensitive to UV-C radiation. The reduction of chlorophyll content is thought to have a negative effect on plant photosynthetic efficiency due to UV-C radiation being too severe. Previous studies have demonstrated the inhibitory effect of UV-C light on chlorophyll breakdown and on the activities of Mg-dechelatase, chlorophyllase and chlorophyll degrading peroxidase in crops [53-55].

Crop	UV-C conditions	Results	Source
Spilanthes acmella (toothache plant)	1.5 kJm^{-2} and storage in field conditions after 3 and 5 days	Chlorophyll *a*, chlorophyll *b*,and total chlorophyll contents were considerably reduced with UV-C treatment.	[29]
Turnera diffusa Willd (*Damiana* medicinal plant)	0.38 $mWcm^{-2}$; 5 min day^{-1}, 10 min day^{-1}, and 20 min day-1 exposure and storage for 10 days	Chlorophyll *a* content significantly decreased in plants exposed to UV-C radiation for 5 and 20 min day^{-1} while chlorophyll *b* content significantly decreased with UV-C 20 min day^{-1} dose relative to control plants.	[37]
Broccoli heads (cv. de Cicco)	4 kJm^{-2}, 7 kJm^{-2}, 10 kJm^{-2}, or 14 kJm^{-2} and storage at 20°C for 6 days	All UV treatments delayed yellowing and chlorophyll degradation. UV-C dose of 10 kJm^{-2} reduced the degradation of both chlorophyll *a* and *b* in broccoli florets and this was correlated to a reduced activity of chlorophyllase and chlorophyll peroxidase in treated florets, which maintained a greener color than the controls. UV-C treated broccoli also maintained lower Mg-dechelatase level than controls.	[53]
Brassica oleracea var. *alboglaba* (Chinese kale)	1.8 kJm^{-2}, 3.6 kJm^{-2}, 5.4 kJm^{-2}, and 7.2 kJm^{-2} and storage at 20°C for 8 days	UV-C doses of 3.6 kJm^{-2} and 5.4 kJm^{-2} delayedleaf yellowing and chlorophyll loss depicted as higher chlorophyll contents and lower activity of chlorophyllase, Mg-dechelatase and chlorophyll-degrading peroxidase as compared to the other treatments.	[54]

Crop	UV-C conditions	Results	Source
Mature Green Tomato (cv. Capello)	3.7 kJm^{-2} and 24.4 kJm^{-2} and storage at 16°C for 28-35 days	There was a significant decrease in chlorophyll degradation, with levels significantly higher in UV-C treated fruit compared to controls with storage time for both UV-C doses.	[55]

Table 4. Summary of changes in chlorophyll content with postharvest UV-C treatment of crops.

5.2.3.2. Carotenoids and lycopene

Carotenoids (β-carotene) or pro-vitamin A are quenching agents that facilitate the return of singlet oxygen to its ground state. Table 5 summarizes changes in Total Carotenoids Content (TCC) and Lycopene content with postharvest UV-C treatment of some crops. In some cases, decreases in β-carotene as a result of UV-C treatment, are due most likely to the phenomenon of photobleaching [37, 45]. These observations appear to be at variance with those of other authors where carotenoid levels increased in medicinal plants [29], tomato [55], and minimally processed carrots [61] with UV-C treatment. The increase in total carotenoids may be part of the antioxidant system where carotenes are involved in protection of the chloroplast against photooxidation.

Crop	UV-C conditions	Results	Source
Spilanthes acmella (toothache plant)	1.5 kJm^{-2} and storage in field conditions after 3 and 5 days	TCC increased with UV-C treatment. Lycopene was not analyzed.	[29]
Yellow Bell Pepper	2.2 kJm^{-2}, 4.4 kJm^{-2}, and 6.6 kJm^{-2} and storage at 12 ± 1°C for 15 days	UV-C illumination at 6.6 kJ/m^2 enhanced TCC.	[32]
Turnera diffusa Willd (*Damiana* medicinal plant)	0.38 $mWcm^{-2}$; 5 min day^{-1}, 10 min day^{-1}, and 20 min day^{-1} exposure and storage for 10 days	TCC significantly decreased in plants exposed to UV-C (20 min day^{-1}) compared to control. Lycopene was not analyzed.	[37]
Light Vine-Ripe Red Tomato	1 kJm^{-2}, 3 kJm^{-2}, and 12.2 kJm^{-2} and storage at RT for 2 days	UV-C doses caused a decrease in β-carotene while UV-C doses at 1 kJm^{-2} and 12.2 kJm^{-2} enhanced the increase in total lycopene (*E*+*Z* isomers), by about 20% over control samples. UV-C 1 h treatment favored an increase in *E*-lycopene, while UV-C 12 h treatment affected mainly *Z*-isomers, which is considered to be better from a nutritional point.	[45]
Mature Green (Breaker stage)Tomato	1 kJm^{-2}, 3 kJm^{-2}, and 12.2 kJm^{-2} and	UV-C doses caused a decrease in β-carotene while total lycopene increased 8-fold at doses of 1.0 kJm^{-2} and 3.0 kJ/m^2	[46]

Crop	UV-C conditions	Results	Source
	storage at RT for 8 days	over those of control. *E*-lycopene decreased with 12.2 kJ/m^2 dose.	
Fresh-cut Mango (cv. Tommy Atkins)	1 min day^{-1}, 3 min day^{-1}, 5 min day^{-1}, and 10 min day^{-1} exposure and storage at 5°C for 15 days	β-carotene content decreased with storage in all treatments including controls with the major reduction observed in fruits treated for 10 mins and 5 mins.	[48]
Mature Green Tomato (cv. Capello)	3.7 kJm^{-2} and 24.4 kJm^{-2} and storage at 16°C for 28-35 days	There was a significant decline in lycopene accumulation with time for both UV-doses. UV-3.7 kJm^{-2} exhibited significantly higher levels of TCC compared to control fruits throughout storage.	[55]
Mature Green (Breaker stage)Tomato	13.7 kJm^{-2} and storage at 12°C-14°C for 21 days	Lycopene content was enhanced by UV-C treatment but the concentration of β-carotene was not affected by UV-C.	[56]
Mature Green Tomato	3.7 kJm^{-2} and storage at 13°C for 30 days	UV treatment significantly reduced the lycopene content.	[57]
Fresh-cut Carrots (cv. Nantes)	0.78-0.36 kJm^{-2} and storage at 5°C for 10 days	A 64% loss in TCC in UV-C treated samples compared to controls was noted just after processing. However, UV samples exhibited a consistent increase in TCC levels with levels reaching 3-fold higher at day 7 than at day 0.	[61]

Table 5. Summary of changes in total carotenoids content (TCC) and lycopene with postharvest UV-C treatment of crops.

5.2.3.3. *α-tocopherol (Vitamin E)*

Alpha-tocopherol is a major lipid-soluble antioxidant that breaks the chain of free radical reactions of polyunsaturated fatty acids (PUFAs) in cell membranes. Alpha-tocopherol protects membrane PUFAs from ROS and free radical damage by reacting with lipid radicals produced in the lipid peroxidation chain reaction [16]. Levels of tocopherol in plants are as a result of synthesis, recycling, and degradation. Changes in α-tocopherol levels during plant responses to environmental stress are characterized by two phases; in the first phase there is an increase in tocopherol synthesis that was followed by a second phase of net tocopherol loss [24]. Endogenous α-tocopherol levels are also severely affected by the extent of its degradation and recycling under stress. As stress is more severe and the amounts of ROS in chloroplasts increase, α-tocopherol levels tend to decrease. Irreversible degradation of α-tocopherol may also occur when α-tocopheroxyl radicals, which result from the scavenging of lipid peroxyl radical by α-tocopherol, are not recycled back by ascorbate. Table 6 summarizes the changes

in α-Tocopherol (Vitamin E) content with postharvest UV-C treatment of some crops. While it has been reported that α-tocopherol levels increase in photosynthetic plant tissues in response to a variety of abiotic stresses, results from a study with UV-C irradiated tomato exocarp demonstrated declining levels and do not support the hypothesis that antioxidants such as α-tocopherol are stimulated for plant defense against oxidative stress caused by UV-C radiation [36]. On the other hand, the increased vitamin E content in medicinal *Damiana* plants exposed to UV-C radiation may provide protection against the oxidative damage induced by UV-C radiation [37].

Crop	UV-C conditions	Results	Source
Tomato (cv. Capello)	3.7 kJm^{-2} and 24.4 kJm^{-2} and storage at 16°C for 28 days	Levels of α-tocopherol were considerably lowered with UV treatment.	[36]
Turnera diffusa Willd (*Damiana* medicinal plant)	0.38 $mWcm^{-2}$; 5 min day^{-1}, 10 min day^{-1}, and 20 min day-1 exposure and storage for 10 days	Levels of α-tocopherol increased with UV treatment.	[37]

Table 6. Summary of changes in α-Tocopherol (vitamin E) content with postharvest UV-C treatment of crops.

5.2.3.4. Ascorbic acid (Vitamin C)

Vitamin C content, considered to be a nutritional quality index for fruits and vegetables occurs as L-ascorbic acid and dehydroascorbic acid (oxidised form of ascorbic acid). Ascorbate is an electron donor and this property explains its function as an antioxidant or reducing agent and is easily destroyed by oxidation, exposure to light or high temperatures. Ascorbic acid scavenges free radicals in the water soluble compartment of the cell and may also regenerate α-tocopherol (vitamin E), an important lipid-phase antioxidant [16]. Table 7 summarizes the changes in ascorbic acid (vitamin C) content with postharvest UV-C treatment of some crops. In most cases, UV-C treatment decreased the vitamin C content of fruits or did not affect ascorbic acid levels.

Crop	UV-C conditions	Results	Source
Yellow Bell Pepper	2.2 kJm^{-2}, 4.4 kJm^{-2}, and 6.6 kJm^{-2} and storage at 12 ± 1°C for 15 days	No effect on ascorbic acid levels with UV-C treatment.	[32]
Tomato (cv. Capello)	3.7 kJm^{-2} and 24.4 kJm^{-2} and storage at 16°C for 28 days	UV treatment decreased ascorbic acid levels.	[36]

Crop	UV-C conditions	Results	Source
Fresh-cut pineapple (cv. Honey), banana (cv. Pisang mas) and Thai seedless guava	10 min day^{-1}, 20 min day^{-1}, and 30 min day^{-1} exposure and storage at RT	UV treatment decreased ascorbic acid levels.	[47]
Fresh-cut Mango (cv. Tommy Atkins)	1 min day^{-1}, 3 min day^{-1}, 5 min day^{-1}, and 10 min day^{-1} exposure and storage at 5°C for 15 days	A reduction in total ascorbic acid content with UV treatment was observed. The lowest values for ascorbic acid occurred in fruits irradiated for 10 min.	[48]
Button mushrooms	0.225 kJm^{-2}, 0.45 kJm^{-2}, and 0.90 kJm^{-2} and storage at 4°C for 21 days	Apart from day 1 where UV treated mushrooms had lower ascorbic acid content than controls, during storage, levels increased to a maximum at day 14 and remained stable until day 21 for both control and UV-treated mushrooms.	[50]
Tomato (cv. Trust)	3.7 kJm^{-2} at and storage at 16°C for 25 days	Ascorbate oxidase activity exhibited a decline during storage for both control and UV-C treated fruit, however the amount of this enzyme was lower in UV-C-treated fruit and could correlate to decreases in levels of ascorbic acid.	[58]
Fresh-cut mature green bell pepper	3 kJm^{-2}, 10 kJm^{-2}, and 20 kJm^{-2} and storage at 10°C for 8 days	The antioxidant capacity of UV-C-treated fruit did not change during storage, but showed a slight increase in the control.	[59]
Fresh fruit juices	Various time intervals of exposure to UV-C	UV treatment decreased ascorbic acid levels. The longer the exposure to UV, the higher the losses of ascorbic acid content in fruit juices.	[60]

Table 7. Summary of changes in ascorbic acid (vitamin C) content with postharvest UV-C treatment of crops.

5.2.4. Glutathione

Thiol (-SH) groups and other functions readily donate hydrogen atoms and are scavengers of hydroxyl radicals. The tri-peptide Glutathione (γ-glutamylcysteinylglycine) is a major free non-protein thiol compound found in plant tissues. In non-stressed cells, 90% of glutathione is mainly present in its reduced form (GSH) while the oxidized GSSG levels are lower. Upon oxidative stress, the glutathione redox status may shift to a more oxidized form, $ROOH + 2GSH \rightarrow ROH + GSSG + H_2O$, due to increased GSH oxidation and/or decreased GSSG reduction. Such a shift in the glutathione status in plants has been reported upon exposure to a variety of environmental factors associated with oxidative stresses [62]. The ratio of reduced GSH to oxidized GSSG within the cells can be used to measure cellular toxicity. Table 8 summarizes the changes in glutathione content with postharvest UV-C treatment of some crops. Although glutathione has been used as an oxidative stress indicator in plants, there is conflicting evidence about glutathione responses under oxidative stress conditions. While some studies have found an increase in glutathione synthesis in response to oxidative stress, others have found no increase in GSH by environmental stresses.

Crop	UV-C conditions	Results	Source
Strawberry	0.43 kJm^{-2}, 2.15 kJm^{-2}, and 4.3 kJm^{-2} and storage at 10°C for 15 days	UV-C enhanced the increase of GSH in strawberry fruit during storage.	[34]
Tomato (cv. Capello)	3.7 kJm^{-2} and 24.4 kJm^{-2} and storage at 16°C for 28 days	UV-C treatment did not enhance GSH levels when compared to non-irradiated fruit.	[36]
Young Pea Plants	9 kJm^{-2} and storage up to 72 h	Apart from the initial increase in free thiols by 20% with UV-C, free thiol levels decreased with UV-treatment.	[63]

Table 8. Summary of changes in glutathione content with postharvest UV-C treatment of crops

5.2.5. *Polyamines(PAs)*

Polyamines (PAs) are implicated in a variety of regulatory processes ranging from regulation of growth and cell division, regulating the activity of ribonucleotides and proteinase to inhibition of ethylene (C_2H_4) production and senescence. The anti-senescent activity of PAs may also be related to their ability to be effective free radical scavengers, as well as stabilizing DNA and membranes by their positively charged cations associating with negative charges on nucleic acids and phospholipids [26]. Changes in plant PA metabolism occur in response to a variety of abiotic stresses and have been shown to enhance the ability of plants to resist environmental stresses. However, the physiological significance of elevated PA levels in abiotic stress responses is still unclear in terms of whether such a response is as a result of stress-induced injury or a protective response to abiotic stress [64]. Table 9 summarizes the changes in PA content with postharvest UV-C treatment of some crops.

Crop	UV-C conditions	Results	Source
Young Pea Plants	9 kJm^{-2} and storage up to 72 h	Exogenous application of PA (spermine) plus UV-C positively affected the plant in maintaining normal plant growth, stabilizing cell membranes and activating non-enzymatic antioxidants.	[63]
Tomato (cv Capello)	3.7 kJm^{-2} and 24.4 kJm^{-2} and storage at 16°C for 21 days	UV-C treatment induced PA (free Putrescine) accumulation in tomato fruit. By day 21, levels were still high in UV-treated fruit compared to controls. Similarly, in another recent study, PA content of UV-C (3.7 kJm^{-2}) treated tomato were higher that controls.	[65-66]
Strawberry	0.72 kJm^{-2} and storage at 4°C	Exogenous application of 2 mM putrescine plus UV-C resulted in positive effects on maintenance of firmness, reduction of weight loss and protection of total antioxidant capacity and vitamin C content against degradation.	[67]

Crop	UV-C conditions	Results	Source
Intact Pea Plants	0.1 kJm^{-2} and 0.3 kJm^{-2} and storage	Endogenous free, conjugated, and bound PAs (Spm, Spd, and Put) in leaves of young pea plants reduced membrane damage as a result of UV-C irradiation.	[68]

Table 9. Summary of changes in polyamine (PA) content with postharvest UV-C treatment of crops.

6. Conclusions

Studies on the benefit of phytochemicals continue to be of interest, as researchers have been exploring alternative medicine in the management of common lifestyle diseases and preventative actions, as well as the promotion of good health and nutrition. Abiotic stresses are significant determinants of quality and nutritional value of crops during their life cycle. Notwithstanding, climate change has created additional environmental variables that may influence postharvest stress susceptibility of crops. While breeding programs are underway for many crops to develop stress resistance that will allow them to adapt to climate change, it is not clear that breeding for stress resistance in the field will also extend to stress resistance characteristics of harvested crops particularly fresh fruits and vegetables and fresh-cut produce. Proper postharvest management of crops can positively influence susceptibility to abiotic stress. It is important to understand the various response networks to abiotic stresses encountered in the field and in the postharvest continuum to better evaluate the benefits that may yield from such stresses. Controlled stresses may be used as tools by the fresh produce and food processing industries to obtain enhanced phytochemical or health promoting components of fresh-cut or whole fresh produce and for growers interested in finding alternative uses for their crops. In the context of the different postharvest handling treatments, the potential of UV-C irradiation has enormous possibilities as a non-chemical treatment to sanitize and reduce microbial loads, delay ripening, and improve shelf-life of fresh fruits and vegetables. Further, the elicitation of health promoting phytochemicals with UV-C is an indication that such an approach may be of benefit to growers, processors, and consumers in enhancing sensory characteristics of fresh fruits and vegetables. The beneficial action of UV-C is thought to be as a result of the activation of multiple defense systems involving secondary stress metabolites such as phytoalexins and enzymic and non-enzymic antioxidants. The intensification of natural defense mechanisms of higher plant so that they can defend themselves against infection or adverse stresses is one alternative approach to controlling postharvest losses. Depending on the experimental conditions including UV-C dose, commodity, maturity stage, storage, and environmental conditions administered, such bioactive compounds have been found to be variable, in some instances may decrease or increase, and this change may impact quality of the crops. In many studies, the strongest responses to UV-C treatment occurred almost immediately after the illumination with some beneficial effects of crops occurring after UV-C treatment with the effects decreasing with time. There is need for further research on the molecular and biochemical mechanisms by which these beneficial responses are derived in UV-C treated fresh fruits and vegetables. This is in order to help guide the development of approaches to reliably confer health and nutritional benefits. The potential

http://dx.doi.org/10.5772/60050

application of UV-C is novel and relevant particularly for the fresh fruit and vegetable and fresh-cut trade industries.

Author details

Rohanie Maharaj

Address all correspondence to: rohanie.maharaj@utt.edu.tt

Biosciences, Agriculture and Food Technologies (BAFT), ECIAF Campus, The University of Trinidad and Tobago, Caroni North Bank Road, Centeno, Piarco, W.I., Trinidad & Tobago

References

[1] Palmer S. Phytochemicals Fight Disease. Environmental Nutrition, 2011; 34(9): 1-4.

[2] Kalam S, Singh R, Mani A, Patel J, Khan FN, Pandey A. Antioxidants: Elixir of Life. International Multidisciplinary Research Journal, 2012; 2(1): 18-34.

[3] Liu RH. Potential synergy of phytochemicals in cancer prevention: mechanism of action. The Journal of Nutrition, 2004;134 (12): 3479S-3485. http://jn.nutrition.org/content/134/12/3479S.full (accessed 20 October 2014).

[4] Schreiner M, Huyskens-Keil S. Phytochemicals in Fruit and Vegetables: Health Promotion and Postharvest Elicitors. Critical Reviews in Plant Sciences, 2006; 25(3): 267-278.

[5] Saxena M, Saxena J, Nema R, Singh, D, Gupta A. Phytochemistry of Medicinal Plants. Journal of Pharmacognosy and Phytochemistry, 2013; 1(6): 168-182.

[6] Sánchez-Rangel JC, Benavides J, Jacobo-Velázquez DA. Abiotic Stress Based Bioprocesses for the Production of High Value Antioxidant Phenolic Compound in Plants: An Overview. Revista Mexicana de Ingeniería Química, 2014; 13(1): 49-61.

[7] González -Aguilar GA, Villa-Rodriguez JA, Ayala-Zavala, JF, Yahia, EM. Improvement of the Antioxidant Status of Tropical Fruits as a Secondary Response to Some Postharvest Treatments. Trends in Food Science & Technology, 2010; 21: 475-482.

[8] Tříska J, Houška M. Physical Methods of Resveratrol Induction in Grapes and Grape Products—A Review. Czech J. Food Sci., 2012; 30(6): 489-502.

[9] Jacobo-Velázquez DA, Cisneros-Zevallos L. An Alternative Use of Horticultural Crops: Stressed Plants as Biofactories of Bioactive Phenolic Compounds-Review. Agriculture, 2012; 2: 257-271.

[10] Tiovonen PMA, Hodges DM. Abiotic Stress in Harvested Fruits and Vegetables. In: Shanker A, (ed.) Abiotic Stress in Plants - Mechanisms and Adaptations. Rijeka: InTech, 2011; p39-58. http://www.intechopen.com/books/abiotic-stress-in-plants-mechanisms-andadaptations/abiotic-stress-in-harvested-fruits-and-vegetables (accessed 22 September 2014).

[11] Ribeiro C, Canada J, Alvarenga B. Prospects of UV Radiation for Application in Postharvest Technology. Emir. J. Food Agric., 2012; 24(6): 586-597.

[12] Cisneros-Zevalios L. The Use of Controlled Postharvest Abiotic Stresses as a Tool for Enhancing the Nutraceutical Content and Adding-Value of Fresh Fruits and Vegetables. Journal of Food Science, 2003; 68(5): 1560-1565.

[13] Ali A, Alqurainy F. Activities of Antioxidants in Plants under Environmental Stress. In: Motohashi N. (ed.). The Lutein-Prevention and Treatment for Age-Related Diseases. Trivandrum: Transworld Research Network, 2006; p187-256. http://www.cabdirect.org/abstracts/20063204010.html;jsessionid=72FCA1820C86D7F95?36027037824D3AB?freeview=true&gitCommit=?4.13.29 (accessed 20 April 2014).

[14] Shama G. Process Challenges in Applying Low Doses of Ultraviolet Light to Fresh Produce for Eliciting Beneficial Hermetic Responses. Postharvest Biology and Technology, 2007; 44(1): 1-8.

[15] Vigneault C, Leblanc DI, Goyette B, Jenni, S. Invited review: Engineering aspects of physical treatments to increase fruit and vegetable phytochemical content. Can. J. Plant Sci., 2012; 92: 373-397.

[16] Gill SS, Tuteja N. Reactive Oxygen Species and Antioxidant Machinery in Abiotic Stress Tolerance in Crop Plants. Plant Physiology and Biochemistry, 2010; 48: 909-930.

[17] Charles MT, Arul J. UV Treatment of Fresh Fruits and Vegetables for Improved Quality. Stewart Postharvest Review, 2007; 3(6): 1-8.

[18] Sharma P, Jha BA, Dubey SR, Pessarakli M. Reactive Oxygen Species, Oxidative Damage and Antioxidative Defence Mechanisms in Plants Under Stressful Conditions. Journal of Botany 2012; 2012: 1-28. doi:10.1155/2012/217037 http://www.hindawi.com/journals/jb/2012/217037/ (accessed 27 September 2012).

[19] Mittler R. Oxidative Stress, Antioxidants and Stress Tolerance. Trends in Plant Science, 2002; 7: 405-410.

[20] Obande MA, Tucker GA, Shama G. I Effect of preharvest UV-C treatment of tomatoes (*Solanum lycopersicon* Mill.) on ripening and pathogen resistance. Postharvest Biol. Technol., 2011; 62: 188-192.

[21] Kowalski W. Ultraviolet Germicidal Irradiation Handbook: UVGI for Air and Surface. Berlin: Springer; 2009. http://link.springer.com/chapter/10.1007/978-3-642-01999-9_1#page-1. (accessed 14 September 2014).

[22] Stapleton AE. Ultraviolet Radiation and Plants: Burning Questions, Review Article. The Plant Cell, 1992; 4: 1353-1358.

[23] Häder DP, Tevini M. General Photobiology. New York: Pergamon Press; 1986; p293-307.

[24] Szarka A, Tomasskovics B, Bánhegyi G. The Ascorbate-Glutathione-α-Tocopherol Triad in Abiotic Stress Response. Int. J. Mol. Sci., 2012; 13: 4458-4483. doi:10.3390/ijms13044458.

[25] Bowler C, Montagu MV, Inze D. Superoxide Dismutase and Stress Tolerance. Annual Review of Plant Physiology and Plant Molecular Biology, 1992; 43: 83-116.

[26] Maharaj R., Mohammed A. Significance of UV-C Hormesis and its Relation to Some Phytochemicals in Ripening and Senescence Process. In: Montanaro G, Dichio B. (eds.) Advances in Selected Plant Physiology Aspects. Rijeka: InTech; 2012; p251-268.

[27] Turtoi M. Ultraviolet Light Treatment of Fresh Fruits and Vegetables Surface: A Review. Journal of Agroalimentary Processes and Technologies, 2013; 19(3): 325-337.

[28] Shama G, Alderson P. UV Hormesis in Fruits: A Concept Ripe For Commercialization. Trends in Food Science and Technology, 2005; 16: 128-136.

[29] Reshmi GR, Rajalakshmi R. Drought and UV stress response in *Spilanthes acmella* Murr., (Tooth-Ache Plant). Journal of Stress Physiology & Biochemistry, 2011; 8(4): 111-129. http://www.jspb.ru/issues/2012/N4/JSPB_2012_4_110-129.pdf (accessed 20 September 2014).

[30] Pongprasert N, Sekozawa YS, Gemma H. The Role and Mode of Action of UV-C Hormesis in Reducing Cellular Oxidative Stress and the Consequential Chilling. International Food Research Journal, 2011; 18: 741-749.

[31] Liu C, Cai L, Han X, Ying T. Temporary Effect of Postharvest UV-C Irradiation on Gene Expression Profile in Tomato Fruit. Gene, 2011; 486: 56-64.

[32] Promyou S, Supapvanich S. Effect of Ultraviolet-C (UV-C) Illumination on Postharvest Quality and Bioactive Compounds in Yellow Bell Pepper Fruit (*Capsicum annuum* L.) During Storage. African Journal of Agricultural Research, 2012; 7(28): 4084-4096.

[33] Li J, Zhang Q, Cui Y, Yan J, Cao J, Zhao Y, Jiang W. Use of UV-C Treatment to Inhibit the Microbial Growth and Maintain the Quality of Yali Pear. Journal of Food Science, 2010; 75(7): M503-M507.

[34] Erkan M, Wang SY, Wang CY. Effect of UV Treatment on Antioxidant Capacity, Antioxidant Enzyme Activity and Decay in Strawberry. Postharvest Biology and Technology, 2008; 48: 163-171.

[35] Mohammadi N, Mohammadi S, Abdossi V, Mashhadi A, Boojar A. Effect of UV-C Radiation on Antioxidant Enzymes in Strawberry Fruit (*Fragaria x ananassa* cv. Camarosa) ARPN Journal of Agricultural and Biological Science, 2012; 7(10): 860-864.

[36] Maharaj R, Arul J, Nadeau P. UV-C Irradiation Effects on Levels of Enzymatic and Non-Enzymatic Phytochemicals in Tomato. Innovative Food Science and Emerging Technologies, 2014; 2: 99-106.

[37] Soriano-Melgar LA, Alcaraz-Meléndez L, Méndez-Rodríguez LC, Puente ME, Rivera-Cabrera F, Zenteno-Savín T. Antioxidant Responses of Damiana *(Turnera diffusa* Willd) to Exposure to Artificial Ultraviolet (UV) Radiation in an *in vitro* Model; Part I; UV-C Radiation. Nutr Hosp. 2014; 29(5): 1109-1115.

[38] Tang K, Zhan JC, Yang HR, Huang WD. Changes of Resveratrol and Antioxidant Enzymes During UV-Induced Plant Defence Response in Peanut Seedlings. J Plant Physiol., 2010; 167(2): 95-102. doi: 10.1016/j.jplph.2009.07.011.

[39] Hernández FA, Robles PA, Gómez PA. Low UV-C Illumination for Keeping Overall Quality of Fresh-Cut Watermelon. Postharvest Biology and Technology, 2010; 55: 114-120.

[40] Chung IM, Park MR, Chun JC, Yun, SJ. Resveratrol Accumulation and Resveratrol Synthase Gene Expression in Response to Abiotic Stresses and Hormones in Peanut Plants. Journal of Food Science, 2003; 68(5): 1560-1565.

[41] D'hallewin G, Schirra M, Pala M, Ben-Yehoshua S. Ultraviolet C Irradiation at 0.5 kJm-2 Reduces Decay without Causing Damage or Affecting Postharvest Quality of Star Ruby Grapefruit (*C. paradisi* Macf.). Journal of Agriculture Food Chemistry, 2000; 48(10): 4571-4575. http://pubs.acs.org/doi/abs/10.1021/jf000559i?journalCode=jafcau (accessed 27 August 2014).

[42] Charles MT, Mercier J, Makhlouf J, Arul J. Physiological Basis of UV-C Induced Resistance to *Botrytis cinerea* in Tomato Fruit: I. Role of Pre- and Post-Challenge Accumulation of the Phytoalexin-Rishitin. Postharvest Biology and Technology, 2008;47(1): 10-20. http://www.sciencedirect.com/science/article/pii/S0925521407001974 (accessed 20 August 2014).

[43] Hasan MM, Cha M, Bajpai VK, Baek, K. Production of a major stilbene phytoalexin, resveratrol in peanut (*Arachis hypogaea*) and peanut products: A mini review. Rev. Environ. Sci. Biotechnol., 2013; 12: 209-221.

[44] Shahidi F, Chandrasekara A, Zhong Y. Bioactive Phytochemicals in Vegetables. In: Sinha NK. (ed.) Handbook of Vegatables and Vegetable Processing. Iowa: Blackwell Publishing Ltd; 201;. p125-158.

[45] Bravo SB, García-Alonso J, Martín-Pozuelo G, Gómez V, García-Valverde V, Navarro-González I, Periago MJ. Effects of Postharvest UV-C Treatment on Carotenoids and Phenolic Compounds of Vine-Ripe Tomatoes. International Journal of Food Science and Technology, 2013; 48: 1744-1749.

[46] Bravo S, García-Alonso J, Martín-Pozuelo G, Gómez V, Santella M, Navarro-González I, Periago MJ. The Influence of Post-harvest UV-C Hormesis on Lycopene, β-Car-

otene, and Phenolic Content and Antioxidant Activity of Breaker Tomatoes. Food Research International, 2012; 49: 296-302.

[47] Alothman M, Bhat R, Karim AA. UV Radiation-Induced Changes of Antioxidant Capacity of Fresh-Cut Tropical Fruits. Innovative Food Science and Emerging Technologies, 2009; 10: 512-516.

[48] González-Aguilar GA, Villega-Ochroa MA, Martinez-Telez MA; Gardea AA, Ayala-Zavala JF. Improving Antioxidant Capacity of Fresh Cut Mangoes Treated with UV-C. Journal of Food Science, 2007; 36: S197-S202.

[49] Wang CY, Chen C-T, Wang S. Changes of Flavonoid Content and Antioxidant Capacity in Blueberries After Illumination with UV-C. Food Chemistry, 2009; 117(3): 426-431.

[50] Guan W, Fan X, Yan R. Effects of UV-C treatment on inactivation of Escherichia coli O157:H7, microbial loads, and quality of button mushrooms. Postharvest Biology and Technology, 2012; 64: 119-125.

[51] Lu X, Liu C, Ying T. Influence of UV-C irradiation on antioxidant contents and antioxidant capacity of Korla fragrant pear (*Pyrus sinkiangensis* Yu). Journal of Food, Agriculture & Environment, 2013; 11(3&4): 324-327.

[52] Matile P, Hortensteiner S, Thomas H. Chlorophyll Degradation. Annu. Rev. Plant Physiol. Plant Mol. Biol., 1999; 50: 67-95.

[53] Costa L, Vincente AR, Civello PM, Chaves AR, Martinez GA. UV-C Treatment Delays Postharvest Senescence in Broccoli Floret. Postharvest Biology and Technology, 2006; 39(2): 204-210.

[54] Chairat B, Nutthachai P, Varit S. Effect of UV-C treatment on Chlorophyll Degradation, Antioxidant Enzyme Activities and Senescence in Chinese Kale (*Brassica oleracea* var. *alboglabra*). International Food Research Journal, 2013; 20(2): 623-628. http://www.ifrj.upm.edu.my/20%20(02)%202013/17%20IFRJ%2020%20(02)%202013%20Varit.pdf (accessed 20 September 2014).

[55] Maharaj R, Arul J, Nadeau P. UV-C Irradiation of Tomato and its Effects on Color and Pigments. Advances in Environmental Biology, 2010; 4(2): 308-315.

[56] Liu LH, Zabaras D, Bennett LE, Aguas P, Woonton BW. Effects of UV-C Red Light and Sunlight on the Carotenoid Content and Physical Qualities of Tomato during Post-Harvest Storage. Food Chemistry, 2009; 115: 495-500.

[57] Jagadeesh, SL, Charles MT, Gariepy Y, Goyette B, Raghavan GSV, Vigneault C. Influence of Postharvest UV-C Hormesis on the Bioactive Components of Tomato during Post-Treatment Handling. Food and Bioprocess Technology, 2011; 4(8): 1463-1472.

[58] Barka EA. Protective Enzymes Against Oxygen Species during Ripening of Tomato Fruits in Response to Low Amounts of UV-C. Australian J. Plant Physiology, 2001; 28: 785-791.

[59] Rodoni LM, Concellón A, Chaves AR, Vicente AR. Use of UV-C Treatments to Maintain Quality and Extend the Shelf Life of Green Fresh-Cut Bell Pepper (*Capsicum annuum* L.) Journal of Food Science, 2012; 77(6): C632-C639.

[60] Rameshkumar A, Sivasudha T, Jeyadevi R, Arul-Ananth D, Pradeepha G. Effect of Environmental Factors (Air and UV-C Irradiation) on Some Fresh Fruit Juices. Eur Food Res Technol, 2012; 234: 1063-1070.

[61] Alegria C, Pinheiro J, Duthoit M, Gonçalves EM,Moldão-Martins M, Abreu M. Fresh-cut carrot (cv. Nantes) quality as affected by abiotic stress (heat shock and UV-C irradiation) pre-treatments. LWT - Food Science and Technology, 2012; 48: 197-203.

[62] Ric De Vos CH, Lieke-Kraak H, Bino RJ. Ageing of Tomato Seeds Involve Glutathione Oxidation. Physiologia Plantarum, 1994; 92: 131-139.

[63] Todorova D, Katerova Z, Shopova E, Nikolova A, Georgieva N, Sergiev I, Mapelli S. Polyamine Spermine Protects Young Pea Plants Against Ultraviolet-C Radiation. Biotechnology & Biotechnological Equipment, 2013; 27(3): 3798-3802, doi: 10.5504/BBEQ.2013.0012.

[64] Alcázar R, Altabella T, Marco F, Bortolotti C, Reymond M, Koncz C, Carrasco P, Tiburcio AF. Polyamines: Molecules with Regulatory Functions in Plant Abiotic Stress Tolerance. Planta, 2010; 231: 1237-1249.

[65] Maharaj R, Arul J, Nadeau P. Effect of Photochemical Treatment in the Preservation of Fresh Tomato (*Lycopersicon esculentum cv. Capello*) by Delaying Senescence. Postharvest Biology and Technology, 1999; 15(1): 13-23.

[66] Tiecher A, Arantes de Paula L, Chaves FC, Rombaldi CV. UV-C Effect on Ethylene, Polyamines and the Regulation of Tomato Fruit Ripening. Postharvest Biology and Technology, 2013; 86: 230-239.

[67] Mortazavi SMH, Siruie BN, Moalemi N, Eshgh S. The Effects of Polyamines and UV-C Irradiation on Postharvest Quality of Strawberry Fruit. ISHS Acta Hort., 2014; 1049: 749-754.

[68] Katerova ZI, Todorova D. Endogenous Polyamines Lessen Membrane Damages in Pea Plants Provoked by Enhanced Ultraviolet-C Radiation. Plant Growth Regul., 2009; 57: 145-152.

Section 5

Biological and Health Significance of Phytochemicals

Chapter 10

Oxidative Stress and Antioxidants in the Risk of Osteoporosis — Role of Phytochemical Antioxidants Lycopene and Polyphenol-containing Nutritional Supplements

L.G. Rao and A.V. Rao

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/60446

1. Introduction

Osteoporosis is a systemic disease that is characterized by low bone mass and deterioration of the microarchitecture of bone, resulting in an increased risk of fracture in postmenopausal women and men over 50 years old. Several factors have been identified that contribute to the risk of osteoporosis, and oxidative stress has now emerged as one of the most important life style risk factor associated with loss of bone mass. Phytochemicals as antioxidants have been shown to counteract the deleterious effects of oxidative stress in the risk of osteoporosis. This review will include an overview on osteoporosis, the deleterious effects of oxidative stress and the beneficial effects of phytochemical antioxidants, with emphasis on the results of our clinical studies on the phytochemical lycopene and polyphenols present in nutritional supplements.

2. Osteoporosis – An overview

Osteoporosis is a metabolic bone disease known as "the silent thief" because the gradual loss of bone associated with this disease usually occurs over the years, and there are usually no noticeable symptoms until the bones are so fragile that a fracture occurs [1]. Osteoporosis is "a major public health threat" that is projected to results to 8.1 million fractures (78 % women, 22 % men) during the period between 2010 and 2050 [2]. Approximately 1 in 2 women and 1 in 5 men older than 50 years will eventually experience osteoporotic fractures [3]. The condition costs our healthcare system $18 billion per year [4]. Newer findings on all aspects of osteoporosis have increased exponentially. The more importantly ones are discovering an ever

© 2015 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

increasing number of risk factors including oxidative stress, opening up new knowledge on the involvement of the bone forming cells osteoblasts and the bone resorbing cells osteoclasts in the development of osteoporosis, the introduction and improvement of more sensitive diagnostic instruments, and finding new drugs and the nutritional alternatives for the prevention and treatment of osteoporosis. Advances in the knowledge on osteoporosis is not without pitfalls. Hormone Replacement Therapy (HRT), once a first line of treatment for osteoporosis has been discontinued due to side effects [5]. It is becoming more evident that the drugs known as bisphosphonates, although effective in stopping the resoption of bone and preventing osteoporosis in women, are associated with a number of side effects [6, 7]. Because of this, a number of women are now resorting to other modes of treatment, including that from natural food components. Our laboratory has carried out studies on the use of phytochemical antioxidants such as lycopene and polyphenols present in nutritional supplements as possible alternatives and/or complementary to drugs in the treatment and prevention of osteoporosis. This chapter will include an overview on osteoporosis, oxidative stress as a risk factor in the development of osteoporosis and a review of studies on the use of antioxidants in counteracting oxidative stress in the prevention of osteoporosis. These topics should put our research in perspective and offer a rationale to our study approaches. Finally we will highlight our pioneering clinical studies on the lipid-soluble phytochemical antioxidant lycopene and the water-soluble antioxidant polyphenols present in a nutritional supplement in the prevention of risk for osteoporosis in postmenopausal women.

2.1. Risk factors for osteoporosis

Some of the risk factors for osteoporosis [8, 9] are presented in Table 1 [10]. The risk factors that are of interest in our studies are the oxidative stress-generating factors, including nutrition deficiency, low antioxidant status, smoking, alcohol intake, excessive sports activity and caffeine intake.

Unmodifiable	Modifiable
Race	Chronic inactivity
Sex	Low body weight
Age	Low lifetime calcium intake
Genetics	Medication used
Body size	Oxidative stress-related
Family History	Factors:
Previous Fractures	Smoking
	High Alcohol intake
	Low antioxidant status
	Nutrition deficiency
	Excessive sports activity
	Excessive caffeine intake

Table 1. Risk Factors for Osteoporosis

http://dx.doi.org/10.5772/60446

2.2. Prevention and treatment of osteoporosis

Until 10 years ago, the first line of treatment for women who have gone through menopause and were diagnosed with osteoporosis was hormone replacement therapy (HRT). However, results of the Women's Health Initiative (WHI) revealed that women taking HRT had higher risks for breast cancer, cardiovascular events, blood clots, cognitive decline, and more [5]. This treatment for osteoporosis has since been discontinued and is prescribed only for a short period of time to alleviate hot flashes in menopausal women [11]. The current treatments which inhibit bone resorption that are approved by the Food and Drug Administration (FDA) include a number of bisphosphonates under specific trademarks [12]. Some are taken daily while others are formulated for weekly, monthly or intermittent oral use [13, 14]. The newer bisphophonates are injectables such as Zoledronate and Ibandronate [14]. Other drugs available include calcitonin; Raloxifene (Evista), the Selective Estrogen Receptor Modulator (SERM) and strontium renalate [15]. Parathyroid hormone, PTH1-34 or teriparatide (Forteo), is the only anabolic agent currently approved for use by the FDA [16, 17]. The new class of osteoporosis drug now approved for use is a human monoclonal antibody (Denosumab) which bind to RANKL, imitating the effects of OPG and acting as an inhibitor of RANKL [18]. Other drugs are still being tested clinically for osteoporotic treatment and prevention [16].

None of the drugs are without side effects. Side effects that emerged in clinical trials include acute phase response with iv treatment or high-dose oral therapy and esophageal irritation with oral administration. Osteonecrosis of the jaw, musculoskeletal complaints, and atypical fractures are some uncommon side effects that have been noted with wide clinical use of bisphosphonate. The number of these events are small, and a clear cause-and-effect relationship between events and bisphosphonate treatment has not been established. Accumulation of Bisphosphonates in the bone create a reservoir leading to continued release from bone for months or years and provide some residual anti-fracture reduction long after treatment is stopped [19]. As a result, there is a recommendation for a drug holiday after 5 –10 yr of treatment with bisphosphonate [7, 19]. The length of the holiday is based on previous duration of treatment, BMD status and fracture risk. Studies with alendronate and risedronate showed that if treatment is stopped after 3–5 yr, there is at least 1–2 yr persisting anti-fracture efficacy. The consensus from expert panels [7] for those who are not on holiday is not to stop the use of drug since the side effects are often rare, and that the benefits outweigh the side effects. In the balance, most individuals who have osteoporosis are much better taking an osteoporosis medication [6].

2.3. Alternative approach to prevention and treatment of osteoporosis

Diet is now recognized as an important life-style factor in the management of bone health [20]. Given that many nutrients have been identified as being beneficial to bone health [21, 22], there is strong scientific support for the potential benefits of incorporating therapeutic nutritional interventions with contemporary pharmaceutical treatments [23]. As a result of the possible adverse side effects of HRT [5] and the ever increasing reports on the side effects of bisphosphonates that are prescribed for the management of postmenopausal osteoporosis [19], complementary and alternative medicine (CAM) is in demand as an alternative for the prevention

and treatment of osteoporosis [24]. CAM is the term for medical practices, services and products that are not a part of standard care. Some of the approaches include exercise, acupuncture, diet, herbs rich in polyphenols and nutritional supplements including calcium, zinc, magnesium boron and other vitamins and minerals [24]. Recent dietary guidelines for the prevention of chronic diseases have recommended an increase in the consumption of fruits and vegetables worldwide [25] that are good sources of dietary antioxidants [26]. The beneficial effects of antioxidants in bone health and osteoporosis are demonstrated epidemiologically and through clinical intervention. As will be reviewed in this chapter, our clinical studies on lycopene treatment and nutritional supplements containing polyphenols and other nutritional components showed positive results on bone health.

3. Oxidative stress and antioxidants – An overview

Oxidative stress is caused by reactive oxygen species (ROS) which are the main by-products formed in the cells of aerobic organisms that can initiate autocatalytic reactions in such a way that the target molecules get converted into free radicals causing a chain of damage [27]. There is ample evidence to show that oxidative stress induces an increase in the rate of bone loss and is therefore a risk factor for osteoporosis. Epidemiological evidence in humans and studies in animals indicate that aging and the associated increase in reactive oxygen species (ROS) are responsible for bone loss [28]. Oxidative stress is associated with the activity and function of both the osteoblasts and osteoclasts cells, the two major bone cells involved in the pathogenesis of osteoporosis [29, 30].

Under normal physiological conditions, the cells can fight free radical attack or oxidative stress by promoting antioxidant defenses. A number of endogenous defense mechanisms are present in the body, including the metal chelating proteins and the endogenous antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) [31]. Exogenous antioxidants come from dietary sources present in fruits and vegetables containing several phytonutrient antioxidants two of which are the potent antioxidant lipid-soluble lycopene and the water-soluble antioxidant polyphenols [32]. In cases where the exogenous antioxidants or antioxidants from diet fail to prevent oxidative damage, the repair antioxidants come into play which include DNA repair enzymes, protease, lipase, and transferase [33]. When antioxidants loses its fight with oxidative stress, diseases associated with oxidative stress develop, which include cardiovascular disease, cancer, diabetes, neurological diseases and osteoporosis [26].

3.1. Lycopene, a lipid-soluble phytochemical antioxidant

The role of lycopene in the prevention of human diseases is supported by a number of evidence and previously reviewed [34, 35]. Since then, there have been several epidemiological as well as clinical intervention studies showing the relationship between lycopene intake and the prevention of cancers at other sites, as well as coronary heart disease, diabetes, hypertension, macular degenerative disease, neurodegenerative disease and male infertility [26]. The role of

lycopene in bone health has so far been based on its potent antioxidant properties, the well-known role of oxidative stress in bone health, the limited studies on the effects of lycopene in bone cells in culture [34, 35] and the results of epidemiological studies [36, 37]. To date our clinical intervention studies at St. Michael's Hospital on the role of lycopene and elucidation of its mechanism in lowering the risk for osteoporosis in postmenopausal women (aged 50 to 60 years) are so far the only clinical studies reported in the literature.

3.2. Polyphenols, the water-soluble antioxidant

Polyphenols are a class of water-soluble molecules naturally found in plants [38]. It is estimated that there are 10,000 different phytonutrients (phyto, meaning from plants). The health benefits associated with fruits, vegetables, tea, red wine, and Mediterranean diets are probably linked to the polyphenol antioxidants [21, 39, 40]. The polyphenols of interest in our study are a mixture of flavonoids such as quercetin, apigenin, kaempferol and luteolin present in the supplement greens+™ [41]. greens+™ in combination with another supplement, bone builder™, were used in our study on osteoblasts cells and in clinical intervention studies on the prevention of risk of osteoporosis in postmenopausal women as will be reviewed below.

4. Studies on the antioxidants polyphenols and lycopene

4.1. Studies on lycopene

The direct role of lycopene in osteoblasts and osteoclasts, the cells involved in the pathogenesis of osteoporosis, is now being unraveled. This involvement is further supported by both epidemiological and clinical intervention with lycopene in postmenopausal women who are at risk of osteoporosis [29, 30].

An epidemiological study to determine the beneficial role of lycopene in the prevention of risk for osteoporosis was carried out by Rao et al [36]. In a cross-sectional study, 33 postmenopausal women aged 50–60 years participants were recruited and asked to provide seven-day dietary records and blood samples for analyses of total antioxidant capacity; oxidative stress parameters including lipid peroxidation and protein oxidation; and bone turnover markers including bone resorption marker NTX and bone formation marker. Their results showed that the estimated dietary lycopene had a significant and direct correlation with serum lycopene, suggesting that lycopene from the diet is bioavailable. Their conclusion that the higher serum lycopene was associated with a low NTx ($p<0.005$) and lower protein oxidation ($p<0.05$) supports the antioxidative properties of lycopene involvement in its mechanisms of action in bone [36].

The overall conclusions that can be derived from the cross-sectional study is that lycopene has a role in the prevention of risk for osteoporosis. Further clinical studies described below support this conclusion.

Mackinnon et al [42] studied whether the elevated dose obtained through lycopene supplementation compared to intakes typically obtained from the usual daily diet was more beneficial in reducing bone turnover markers. Serum lycopene, bone turnover markers and oxidative stress parameter data were compared between postmenopausal women who were supplemented with lycopene and those who obtained both a low and high intake lycopene from daily food diet. Results showed that women who consumed lycopene supplement had significantly lower TBARS values than participants who obtained a low intake or high intake lycopene through their usual daily diets. These differences in TBARS value may be attributed to a significantly higher concentration of serum 5-*cis* in lycopene-supplemented participants compared to participants who obtained their lycopene from low or high usual daily diet. This suggests that it is the 5-*cis* isomer, with the most potent antioxidant capacity which, at higher concentrations, decreases bone turnover markers due to its ability to provide the greatest protection against oxidative stress. It also appears to show that supplementation with lycopene may be necessary in spite of the daily intake of lycopene [42].

Another study was carried out to determine the effects of a lycopene-restricted diet on oxidative stress parameters and bone turnover markers in postmenopausal women [43]. Results showed that restricting the participants from consuming lycopene-containing products resulted in significant decreases in serum lycopene, α-/β-carotene and lutein/zeaxanthin, with the overall change in the serum carotenoids being lower than that seen for lycopene. All configurations of lycopene (all trans, 5-cis- and other cis lycopene) were found to be decreased and the antioxidant enzymes SOD and CAT were also significantly depressed after lycopene restriction. These changes were accompanied by a significant increase in the bone resorption marker NTx. The important conclusion from this study is the possibility that the significant increase in the bone resorption marker NTx could lead to a long-term decrease in BMD and increased fracture risk as was observed by Brown et al. [44]; longer restriction period may be detrimental to a group of postmenopausal women who were already at high risk for osteoporosis. Therefore, shorter wash-out periods of no lycopene consumption is all that is needed in clinical trials examining the effects of lycopene on bone health [43].

A clinical fully randomized controlled intervention study was next carried out by Mackinnon et al [45] to investigate directly the effects of lycopene supplementation on decreasing the risk for osteoporosis. Lycopene supplements include lycopene capsules, tomato juice with normal amount of lycopene, tomato juice with high amount of lycopene. They have shown that after the 4-month duration, the LYCOPENE-supplemented group had a significant increase in total antioxidant capacity, decrease in oxidative stress parameters protein oxidation as shown by increase in thiol values and lipid peroxidation as shown by TBARS which correlated to a decrease in NTx; all changes were significantly different from the PLACEBO group. These findings suggest that lycopene obtained in the form of tomato juice or capsule exerted equivalent antioxidant potency in reducing the risk of osteoporosis in postmenopausal women [45].

Mackinnon et al studied whether polymorphism plays a role in the development of osteoporosis [46]. To do this, Mackinnon et al. studied the role of 172T→A or 584A→G polymorphisms

of the paraoxonase 1 (PON 1) in modulating the effects of serum lycopene on antioxidant capacity, oxidative stress parameters and bone turnover markers, and in women between the ages of 25-70 years). Their results showed that the PON1 polymorphism modified the association between lycopene and NTx and BAP, an interaction that may also moderate the risk of osteoporosis [46].

In another study, they showed that there was a significant interaction between PON1 genotype and change in TBARS ($p<0.05$) suggesting that supplementation with lycopene resulted in decreased lipid peroxidation, which interacted with the PON1 genotype to decrease bone resorption markers in postmenopausal women. These results provided a mechanistic evidence of how intervention with lycopene may act to decrease lipid peroxidation and thus the risk of osteoporosis in postmenopausal women [45, 47].

In conclusion, the demand for the use of other natural food components in the management of postmenopausal osteoporosis has increased due to reports on the adverse side effects of the conventional therapy (eg, HRT and bisphosphonates). The studies reviewed above revealed evidence that antioxidants such as lycopene can counteract the damaging effects of oxidative stress brought about by ROS that lead to the development of osteoporosis. The results of studies reviewed here indicate that lycopene maybe useful either as a dietary alternative to drug therapy or as a complement to the drugs presently approved for used by women at risk of osteoporosis.

4.2. Studies on polyphenols

It is well known that polyphenols have a role in the prevention of chronic diseases such as cancers, diabetes, cardiovascular diseases, neurodegenerative diseases, and osteoporosis. Interest on polyphenols and bone health has increased in the last 10 years [48-51]. The anabolic role of phytonutrients and especially polyphenols in bone was reviewed by Horcajada [49], the mechanisms of action of polyphenol in osteoblast function and its interaction with osteoclasts was reviewed by Trzeciakiewicz [50] and the beneficial effects of green tea polyphenols has been reviewed [52, 53].

Currently, most of the research on polyphenols and their effects have emerged from *in vitro* and *in vivo* animal studies with only a few clinical studies available. In our recent review, we have included tables listing all the studies on polyphenols *in vitro* bone cell culture and the epidemiologic studies on the protective effects of polyphenol consumption against osteoporosis [54].

Combinations of polyphenols have also been studied. One such source is the nutritional supplement greens+™, a blend of several herbal and botanical products containing a substantial amount of polyphenols including quercetin, apigenin and luteolin [41] which act as antioxidants and therefore should be able to counteract oxidative stress. Thus, Rao et al [55] have shown that greens+*™*, is more effective in stimulating osteoblasts to form more bone nodules in a dose-dependant manner than epicatechin, the main polyphenol found in green

tea. We have further shown that this stimulatory effect is accompanied by decreases in the reactive oxygen species H_2O_2 [56].

Two additional nutritional supplements have since been formulated which may prove to be good for bone health. These are the bone builder™ and the greens+bone builder™ ; the latter is the original greens+™ product that has been supplemented with the bone builder™ formula which contains several compounds including vitamins, minerals, and antioxidants. These various components have been separately shown to have some beneficial effect on bone [57]. Using the human osteoblast SaOS-2 cells, Rao et al showed that similarly to the greens+™, the water-soluble bone-builderTM extract had a significant dose-dependent stimulatory effect on bone nodules formation [58]. It was additionally shown that similarly to the greens+™, the watersoluble bone-builder™ extract had a significant dose-dependent stimulatory effect on bone nodules formation [58]. In a later study, they have shown that when the two supplements, greens+*™* and bone builder*™*, were tested as combination, the effects were six times more effective than either one alone [59]. This led Rao et al to believe that synergistic effects of greens +*™* and bone builder*™* may have a beneficial effect on osteoporosis. A product called greens +bone builder™ is available commercially [57].

A clinical study was then carried out to evaluate the effect of the nutritional supplement greens + bone builder*™* for 8 weeks on the risk for osteoporosis in postmenopausal women compared to placebo control [60, 61]. Results have shown that there was an increase in total antioxidant capacity, as well as a decrease in both lipid and protein oxidation over a 4 and 8-weeks of intervention with greens+ bone builder*™* compared to placebo [60] suggesting that the nutritional supplement may have a beneficial effect on bone health by mitigating the effects of oxidative stress. In order to test whether the antioxidant properties of greens+bone builder™ can prevent the risk of osteoporosis in postmenopausal women. Kang et al [60] also measured the serum bone turnover markers, C-terminal telopeptide of type I collagen (CTX) as indicator of bone resorption, and procollagen type I N-terminal propeptide (PINP) as indicator of bone formation and determined their correlations with the serum antioxidant capacity, and the oxidative stress parameters lipid peroxidation, protein oxidation. Statistical analysis showed that at 8 weeks, the greens +bone builder™ supplement group significantly decreased the bone resorption marker CTX, while the Placebo group showed no significant changes. The supplement group was also significantly different from that of the Placebo group in all parameters measured. This decrease CTX correlated to the increase in their serum total antioxidant capacity and decreases in oxidative parameters protein oxidation lipid peroxidation [61]. These results suggest that a daily supplementation with polyphenols and micronutrients may be important in reducing oxidative damage by reducing bone resorption, thereby reducing the risk of osteoporosis in postmenopausal women [60, 61].

In summary, studies reported in the literature on the role of polyphenols in bone health have exploded in the last 10 years, but most of the reports involved in vitro studies in osteoclasts and osteoblasts, animal studies and epidemiologicai studies. There is little doubt from the excellent studies reported that oxidative stress is one of the primary culprits responsible for

http://dx.doi.org/10.5772/60446

the pathogenesis of osteoporosis via its role in osteoclastic resoption and the detrimental effects on the bone-forming osteoblasts. To date, only four clinical intervention studies have been reported, including ours. It is easy to see why it is very difficult to evaluate the role of polyphenols since, as we learned from this review, there are at least 8,000 different polyphenols identified to date, and each one probably having different effects on humans. Additionally, polyphenols are present in food with other constituents that may also be beneficial to bone health. In our clinical study, we combined the effects of a combination of polyphenols present in the nutritional supplement from greens+™ with the nutritional components present in bone builder™ such as minerals, vitamins and other nutrients. It is possible that the effects of greens +bone builder™ in increasing total antioxidant capacity, decreasing the oxidative stress markers protein oxidation and lipid peroxidation which correlated to the decrease in bone turnover marker for bone resorption is a result of the combined effects of the different polyphenols it contained with those of the other nutritional components present in the bone builder™. It remained for future studies to zero in on specific component that is responsible for its beneficial effect.

In conclusion, we showed that oxidative stress due to ROS that are shown to cause the development of osteoporosis may be prevented by supplementation with the antioxidants lycopene and polyphenols. Results of in vitro studies in osteoblasts and osteoclasts, animal intervention studies, epidemiological studies and clinical intervention studies on lycopene and polyphenols are evidence for their potential use as alternative or complementary agent with other established drugs approved for the prevention or treatment of osteoporosis in postmenopausal women.

Acknowledgements

Funding for this research into Oxidative Stress, Antioxidants and Bone Health is shared by H.J. Heinz Co (Canada), Kagome Co. (Japan), LycoRed Natural Product Industries, Ltd. (Israel), Genuine Health Ltd (Canada), Millenium Biologix Inc. (Canada), and matched by the Canadian Institutes of Health Research (CIHR). We sincerely thanked the valuable contributions to this research by the following students/graduate students and staff at the Calcium Research Laboratory, Department of Medicine at St Michael's Hospital and the University of Toronto and Department of Nutritional Sciences, University of Toronto: Dr. Bala Balachandran, Jaclyn Beca, Dawn Snyder, Loren Chan, Honglei Shen, Salva Sadeghi, Ayesha Quireshi, Dr. Erin Mackinnon and Nancy Kang. Their contributions were based on their experimental data, written reports published/in press manuscripts/theses. We would also like to thank to Dr. R.G. Josse for providing us with his medical expertise as well as allowing us access to his list of patients we were able to recruit. Special thanks to Dr. H. Vandenberghe for carrying out the CTX assay and for her valuable suggestions.

Author details

L.G. Rao[1*] and A.V. Rao[2]

*Address all correspondence to: leticia.rao@utoronto.ca

1 Department of Medicine, St Michael's Hospital and University of Toronto, Canada

2 Department of Nutritional Sciences, University of Toronto, Canada

Parts of this chapter are © [2013] IEEE. Reprinted, with permission, from Rao LG and Rao AV Oxidative stress and antioxidants in the risk of osteoporosis - Role of the antioxidants lycopene and polyphenols. Chapter 5, pages 117-161. In: Topics in Osteoporosis. Ed. M. Vades-Flores.Intech Publishing. 2013.

References

[1] Ahmed S, Elmantaser M. Secondary osteoporosis. Endocr Dev. 2009;16:170-90.

[2] Bleibler F KA, Benzinger P, Rapp K, König H.. The health burden and costs of incident fractures attributable to osteoporosis from 2010 to 2050 in Germany-a demographic simulation model. Osteoporos Int Epub July 14 2012[Epub ahead of 2012.

[3] Coxam V. New advances in osteoporosis nutritional prevention. Med Sci (Paris). 2005;21(3):297-301.

[4] Lindsay R, Burge R, Strauss D. One year outcomes and costs following a vertebral fracture. Osteoporosis Int. 2005;16:78-85.

[5] Rossouw J, Anderson G, Prentice R, LaCroix A, Kooperberg C, Stefanick M, et al. Risks and Benefits of Estrogen Plus Progestin in Healthy Postmenopausal Women Principal Results From the Women's Health Initiative Randomized Controlled Trial. JAMA 2002;288. 2002;3:321-33.

[6] Schmidt G, Horner K, McDanel D, Ross M, Moores K. Risks and benefits of long-term bisphosphonate therapy. Am J Health Syst Pharm. 2010;67(12):994-1001.

[7] Diab D, Watts N. Bisphosphonates in the treatment of osteoporosis. Endocrinol Metab Clin North Am (Epub 41 Epub 2012 Jun 9). 2012;3:487-506.

[8] Monti J. Osteoporosis Risk Factors http://askhealthlinecom/health/osteoporosis-risk-factors. 2010.

[9] Stetzer E. Identifying Risk Factors for Osteoporosis in Young Women. The Internet Journal of Allied Health Sciences and Practice. 2011;9(4):1-8.

[10] Rao L. Will tomatoes prevent osteoporosis. Snell Endocrine Rounds 2005;5(2):1-6.

[11] Furness S, Roberts H, Marjoribanks J, Lethaby A. Hormone therapy in postmenopausal women and risk of endometrial hyperplasia. Cochrane Database of Systematic Reviews (Epub 15 AUG 2012) 2012;8.

[12] Bell NH, Johnson RH. Bisphosphonates in the treatment of osteoporosis: virtual symposium on osteoporosis. Endocrine. 1997;6(2):203-6.

[13] Dempster DW, Bolognese MA. Ibandronate: The Evolution of a Once-a-Month Oral Therapy for Postmenopausal Osteoporosis. Journal of Clinical Densitometry. 2006;9(1):58-65.

[14] Sunyecz J. Optimizing dosing frequencies for bisphosphonates in the management of postmenopausal osteoporosis: patient considerations. Clin Interv Aging (Epub 2008 December). 2008;3(4):611-27.

[15] Lyritis GP. Fracture healing and antiosteoporotic treatments. Medicographia. 2010;32:79-85.

[16] Hegge K, Fornoff A, Gutierres S, Haack S. New therapies for osteoporosis. Journal of Pharmacy Practice. 2009;22:53-64.

[17] Han S, Wan S. Effect of teriparatide on bone mineral density and fracture in postmenopausal osteoporosis: meta-analysis of randomised controlled trials. International Journal of Clinical Practice. 2012;66(2):199-209.

[18] Adler RA, Gill RS. Clinical utility of denosumab for treatment of bone loss in men and women. Clin Interv Aging (Epub 2011 May 24). 2011;6:119-24.

[19] Watts N, DL D. Long-Term Use of Bisphosphonates in Osteoporosis. The Journal of Clinical Endocrinology & Metabolism. 2010;95(4):1555-65.

[20] Palacious C. The role of nutrients in bone health, from A to Z. Critical Reviews in Food Science & Nutrition. 2006;46:621-8.

[21] New S. Intake of fruit and vegetables: Implications for bone health. The Proceedings of the Nutrition Society 2003;62(4):889-99.

[22] Lister C, Skinner M, D. H. Fruits, vegetables and their phytochemicals for bone and joint health. Curr Top Nutraceut Res. 2007;5:67-82.

[23] Tucker K, Hannan M, Chen H, Cupples L, Wilson P, Kiel D. Potassium, magnesium, and fruit and vegetable intakes are associated with greater bone mineral density in elderly men and women. The American Journal of Clinical Nutrition. 1999;69(4): 727-36.

[24] Rees M. Management of the menopause: integrated health-care pathway for the menopausal woman. Menopause Int. 2011;17(2):50-4.

[25] Pomerleau J, Lock K, Knai C, McKee M. Effectiveness of intervention and programmes promoting fruit and vegeta ble intake. WHO. 2005.

[26] Rao A, Rao LG. Carotenoids and human health (Review). Pharmacological Research. 2007;55(3):207-16.

[27] Raman W, Khalid A, Right N. Studies on free radicals, antioxidants, and co-factors. Clinical Interventions in Aging. 2007;2(2):219-36.

[28] Manolagas S, A. P. What old means to bone. Trends Endocrinol Metab (Epub 2010 Mar 11). 2010;21(6):369-74.

[29] Rao LG, Rao AV. Oxidative stress and antioxidants in the risk of osteoporosis - role of the antioxidants lycopene and polyphenols. In: M V-F, editor. Topics in Osteoporosis. Croatia: InTech. p. 117-61.

[30] Rao LG, Kang NN, AV R. Lycopene and Other Antioxidants in the Prevention and Treatment of Osteoporosis in Postmenopausal Women. In: V P, editor. Aging: Oxidative Stress and Dietary Antioxidants: Elsevier Inc; 2014. p. 247-58.

[31] Mate JM, Perez-Gomez C, I. NdC. Antioxidant Enzymes and Human Diseases. Clinical Biochemistry. 1999;32(8):595-603.

[32] Rao L. Lycopene and the Prevention of Osteoporosis.. In: AV R, editor. Lycopene. Scotland: Caledonia Science; 2006.

[33] Willcox J, Ash S, Catignani G. Antioxidants and prevention of chronic disease. Crit Rev Food Sci Nutr. 2004;44:275-95.

[34] Rao A, Rao L. Lycopene and human Health. Current Topics in Nutraceutical Research. 2004;2.

[35] Giovannucci E. Tomatoes, tomato-based products, lycopene, and cancer: Review of the epidemiologic literature. J Natl Cancer Inst. 1999;91:317-31.

[36] Rao L, Mackinnon E, Josse R, Murray T, Strauss A, Rao A. Lycopene consumption decreases oxidative stress and bone resorption markers in postmenopausal women. Osteoporosis International 2007. 2007;18(1).

[37] Sahni S, Hannan M, Blumberg J, Cupples L, Kiel D, Tucker K. Protective effect of total carotenoid and lycopene intake on the risk of hip fracture: a 17-year follow-up from the Framingham Osteoporosis Study. Journal of Bone & Mineral Research. 2009;24(6):1086-94.

[38] Quideau S, Deffieux D, Douat-Casassus C, Pouysegu L. Plant polyphenols: chemical properties, biological activities, and synthesis. Angew Chem Int Ed Engl (Epub 2011/01/13) 2011;50(3):586-91.

[39] Puel C, Coxam V, Davicco M. Mediterranean diet and osteoporosis prevention. Med Sci (Paris). 2007;23:756-60.

[40] Urquiaga I, Strobel P, Perez D, Martinez C, Cuevas A, Castillo O, et al. Mediterranean diet and red wine protect against oxidative damage in young volunteers. Atherosclerosis (Epub 2010 Apr 21). 2010;211(2):694-9.

[41] Rao A, Balachandran B, Shen H, Logan A, Rao L. In Vitro and in Vivo Antioxidant Properties of the Plant-Based Supplement Greens+. Int J Mol Sci. 2011;12:4896-908.

[42] Mackinnon E, Rao A JR, L. R. Supplementation with the antioxidant lycopene significantly decreases oxidative stress parameters and the bone resorption marker N-telopeptide of type I collagen in postmenopausal women. Osteoporosis International. 2011;22(4):1091-101.

[43] Mackinnon E, Rao A, Rao L. Dietary restriction of lycopene for a period of one month resulted in significantly increased biomarkers of oxidative stress and bone resorption in postmenopausal women Journal of Nutrition, Health & Aging. 2011;15(2):133-8.

[44] Brown J, Albert C, Nassar B, Adachi J, Cole D, Davison K, et al. Bone turnover markers in the management of postmenopausal osteoporosis (review). Clin Biochem. 2009;42(10-11):929-42.

[45] MacKinnon E. The Role of the Carotenoid Lycopene as an Antioxidant to Decrease Osteoporosis Risk in Women: Clinical and in vitro Studies [PHD [dessertation]. Toronto, Ontario: : University of Toronto; 2010.

[46] Mackinnon E, El-Sohemy A, Rao A, L. R. Paraoxonase 1 polymorphisms 172T->A and 584A->G modify the association between serum concentrations of the antioxidant lycopene and bone turnover markers and oxidative stress parameters in women 25-70 years of age. Journal of Nutrigenetics & Nutrigenomics. 2010;3(1).

[47] Rao L, Mackinnon E, El-Sohemy A, V. R, editors. Postmenopausal Women with PON1 172TT Genotype Respond to Lycopene Intervention With a Decrease in Oxidative Stress Parameters and Bone Resorption Marker NTx Annual Meeting of the American Society for Bone and Mineral Research (ASBMR); 2010; Toronto, Ontario.

[48] Everitt A, Hilmer S, Brand-Miller J, Jamieson H, Truswell A, Sharma A, et al. Dietary approaches that delay age-related diseases. Clin Interv Aging. 2006;1(1):11-3.

[49] Horcajada M, Offord E. Naturally plant-derived compounds: role in bone anabolism. Curr Mol Pharmacol. 2012;5(2):205-18.

[50] Trzeciakiewicz A, Habauzit V, Horcajada M. When nutrition interacts with osteoblast function: molecular mechanisms of polyphenols. Nutr Res Rev (Epub 2009 Feb 26). 2009;22(1):68-81.

[51] Scalbert A, Manach C, Morand C, Rémésy C, Jiménez L. Dietary polyphenols and the prevention of diseases. Crit Rev Food Sci Nutr. 2005;45(4):287-306.

[52] Cabrera C, R A, Giménez R. Beneficial effects of green tea--a review. J Am Coll Nutr. 2006;25(2):79-99.

[53] Yang C, Landau J. Effects of tea consumption on nutrition and health. J Nutr. 2000;130(10):2409-12.

[54] Rao L, Kang N, Rao A. Polyphenols and bone health: A review.. In: Rao A, editor. Phytochemicals. Rijeka, Croatia: In Tech Open Access Publisher 2012. p. 958-73.

[55] Rao L, Balachandran B, A. R. Polyphenol Extract of Greens+™ Nutritional Supplement Stimulates Bone Formation in Cultures of Human Osteoblast-like SaOS-2 Cells. J Diet Suppl. 2008;5(3):264-82.

[56] Rao L, Balachandran B, A. R. The stimulatory effect of the polyphenols in the extract of Greens+TM herbal preparation on the mineralized bone nodule formation (MBNF) of SaOS-2 cells is mediated via its inhibitory effect on the intracellular reactive oxygen species (iROS).. 27th Annual Meeting of the American Society of Bone and Mineral Research; September 23-27, 2005; 2005.; Nashville, Tennesse2005.

[57] Graci S. The Bone Building Solution. Mississauga, Ontario: John Wylie And Sons Canada, Ltd; 2006.

[58] Rao L, Snyder D, Balachandran B, Beca B, Shen H, Sedeghi S, et al. Herbal extract and nutritional bone-building supplement synergistically stimulate bone formation in human osteoblast cells in vitro. IOF Regionals - 1st Asia-Pacific Osteoporosis Meeting; December 10-13, 2010; Singapore2010.

[59] Snyder D, Rao A, Balachandran B, Beca J, Shen H, Sadeghi S, et al. Extracts of the Nutritional Supplement, bone builderTM, and the Herbal Supplement, greens+TM, Synergistically Stimulate Bone Formation by Human Osteoblast Cells in VitroOntario2010. Annual Meeting of the American Society for Bone and Mineral Research (ASBMR; October 13 - 16, 2010; Toronto, Ontario2010.

[60] Kang N, Rao A, De Asis K, Chan L, Rao L. Antioxidant effects of a nutritional supplement containing polyphenols in postmenopausal women: a randomized controlled study. Journal of Aging: Research and Clinical Practice. 2012;1(3):183-87.

[61] Kang NN, Rao AV, Josse R, Vandenberghe H, De Asis K, Chan LA. Dietary polyphenols combined with micronutrients may be a good alternative to or complement with approved drugs for the prevention of risk of osteoporosis in postmenopausal women. The Journal of Phytochemistry. 2014;115:291300.

Chapter 11

Phytochemicals and Cancer – Possible Molecular Targets of Phytochemicals in Cancer Prevention and Therapy

Victor P. Bagla, Matlou P. Mokgotho and
Leseilane J. Mampuru

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/59873

1. Introduction

Phytochemicals and their synthetic derivatives have, over the decades, attracted huge attention and made significant contribution in modern drug discovery programs for their relevance in leveraging the severity or cure of several human diseases, including cancer. These natural products and their derivatives thereof have demonstrated immense pharmacological and biological properties. Although the molecular mechanisms of action of a majority of these phytochemicals are yet to be elucidated, cumulative evidence and the continued generation of new scientific data on their health benefits in disease prevention and cure have accrued over the years. Recent advancement in molecular biology, high throughput screening, biomarker identifications, target selection and genomic approaches have enabled researchers to understand salient interactions of natural products or their derivatives with cancer cells.

Most phytochemicals exhibit their pharmacologic effects in nature through a multi-targeted approach; a property that is highly desirable since therapy for carcinomas invariably involves dysregulation of multiple genes and associated cell-signalling pathways at various stages of initiation, progression and metastasis. On the other hand, in cancer initiation and progression, acquired genetic alterations, microenvironment-mediated epigenetic (heritable changes in gene activity and expression that occur without alteration in DNA sequences and are sufficiently powerful to regulate the dynamics of gene expression) perturbations have primarily been considered to play an important role in neoplastic development [9]. Genetic factors which control epigenetic modifications have been extensively documented [53].

One of the most widely studied phytochemical with anticancer properties is curcumin. Indeed, curcumin together with a number of related chemically-defined derivatives have been used

© 2015 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

extensively in the treatment of a number of malignant growths, such as breast cancer (Figure 1). The rhizome of the plant *Curcuma longa* L., commonly known as turmeric, has been used for centuries as a spice and colouring agent. The dry rhizome of turmeric contains curcumin, the main bioactive component. Curcumin displays a diverse range of molecular targets, supporting the concept that it acts upon numerous biochemical and molecular cascades (Figure 2). Although the precise mode of action of this compound is yet to be fully elucidated, studies have shown that the chemopreventive action of curcumin might be due to its ability to induce apoptosis by several pathways. Curcumin physically binds to as many as 33 different proteins, including thioredoxin reductase, cyclooxygenase-2, (COX-2), protein kinase C, 5-lipoxygenase (5-LOX), and tubulin. Various molecular targets modulated by this agent include transcription factors, growth factors and their receptors, cytokines, enzymes, and genes regulating cell proliferation, and apoptosis. Since phytochemicals exhibit their therapeutic effect through multi-mechanism of action, research into the mechanism of action of curcumin in cancer has demonstrated its relevance in various biochemical pathways. The modulation of anti-apoptotic and survival pathways by curcumin as a strategy to induce apoptosis in cancer cells is well documented as well as its ability to inhibit proliferation, invasion, angiogenesis and metastasis of different cancers through interaction with multiple cell-signalling proteins [29, 48]. A list of some of apoptotic and growth inhibitory pathways activated by curcumin in tumour cells has been well documented [47].

Several reports indicate conflicting evidence on the sensitivity of different cancer cells to the effect of the same phytochemical content of extracts in exhibiting their anti-proliferative effect. This review will therefore focus on recent research developments in anticancer therapy using curcumin, as a representative plant-derived compound, against breast, lung, colorectal, cervical and prostate cancers, generated between 2008 and 2014.

2. Curcumin and breast cancer

Tumour progression is characterised by a mass formed by multiple populations of cells with mechanisms capable of inhibiting apoptosis, while promoting survival pathways and the invasion of healthy tissues through the blood and lymphatic circulation. The manifestation of breast cancer can be determined by the expression of receptors to oestrogen (ER) and to progesterone (PR) and of Her2 (c-erbB2, Her2/neu). Triple-negative breast cancer (TNBC) lacks the expression of the oestrogen receptor (ER), progesterone receptor (PR) and epidermal growth factor receptor 2 (HER2/EGFR2) and is an aggressive breast cancer phenotype with a poor prognosis. The p27, a CIP/KIP member, is a cyclin-dependent kinase inhibitor that causes G_1 arrest by inhibiting G_1 cyclin-CDK activities and the reduction of p27 is initiated by enhanced ubiquitin-mediated degradation, in which the Her2/Grb2/MAPK pathway has been implicated in the decrease of p27 stability. The effect of curcumin has been reported to stabilise p27 levels, a lack of which is associated with poor prognosis in breast cancer. In order to investigate whether this effect is mediated through changes in the S-phase kinase-associated protein 2 (Skp2) or Her2 expression, Sun and co-workers determined the inhibitory effect of curcumin on Skp2-mediated p27 ubiquitination in Her2/Skp2-overexpressing cancer cell lines

(MDA-MB-231/Her2 cells) [57]. Their findings revealed that curcumin represses cell proliferation, induces G_1 arrest at a low dosage, and triggers apoptosis at a higher dosage and blocks cell migration in MDA-MB-231/Her2 cells. Curcumin at low dose was also shown to increases p27 and decreases Skp2, Her2, Cyclin E, CDK kinases in a time- and dose-dependent manner, a finding that is suggestive that p27, Skp2 and Her2 may be involved in the curcumin-induced growth inhibition in MDA-MB-231/Her2 cells. On the contrary, higher doses of curcumin produce a dose-dependent apoptotic death in MDA-MB-231/Her2 cells, an event that was observed to be related to cleaved forms of PARP and caspase-3 [57]. [16] on the other hand observed that since the F-box protein S-phase kinase-associated protein 2 (Skp2), which acts as an oncogene through targeting p27 for degradation, is overexpressed in many different human cancers; and that since curcumin induces p27 expression and growth arrest through the inhibition of Skp2 in MDA-MB-231 cells, a therapeutic strategy that could be designed to reduce Skp2 may play a central role in the treatment of ER/HER2 negative breast cancers. In another study, curcumin has been shown to exhibit an inhibitory effect on the proliferation of MDA-MB-231 cells and induced G_2/M arrest in a dose-dependent manner. The study further demonstrated curcumin to increase the protein expression levels of p21 and Bax and decreased the levels of p53 and Bcl-2, a finding that suggests that one molecular mechanism by which curcumin inhibits the proliferation of MDA-MB-231 cells could be either through the up-regulation of p21 expression for apoptosis to occur or through the up-regulation of the Bax-to-Bcl-2 ratio [8].

The effect of curcumin in inducing paraptosis in malignant breast cancer cell lines, including MDA-MB-435S, MDA-MB-231, and Hs578T cells has also been demonstrated. Apoptosis was demonstrated to be promoted by vacuolation that results from swelling and fusion of mitochondria and/or the endoplasmic reticulum (ER) of the cell. The importance of protein synthesis in the process was tested by the use of cycloheximide. Cycloheximide was shown to block curcumin-induced vacuolation and subsequent cell death. AIP-1/Alix protein levels, an inhibitor protein of paraptosis, remained increasingly down-regulated in curcumin-treated malignant breast cancer cells while their overexpression decreased curcumin-induced cell death. ERK2 and JNK activation were shown to be associated with curcumin-induced cell death. It was also shown that mitochondrial superoxide acts as a critical early signal in curcumin-induced paraptosis, whereas proteasomal dysfunction was mainly responsible for the paraptotic changes associated with oestrogen receptor (ER) dilation [69]. Other authours have focused on the testing of the potency of curcumin analogues in comparison with curcumin. In one such study, an *ortho*-hydroxy substituted analogue of curcumin (BDMC-A) was analysed for its cytotoxicity. The analogue inhibited MCF-7 cells at a dose equivalent to that of curcumin. Further analysis of the apoptotic mechanism of the analogue, in comparison with curcumin, demonstrated that the analogue exerted more potent effect on the modulation of selective apoptotic markers of the intrinsic pathway: p53, Bcl-2, Bax, cytochrome c, Apaf-1, caspases-9, -3, PARP and those of the extrinsic pathway: FasL, caspase-8, as compared to curcumin. mRNA expression studies for Bcl-2/Bax also buttressed the efficacy of the analogue. An *in silico* molecular docking study with PI3K revealed that the docking of the analogue was more potent compared to curcumin. Increased apoptotic induction by the analogue was also

demonstrated using different techniques in which characteristic apoptotic features such as nuclear fragmentation and chromatin condensation were exhibited [39].

On the other hand, a major metabolite of curcumin tetrahydrocurcumin (THC) has been investigated for its efficacy and associated mechanism of action in MCF-7 cells. The metabolite was shown to exhibit significant cell growth inhibition by inducing MCF-7 cells to undergo mitochondrial apoptosis and G_2/M arrest, while co-treatment of cells with THC and p38 MAPK inhibitor was observed to effectively reverse the dissipation in mitochondrial membrane potential, and was also shown to block THC-mediated Bax up-regulation, Bcl-2 down-regulation, caspase-3 activation as well as p21 up-regulation. This finding thus highlights the role of p38 MAPK in THC-induced mediated apoptosis and G_2/M arrest, and its relevance, following the biotransformation of curcumin *in vivo* in the treatment of breast cancer [20].

Maspin is a serine protease inhibitor, which suppresses tumour growth and metastasis *in vivo* and tumour cell motility and invasion *in vitro*. In another study, curcumin was shown to up-regulate the expression of miR-15a and miR-16 and down-regulate the expression levels of Bcl-2 in MCF-7 treated cells, while silencing of miR-15a and miR-16 expression by specific inhibitors was shown to restore the expression of Bcl-2 levels. It was concluded from that study that curcumin can reduce the expression of Bcl-2 by up-regulating the expression of miR-15a and miR-16 in MCF-7 cells [44, 66].

Chronic inflammation is considered a major risk factor in the development and metastatic progression of cancers, while obesity on the other hand increases the risk of breast cancer in postmenopausal women. In obese individuals, there are increased levels of growth factors including insulin and insulin-like growth factors (IGFs). High insulin levels lead to an increase in the secretion of oestrogen, by binding to the circulating sex hormone binding globulin (SHBG). Consequently, the increased oestrogen-mediated downstream signalling favours breast cancer development.

Recent reports have shown that curcumin inhibits the expression of the pro-inflammatory cytokines CXCL-1 and CXCL-2, thereby enhancing the diminished formation of breast cancer metastasis [28]. In one study, the authours analysed the correlation between the effects of curcumin on miRNA expression using microarray miRNA expression analyses. Their findings revealed curcumin to modulate the expression of a series of miRNAs, including miR181b, in metastatic breast cancer cells while miR181b was observed to down-modulate CXCL-1 and CXCL-2 through a direct binding to their 3′-UTR [28]. [2] have also demonstrated that reduction of CXCL-1 and CXCL-2 messenger RNA levels is NFκB dependent and requires intact IκBα expression. Furthermore, the silencing of CXCL-1 and CXCL-2 was observed to result in down-regulation of several metastasis-promoting genes among which was the cytokine receptor CXCR4. The ability of curcuminoids to prevent transforming growth factor (TGF-β) induction of parathyroid hormone-related protein (PTHrP) and to reduce osteolytic bone destruction by blockade of Smad signalling in breast cancer cells has also been investigated [63]. To further understand the underlying mechanism, the effects of curcuminoids on breast cancer cell secretion of the bone-resorptive peptide PTHrP and on lytic breast cancer bone metastasis were evaluated in the study. Curcumin was shown to inhibit TGF-β-stimulated PTHrP secretion in MDA-MB-231 human breast cancer cells independent of effects on

cell growth inhibition *in vitro*. The effect on TGF-β signalling, reveal decreases in phospho-Smad2/3 and Ets-1 protein levels with no effect on p-38 MAPK-mediated TGF-β signalling [63].

On the other hand, the mechanism of action of EF24, a novel curcumin analogue, in comparison with curcumin has been evaluated on MDA-MB231 breast cancer cells. EF24 and/or curcumin were shown to inhibit HIF-1α protein levels and, the subsequent inhibition of HIF transcriptional activity. The induction of HIF inhibition was demonstrated to occur in a VHL-dependent but proteasome-independent manner. While curcumin was seen to inhibit HIF-1α gene transcription, EF24 on the other hand exerted its activity by inhibiting HIF-1α post-transcription. EF24 was also shown to induce microtubule stabilisation in cells, although it had no stabilising effect on tubulin polymerisation in an *in vitro* assay using purified bovine brain tubulin, a finding that suggests that EF24-induced cytoskeletal disruption in cells may be related to an upstream signalling event rather than the direct binding of EF24 direct to tubulin [59]. In triple negative breast cancer cells however, curcumin was shown to induce DNA damage in association with phosphorylation, increased expression, and cytoplasmic retention of the BRCA1 protein and was shown to promote apoptosis and prevents anchorage-independent growth and migration of the cells [49].

TNF-related apoptosis inducing ligand (TRAIL) has also shown promising anti-cancer therapeutic activity and natural compound such as curcumin could potentially sensitise resistant cancer cells to TRAIL. Although significant percentage of primary tumours resistant to TRAIL-induced apoptosis remains an obstacle to the extensive use of TRAIL-based monotherapies, the combination of TRAIL with curcumin treatment has been investigated in an effort to induce apoptosis in TRAIL-resistant breast cancer cells. Findings revealed the combination to synergistically induce apoptosis in three TRAIL- resistant breast cancer cells due to the effect of curcumin on the expression and activation of TRAIL-associated cell death proteins to be related to differential effects of curcumin on the expression of Mcl-1 and activities of ERK and Akt. Although curcumin-induced production of reactive oxygen species was not observed to affect total expression of DR5 in this study, it was shown to enhance mobilisation of DR5 to the plasma membrane as well as induce the down-regulation of IAP proteins [43].

Another pathway by which curcumin has been shown to induce apoptosis in various malignant cancer cell lines is the induction of apoptosis through the PI3K/Akt signalling pathway. Protein kinase B (PKB) (Akt) is a member of the family of phosphatidylinositol 3-OH-kinase regulated Ser/Thr kinases. When active, Akt regulates cell survival and proliferation in addition to inhibition of apoptosis. At apoptotic concentration, curcumin has been shown to induce Akt phosphorylation, complemented by an increase in phosphorylation of glycogen synthase kinase 3-β (GSK3β), a pro-growth signalling molecule. In the study, the combination of curcumin with a PI3K inhibitor (LY290042) was shown to exhibit synergistic effect in inducing apoptosis while the inhibitor, on the other hand, was shown to attenuate curcumin-induced Akt phosphorylation and activation of GSK3β [27]. Other mechanisms have shown curcumin-treated MDA-MB-435 human breast cancer cells to accumulate in the G_1 phase of the cell cycle, accompanied by the suppression of the expression of Enhancer of Zeste Homolog 2 (EZH2) gene via the stimulation of three major members of the mitogen-activated protein

kinase (MAPK) pathway: c-Jun NH2-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), and p38 kinase [15].

Recently, a new type of non-apoptotic cell death, termed paraptosis has been reported to be induced by insulin-like growth factor-1 receptor, epidermal growth factor, and TAJ/TROY, a member of the tumour necrosis factor (TNF) receptor superfamily. Some authours have demonstrated the ability of curcumin to induce paraptosis in malignant breast cancer cell lines, including MDA-MB-435S, MDA-MB-231, and Hs578T cells, by promoting vacuolation consequent of the swelling and fusion of mitochondria and/or the endoplasmic reticulum (ER). Inhibition of protein synthesis was demonstrated to block curcumin-induced vacuolation and subsequent cell death, a finding that underscores the relevance of protein synthesis in the process of paraptosis. The levels of AIP-1/Alix protein, a known paraptosis inhibitor protein complex, were progressively down-regulated in malignant breast cancer cells exposed to curcumin, and AIP-1/Alix overexpression was shown to attenuate curcumin-induced cell death, while ERK2 and JNK activation were positively associated with curcumin-induced cell death [69].

The different mechanisms through which curcumin inhibits cancer cell functions such as cell growth, survival and motility, continue to be widely explored. In one study, the effect of curcumin on the function of integrin α6β4, a laminin adhesion receptor that plays an important role in the invasion and migration of cancer cells, was assessed. The study revealed curcumin to considerably reduce α6β4-dependent breast cancer cell motility and invasion in a concentration-dependent manner, without affecting apoptosis in MDA-MB-435/ β4β4-integrin transfectants and MDA-MB-231 breast cancer cell lines. Curcumin was also shown to selectively reduce the basal phosphorylation of β4 integrin (Y1494), which is essential in mediating α6β4-dependent phosphatidylinositol 3-kinase activation and cell motility as well as the blocking of α6β4-dependent Akt activation and expression of the cell motility-promoting factor ENPP2 in MDA-MB-435/β4 cell line [24]. The control of matrix metalloproteinases (MMP) and tissue inhibitor of metalloproteinases (TIMP) activity in recent years has also come to great significance. Matrix metalloproteinases play an important role in remodelling the extracellular matrix and their activities are regulated by tissue inhibitor of metalloproteinases (TIMPs) family. To investigate the role of curcumin in regulating cell metastasis, the effect of curcumin on metastatic MMPs and anti-metastatic TIMPs genes on MDA breast cancer cells has been assessed and was shown at high concentration to up-regulate TIMP-1, -2, -3 and -4 genes after 48 hours of treatment, accompanied by down-regulation of MMP-2 and MMP-9 gene expression levels in a concentration- and time-dependent manner [13]. This finding highlights the role of curcumin in regulating cell metastasis by inhibition of MMP-2 and MMP-9 and the up-regulation of TIMP-1 and TIMP-4 gene expression in breast cancer cells. In another study, the authours tested the comparative effect of the major component of turmeric (curcumin, demethoxycurcumin, *bis*demethoxycurcumin) in the modulation of MMP-3 activity and its secretion in MDA-MB-231 breast cancer cells. Analysis of MMP-3 expression by casein zymography exhibited high expression in MDA-MB-231 invasive breast carcinoma cells, but not in MCF-7 non-invasive breast cancer cells. In the ELISA assays however, MMP-3 levels were shown to be significantly decreased in all curcuminoid treat-

ments while in using zymography, exposure to non-toxic doses of curcuminoid compounds except curcumin, was shown to reduce MMP-3 levels [3].

Other studies have shown that demethoxycurcumin (DMC) inhibits the adhesion, migration and invasion of MDA-MB-231 human breast cancer cells, by decreasing levels of extracellular matrix (ECM) degradation-associated proteins including matrix metalloproteinase-9 (MMP-9), membrane type-1 matrix metalloproteinase (MT1-MMP), urokinase plasminogen activator (uPA) and uPA receptor (uPAR), while those of uPA inhibitor (PAI-1) have been shown to be up-regulated. Demethoxycurcumin was also shown to reduce the expression of intercellular adhesion molecule-1 (ICAM-1) and chemokine receptor 4, (CXCR4), which is involved in the modulation of the tumour metastasis process. The authours also demonstrated that DMC treatment inhibits DNA binding activity of nuclear factor-kappa B (NF-κB), which is known to mediate the expression of MMPs, uPA, uPAR, ICAM-1, and CXCR4, a finding that is suggestive that the mechanism of DMC mediated anti-invasive activity may involve the modulation of the expression of invasion-associated proteins, possibly by targeting NF-κB in MDA-MB-231 cells [68]. Similarly, the effect of curcumin on NF-κB, cell cycle regulatory proteins and matrix metalloproteinases (MMPs) in two breast cancer cell lines (MDA-MB-231 and BT-483) were evaluated. It was shown that Curcumin exhibited its anti-proliferation effect on MDA-MB-231 and BT-483 cells in a time- and dose-dependent manner, while the expression of cyclin D1 in MDA-MB-231 and the expression of CDK4 in BT-483 were shown to decline. MMP1 mRNA expression in BT-483 and MDA-MB-231 significantly decreased in curcumin treatment group when compared with untreated control group [35]; a finding that further buttresses the notion of the involvement of the regulation of the NF-kB inducing gene by curcumin in breast cancer cell proliferation and invasion. The effect of curcumin (diferuloylmethane) on 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced MMP-9 expression, cell invasion and the molecular mechanisms involved in MCF-7 cells invasion has also been reported. Curcumin was shown to inhibit TPA-induced MMP-9 expression and cell invasion through suppressing NF-κB and AP-1 activation. It was also revealed to repress TPA-induced phosphorylation of p38 and JNK, and inhibits TPA-induced translocation of PKCα from the cytosol to the membrane, suggesting that curcumin-mediated inhibition of TPA-induced matrix metalloproteinase (MMP)-9 expression and cell invasion is associated with the suppression of the PKCα, MAPK and NF-κB/AP-1 pathway in MCF-7 cells [26]. In another study to evaluate the effects of curcumin on matrixmetalloproteinase-9 (MMP-9) and invasion ability induced by transforming growth factor-β1 (TGF-β1) in MDA-MB-231 cells, it was shown that low doses of curcumin had no obvious toxicity on cells while a change in concentration resulted in a concentration-dependent reduction in cell invasion provoked by TGF-β1. Curcumin was also shown to markedly inhibit TGF-β1-regulated MMP-9 and activation of Smad2, ERK1/2 and p38 in a dose- and time-dependent manner, a mechanism that maybe associated with TGF-β/Smad and TGF-β/ERK signalling [38].

The effect of low concentrations of curcumin has also been tested on patient-derived primary breast cancer-associated fibroblasts (CAF) cells. Cancer-associated fibroblasts actively participate in tumour growth, invasion, and metastasis. This involves many chemokines, growth factors, and matrix metalloproteinases (MMPs), which transmit the message in both directions,

thus allowing cooperative crosstalk between cancer cells and their stroma. Recent reports show that curcumin treatment up-regulates p16INK4A and other tumour suppressor proteins and inactivates the JAK2/STAT3 pathway which results in the reduction level of alpha-smooth muscle actin (α-SMA) and the migration/invasion abilities of CAF cells. Curcumin was also demonstrated to further suppress the expression/secretion of stromal cell-derived factor-1 (SDF-1), interleukin-6 (IL-6), matrix metalloproteinase-2 (MMP-2), MMP-9, and transforming growth factor-β, thereby impeding their paracrine procarcinogenic potential. Intriguingly, these effects were sustained even after curcumin withdrawal and cell splitting. Curcumin-related senescence in this study was shown to be p16INK4A-dependent and occurred with no associated inflammatory secretory phenotype and that curcumin can trigger DNA damage-independent and safe senescence in stromal fibroblasts [14].

The efficacy of curcumin in blocking *Recepteur d'Origine Nantais* (RON) tyrosine kinase–mediated invasion of breast cancer cells has also been analysed. Curcumin-mediated inhibition of RON expression has been shown to result in the blockade of RON ligand, MSP-induced invasion of breast cancer cells and reduced RON expression by distorting p65 protein expression and transcriptional activity. The treatment of MDA-MB-231 cells with pyrrolidine dithiocarbamate, an inhibitor of p65, or small interfering RNA knockdown of p65, leads to the blockage of RON gene expression and MSP-mediated invasion of MDA-MB-231 cells which further highlights the role of curcumin in blocking RON tyrosine kinase-mediated invasion of carcinoma cells [42]. Other studies have focused on the role of curcumin in preventing or delaying the progression of cancer by disruption of epithelial-mesenchymal transition (EMT), a key event in cancer cell invasion and metastasis. The authours showed that curcumin inhibits LPS-induced morphological changes, decreased the expression of LPS-induced markers of EMT such as vimentin, and increased the expression of E-cadherin and as a consequence, the inhibition of motility and invasiveness of MCF-7 and MDA-MB-231 breast cancer cell lines *in vitro*, mediated through the inactivation of NF-κB-Snail signalling pathways [17].

Of equal importance is the frequent association of obesity with breast cancer, an association that is possibly mediated by adipokines. Visfatin, an adipokine, has recently been shown to be related to the development and progression of breast cancer. Hence the consideration that its down-regulation may be a novel strategy for breast cancer therapy has been explored. To investigate this, the effect of curcumin on visfatin gene expression and the characterisation of its functional role in breast cancer have been assessed. It was found that the mRNA and protein levels of visfatin were down-regulated by curcumin in MDA-MB-231, MDA-MB-468, and MCF-7 breast cancer cells, along with decreased activity of constitutive NF-κB which highlights the effect of curcumin to down-regulate visfatin gene expression in human breast cancer cells by a mechanism that is, at least in part, NF-κB dependent [26]. On the other hand obesity has also been shown to results in change in the expression profiles of several adipokines and cytokines including leptin, adiponectin, IL-6, TNF-α and IL-1β. Increased levels of leptin and decreased adiponectin secretions are directly associated with breast cancer development while increased levels of pro-inflammatory cytokines within the tumour microenvironment promote tumour development. The cumulative evidence of different adipokine- and cytokine-mediated

molecular signalling pathways involved in obesity-associated breast cancer have been documented [22].

Furthermore, the development and progression of malignant tumours depends on the formation of new blood vessels inside the tumour through a process termed angiogenesis. It is a vital process that ensues during cancer progression, and depends on the expression and activation of various angiogenic molecules, cytokines, growth factors, kinases and transcription factors. It had been previously demonstrated that the chemokine-like ECM-associated protein osteopontin (OPN) ignites the angiogenic switch by up-regulating the expression of vascular endothelial growth factor (VEGF) in a human breast cancer model. In this study [4], the authours demonstrated that curcumin (diferuloylmethane) abolishes OPN-induced VEGF expression and controls OPN-induced VEGF-dependent breast tumour angiogenesis *in vivo*. It was also observed that curcumin, in combination with anti-VEGF or anti-neuropilin (NRP)-1 antibody, was able to boost anti-angiogenic activity when compared to curcumin alone.

Furthermore, the over-expression of Flap endonuclease 1 (Fen1), a DNA repair-specific nuclease, has been implicated in the development of breast cancer. Nrf-2 is a leading regulator of cellular antioxidant defence systems and its inhibition of proliferation of breast cancer cells through its-mediated down-regulation of Fen1 expression by curcumin has been reported. Curcumin has been demonstrated to inhibit Fen1-dependent proliferation of MCF-7 cells, significantly induce Nrf-2 protein expression and inhibit Fen1 protein expression. It has also been shown to down-regulate Fen1 gene expression in an Nrf-2-dependent manner, as well as causing Nrf-2 translocation from the cytoplasm to the nucleus and to decrease Fen1 promoter activity by decreasing the recruitment of Nrf-2 to the Fen1 promoter [5]. While the abnormal activation of the Wnt/β-catenin signalling pathway and subsequent up-regulation of β-catenin driven downstream targets c-Myc and cyclin D1 is said to be associated with development of breast cancer in another study, the possibility that the efficacy of curcumin in the inhibition of cell proliferation and induction of apoptosis occur through modulation of β-catenin pathway in human breast cancer cells have also been suggested [45].

In order to help circumvent the problem associated with the low bioavailability of curcumin, the activities of analogues of curcumin have been tested in comparison with curcumin. In one study, an analogue of curcumin, GO-Y030, was tested for its efficacy in human breast MDA-MB-231 cell line. Both compounds were shown to reduce cell viability and induce apoptosis, although GO-Y030 was substantially more potent. It was also demonstrated that GO-Y030 was capable of interfering with STAT3 by inhibiting its phosphorylation and transcriptional activity, whereas comparable dosages of curcumin had little or no effect [18]. STAT3 is a persistently activated transcription factor in many cancer types. With regard to STAT3 phosphorylation, another curcumin analogue, FLLL12 was found to be a more potent inhibitor than the other, FLLL11. The reduction of phosphorylation of STAT3 was observed to correlate with the induction of apoptosis (determined by cleavage of PARP and caspase-3) [32]. Similarly, another synthetic curcumin analogue (hydrazinocurcumin) was shown to be more effective than curcumin in inhibiting STAT3 phosphorylation and down-regulation of an array of STAT3 downstream targets which contribute to suppression of cell proliferation, loss of colony formation, depression of cell migration and invasion as well as induction of cell

apoptosis [61]. Inhibition of IkB kinase-nuclear factor-kB signalling pathway by 3,5-*bis*(2-flurobenzylidene)piperidin-4-one (EF24), a novel monoketone analogue of curcumin has also been demonstrated. EF24 has been shown to potently suppress the NF-κB signalling pathway through direct action on IκB kinase (IKK) [21].

Hypoxia-inducible factors (HIFs) are transcription factors that play a central role in the adaptation and response to low oxygen levels in metazoan cells. Curcumin has been attributed with tumour growth inhibiting effects, possibly mediated by promoting hypoxia-inducible factor (HIF) degradation and also as exhibiting properties of an iron chelator, suggesting its potential of inhibiting HIF-α prolyl hydroxylase (PHD) activity. In order to clarify the divergent action of curcumin, researchers studied the concentration- and time-dependent effects of curcumin on HIF-α and -β protein levels and activity in hepatoma and breast carcinoma cell cultures under normoxic and hypoxic conditions. HIF-1α was shown to accumulate in normoxia after the application of higher doses of curcumin. The effect of curcumin was shown to lower HIF-1α and HIF-2α protein levels in hypoxia. HIF-1β (ARNT; arylhydrocarbon nuclear translocator) protein levels and HIF transcriptional activity were also reduced in normoxia and hypoxia after 4 h and 24 h of exposure. Furthermore, curcumin treatment was shown to negatively impact on clonogenic cell survival of Hep3B hepatoma and MCF-7 breast carcinoma cells. Effects of curcumin on cell growth and survival factor expression was suggested to be of potential benefit in the treatment of cancer without a direct radiosensitising influence of the drug on these cells [56].

The effects of curcumin on triple-negative breast cancer (TNBC) cells and the possible molecular mechanisms have been evaluated in MDA-MB-231 cells [57]. The authours examined the anti-proliferative effect of curcumin, its ability to induce apoptosis and the expression levels of extracellular regulated protein kinase (ERK1/2), pERK1/2, EGFR and pEGFR and concluded that the inhibition of the epidermal growth factor receptor (EGFR) signalling pathway is the likely underlying molecular mechanism of curcumin action in these cells. Since the functional interaction between integrin α6β4 and growth factor receptors has been implicated in key signalling pathways important for cancer cell function, the functional interaction between α6β4 and the epidermal growth factor receptor (EGFR) has also been examined. Findings revealed that curcumin is able to disrupt the functional and physical interactions between α6β4 and EGFR, as well as block α6β4/EGFR-dependent functions of carcinoma cells expressing the signalling competent form of α6β4. It has also been established that curcumin inhibits EGF-dependent mobilisation of α6β4 from hemi-desmosomes to the leading edges of migrating cells such as lammelipodia and filopodia, and thereby preventing α6β4 distribution to lipid rafts where functional interactions between α6β4 and EGFR occur. This finding highlights a novel paradigm in which curcumin inhibits α6β4 signalling and functions by altering intracellular localisation of α6β4, preventing its association with signalling receptors such as EGFR [55].

http://dx.doi.org/10.5772/59873

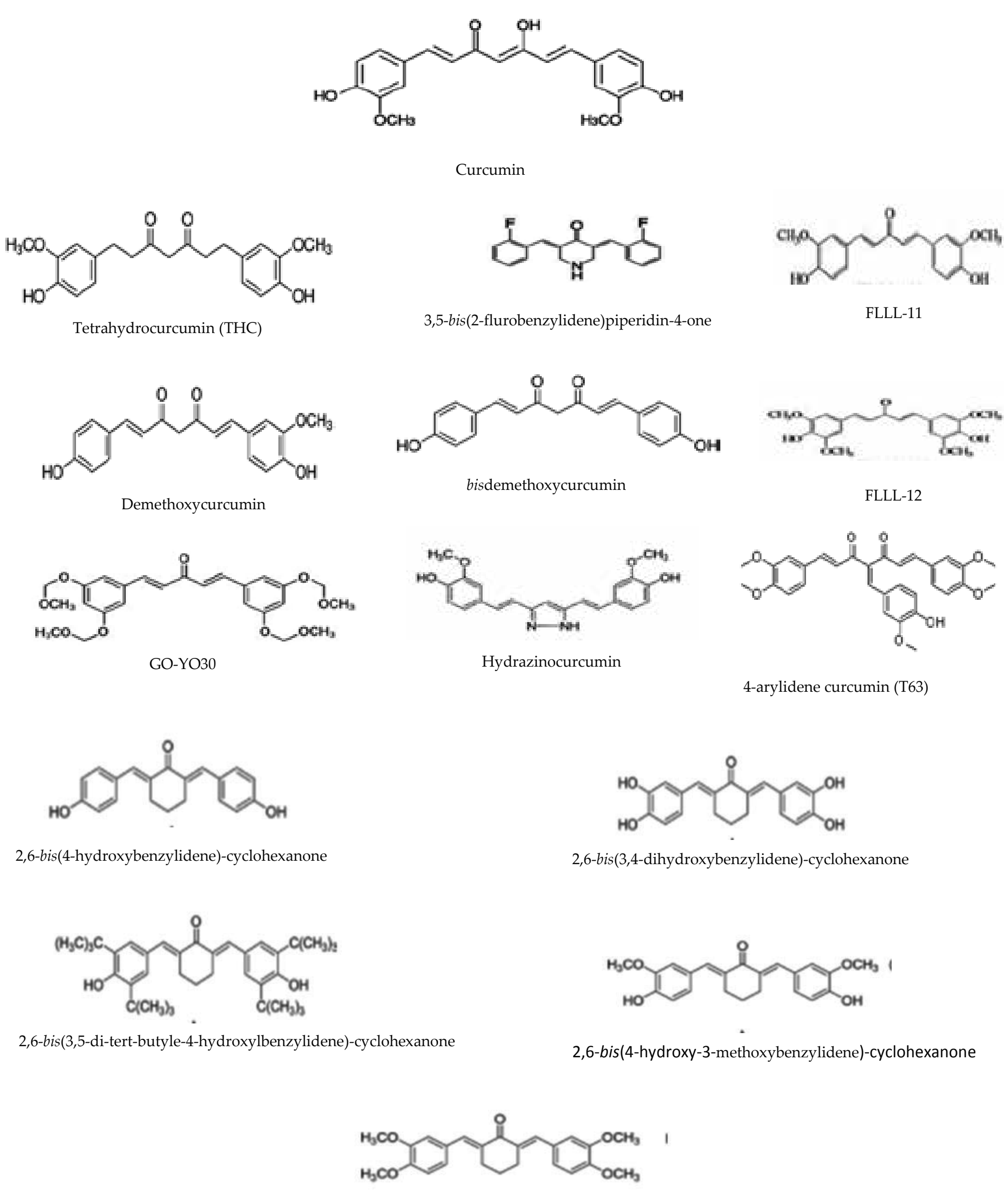


Figure 1. Chemical structure of curcumin and related derivatives/analogues widely used in the treatment of various forms of cancers.

The use of curcumin as potential candidate in the treatment for HER-2-overexpressed breast cancer has also been reported [30]. HER-2 is an important oncoprotein that is overexpressed in about 15–25% of breast cancers. In one particular study, cell growth, cell cycle change, the

anti-mobility effect, signal transduction, and xenograft volume analysis between groups treated with herceptin and/or curcumin were examined. Curcumin was shown to effect a decrease in cell growth of MCF-7, MDA-MB-231, MCF-10A, BT-474, and SK-BR-3-hr breast cancer cell lines while phosphorylation of Akt, MAPK, and expression of NF-κB were shown to be reduced in BT-474 cells, but not in SK-BR-3-hr cells, after treatment with herceptin. Following treatment with curcumin, the HER-2 oncoprotein, phosphorylation of Akt, MAPK and expression of NF-κB were shown to decrease in both BT-474 and SK-BR-3-hr cells [30]. Evidence indicates that curcumin reverses chemo-resistance and sensitises cancer cells to chemotherapy and targeted therapy in breast cancer. Studies have therefore been undertaken to explore curcumin's potential anti-proliferation effects and resistance reversal in anti-oestrogen-resistant breast cancer cell line MCF-7/LCC2 and MCF-7/LCC9. The effect of curcumin treatment revealed anti-proliferative and pro-apoptotic activities and induction of cell cycle arrest at G_2/M interphase. Moreover, the combination of curcumin and tamoxifen exhibited a synergistic survival inhibition in MCF-7/LCC2 and MCF-7/LCC9 cells. It was also revealed that curcumin targets multiple signals that are involved in growth maintenance and resistance acquisition in endocrine resistant cells. In the cell types used, curcumin was shown to suppress the expression of pro-growth and anti-apoptosis molecules, induce inactivation of NF-κB, Src and Akt/mTOR pathways and down-regulates the key epigenetic modifier EZH2 [19]. The chemosensitisation of breast cancer cell by curcumin to 5-fluorouracil (5-FU) has also been demonstrated. 5-Fluorouracil is an antimetabolite which achieves its therapeutic efficacy through inhibition of the enzyme thymidylate synthase (TS), essential for the synthesis and repair of DNA. Prolonged exposure to 5-FU induces TS overexpression, which leads to 5-FU resistance in cancer cells. Curcumin was demonstrated to sensitise the breast cancer cells to 5-FU through TS-dependent down-regulation of NF-κB. Silencing of TS was shown to suppress 5-FU-induced NF-κB activation, whereas inactivation of NF-κB was not shown to affect 5-FU-induced TS up-regulation, a finding that indicates that TS is upstream of NF-κB and is responsible for the regulation of the activation of NF-κB in 5-FU-induced signalling pathway. Although Akt/PI3 kinase and mitogen-activated protein kinase pathways were activated by 5-FU and down-regulated by curcumin, they were not shown to play a role in regulating the synergism in the study [60].

3. Curcumin and lung cancer

The molecular antitumour mechanism of a new 4-arylidene curcumin analogue (T63) has recently been reported to significantly inhibit the proliferation of A549 and H460 human lung cell lines via induction of G_0/G_1 cell cycle arrest and apoptosis. The study implicated reactive oxygen species (ROS)-activated FOXO3a cascade to be responsible to playing a central role in T63-induced cell proliferation inhibition. Enhancement of ROS production by T63 was shown to induce FOXO3a expression and nuclear translocation through activation of p38MAPK and inhibition of AKT, with subsequent elevation of the expression of FOXO3a target genes, including p21, p27, and Bim, as well as increasing the levels of activated caspase-3 and decreased levels of cyclin D1. N-acetylcysteine, an antioxidant, was shown to noticeably block

the above effects, while small interfering RNA-mediated knockdown of FOXO3a significantly decreased T63-induced cell cycle arrest and apoptosis [34]. Evident in another study, was the ability of curcumin to cause DNA damage and endoplasmic reticulum (ER) stress and mitochondrial-dependent-induced apoptosis through the activation of caspase-3 at a treatment concentration of 30 μM in human lung cancer A549 cells. In contrast, lower concentrations of 5–10 μM curcumin showed no significant apoptotic inducing effect but rather induced G_2/M-phase arrest in A549 cells. It was also shown to increase intracellular oxidative stress, indicators of ER stress, Ca^{2+} levels and the mitochondrial membrane potential in A549 cells thereby highlighting the role of curcumin in the activation of pathways involved in inducing G_2/M-phase arrest and apoptosis [33].

In another effort to finding novel putative intervention sites as chemo-protective and chemotherapeutic target for curcumin in squamous cell lung carcinoma, Sen and co-authours, demonstrated that curcumin induces apoptosis in these cells, while microarray analysis revealed about 34 and 31 genes to be up- and down-regulated, respectively, following curcumin treatment. Likewise, growth arrest and DNA damage genes, GADD45a and peroxiredoxin-I was also shown to be up-regulated more than 2-folds (Sen *et al.*, 2008).

The effect of curcumin in non-small cell lung cancer (NSCLC), the leading cause of cancer-related mortality, has also been evaluated. High expression of Rad51, a key protein in the homologous recombination (HR) pathway of DNA double-strand break repair, plays a key role in chemo- or radio-resistant carcinomas and thus HR represents a novel target for cancer therapy. Studies to evaluate the effect of curcumin in enhancing the effect of mitomycin C (MMC), a DNA inter-strand cross-linking agent, to induce cytotoxicity by decreasing Rad51 expression have been reported. Findings revealed curcumin treatment of non-small lung cancer (NSCLC) cell lines (A549 and H1975) was capable of suppressing MMC-induced MKK1/2–ERK1/2 signal activation and Rad51 protein expression. On the other hand, enhancement of ERK1/2 activation by constitutively active MKK1/2 (MKK1/2-CA) was shown to increase Rad51 protein levels in curcumin and MMC co-treated human lung cells. The synergistic cytotoxic effect induced by curcumin–MMC treatment was established to be decreased by MKK1-CA-mediated enhancement of ERK1/2 activation by a significant degree. On the contrary, the MKK1/2 inhibitor, U0126 was shown to augment the cytotoxicity of curcumin and MMC through down-regulation of ERK1/2 activation and Rad51 expression, while depletion of endogenous Rad51 expression by siRad51 RNA transfection was demonstrated to significantly enhance MMC and/or curcumin-induced cell death and cell growth inhibition. In contrast, an overexpression of Rad51 protected the lung cancer cells from synergistic cytotoxic effects induced by curcumin and MMC. It was thus concluded that Rad51 inhibition may be an additional mechanism of action for enhancing the chemosensitisation of MMC by curcumin in NSCLC [27].

In a similar study, the effect of curcumin treatment on the expression of nuclear factor κB-related proteins *in vitro* and *in vivo* and on growth and metastasis in an intra-lung tumour mouse model alone or in combination with gemcitabine or cisplatin has been assessed. Western blot analyses showed that the expressions of IkB, nuclear p65, cyclooxygenase-2 (COX-2) and p-ERK1/2 were down-regulated by curcumin *in vitro*. In *in vivo*, curcumin was shown to

potentiate gemcitabine- or cisplatin-mediated antitumour effects and was also capable of reducing COX-2 expression in subcutaneous tumours with a decrease in weight of intra-lung tumours accompanied by a significant survival rate increase. The effect of curcumin in the inhibition of COX-2, p65 expression and ERK1/2 activity in NSCLC cells was observed to be associated with decreased survival and increased induction of apoptosis [32].

The anti-metastasis effects and mechanism of curcumin action in lung cancer have also been elucidated. Rac1 is an important small Rho GTPases family protein and has been widely implicated in cytoskeleton rearrangements and cancer cell migration, invasion and metastasis. In order to investigate its role, [5] examined the influence of curcumin on *in vitro* invasiveness of human lung cancer cells and the expression pattern of Rac1. Their findings revealed that curcumin at 10 μM was capable of slightly reducing the proliferation of 801D lung cancer cells with a pronounced inhibitory effect on epidermal growth factor or transforming growth factor β1-induced lung cancer cell migration and invasion. The suppression of invasiveness correlated with the inhibition of Rac1/PAK1 signalling pathways and matrix metalloproteinases (MMP) -2 and -9 protein expression when curcumin treatment was combined with the methods of Rac1 gene silencing and overexpression in lung cancer cells. It was also revealed by laser confocal microscopic analysis that Rac1-regulated actin cytoskeleton rearrangement may be involved in anti-invasion effect of curcumin on lung cancer cell. The authours concluded that low-toxic levels of curcumin could efficiently inhibit migration and invasion of lung cancer cells through inhibition of Rac1/PAK1 signalling pathway and MMP-2 and MMP-9 expression [5].

The effect of curcumin as a chemosensitiser in lung cancer has also been examined on HIF-1α in cisplatin (DDP) sensitive A549 and resistant A549/DDP cell lines. Findings revealed HIF-1α in A549/DDP cells to be overexpressed at both mRNA and protein levels together with a poor response to DDP. It was also shown that HIF-1α abnormality contributes to DDP resistance in A549/DDP lung cancer cells while combined curcumin and DDP treatment was observed to markedly inhibited A549/DDP cells proliferation, reversed DDP resistance and triggered apoptotic death by promoting HIF-1α degradation and activation of caspase-3, respectively. The expression of HIF-1α-dependent P-gp was also observed to decrease in response to curcumin in a dose-dependent manner; a finding that highlights the drug resistant reversing effect of curcumin in lung cancer cells by inhibiting HIF-1α expression and activation of caspase-3 [67].

A novel inflammation-related mechanism for curcumin-induced inhibition of lung tumour growth has also been reported. Neutrophil elastase, an important regulator of inflammatory processes has been found to directly triggered tumour cell proliferation in human lung adenocarcinoma A549 cells. Alpha1-antitrypsin synthesised by tumour cells is a natural inhibitor of neutrophil elastase and curcumin has been shown to counter the decrease of α1-antitrypsin induced by neutrophil elastase by prompting the promoter activity of α1-antitrypsin, thereby promoting its expression in A549 cells. The inhibition of neutrophil elastase-induced proliferation was shown to be dependent on the PI3K/Akt pathway. Knockdown of α1-antitrypsin by siRNA was demonstrated to further enhance the tumour cell proliferation induced by neutrophil elastase and significantly blocked the anti-proliferative effect of curcumin against neutrophil elastase. In *in vivo*, curcumin was also observed to remarkably

inhibit the primary tumour growth of Lewis lung carcinoma (LLC) in C57BL/6 mice. The authours also demonstrated that curcumin up-regulates the level of α1-antitrypsin in primary tumour tissue by promoting its local expression, while the protein level of neutrophil elastase in tumour tissue was observed to decrease in mice treated with curcumin. This finding further highlights the roles of neutrophil elastase and α1-antitrypsin in modulating lung tumour proliferation in inflammatory microenvironment and the effect of curcumin in inhibiting neutrophil elastase-induced tumour proliferation via the up-regulation of α1-antitrypsin expression *in vitro* and *in vivo* [64].

4. Curcumin and colorectal cancer

Insulin resistance and obesity are associated with increased colorectal cancer (CRC) risk and high reoccurrence rates. The effect of dietary compounds in reducing insulin-induced cell proliferation in normal and metastatic colon epithelial cells has been demonstrated. Murine colon epithelial cells (YAMC) and adenocarcinoma cells (MC38) were treated with docosahexaenoic acid (DHA) or curcumin alone, followed by the combination of co-treatments of the diet-derived compound and insulin. Insulin-stimulated MAPK and MEK phosphorylation was shown to be inhibited by DHA and curcumin in MC38 cancer cells, suggesting that curcumin and DHA are capable of blocking insulin-induced colon cancer cell proliferation *in vitro* via a MEK-mediated mechanism [12]. Other indicators implicate non-steroidal anti-inflammatory drugs (NSAIDs), particularly the highly selective COX-2 inhibitors in the prevention of colon cancer. As such, some authours have demonstrated the effects of diclofenac, a preferential COX-2 inhibitor, and curcumin in inducing apoptosis in colon cancer cells. Both diclofenac and curcumin were shown to lower COX-2 activity and PGE-2 levels while the expression of IκBα was shown to be higher, with a lowered IKK activity suggesting that these agents may suppress the transfer of NF-κB to the nucleus and its pro-inflammatory gene transcription. Both drugs were also shown to down-regulate the level of pro-inflammatory cytokines, TNF-α, IL-1β and IL-2, through the inhibition of NF-κB and subsequent induction of apoptosis, thus confirming the regulatory role of NF-κB in the process [46]. Similarly, targeting nutraceuticals to tumours can enhance their effectiveness. Nanoparticles encapsulating curcumin have been shown to be more effective than the free curcumin, eventually inhibiting NF-κB regulated transcription and angiogenesis [41].

In a study, [50] exposed human colorectal cancer cells to clinically relevant doses of gamma rays, in an attempt to elucidate the mechanism of their radio-resistance. The authours characterised NF-κB activation as a mechanism of inducible radio-resistance in colorectal cancer. Curcumin was shown to inhibit the proliferation and the post-irradiation clonogenic survival of multiple colorectal cancer cell lines. Radiation was observed to stimulate NF-κB activity in a dose- and time-dependent manner, whereas curcumin was shown to suppress this radiation-induced NF-κB activation via inhibition of radiation-induced phosphorylation and degradation of inhibitor of κB alpha, inhibition of κB kinase activity, and the inhibition of Akt phosphorylation. Curcumin was also shown to suppress NF-κB–regulated gene products (Bcl-2, Bcl-xL, inhibitor of apoptosis protein-2, COX-2, and cyclin D1). The authours concluded

that transient inducible NF-κB activation provides a pro-survival response to radiation that may account for development of radio-resistance and that curcumin blocks this signalling pathway and further potentiates the antitumour effects of radiation therapy.

The induction of cellular senescence of human colon cancer cells HCT116 upon curcumin treatment, demonstrating a functional link between senescence and autophagy in curcumin-treated cells, has been reported. Activation of SA-β-galactosidase activity following curcumin treatment has been observed in p53+/+ and p53−/− cells, although the later was less sensitive to the pro-senescent activity. The authours also demonstrated the up-regulation of p53 and p21 proteins in p53+/+ HCT116 cells, while p53-independent induction of p21 was observed in p53−/− HCT116 cell. Senescence of HCT116 cells was shown to be accompanied by autophagy that was confirmed by electron microscopy observations of autophagosomes in the curcumin-treated cells as well as LC3-II expression, puncture staining of LC3 and increased content of acidic vacuoles. Inhibition of autophagy, due to the diminished expression of ATG5 by RNAi, was observed to decrease the number of senescent cells induced by curcumin, but was not shown to lead to increased cell death [40].

5. Curcumin and cervical cancer

Human papillomavirus (HPV) infections remain a leading cause of mortality worldwide and cervical cancer is associated with infection with high risk human papillomaviruses (HPVs). Cervical cancer is the second leading cause of cancer death for women in the world. The effect of low concentration of curcumin on human cervical cancer cell line (HeLa) has been shown to mediate decrease in the cell number and viability, and increase in apoptotic events and superoxide level. Treatment of cells with curcumin was revealed to be toxic even at concentrations as low as 1 μM even though no genotoxic effect was observed in these cells. Since argyrophilic nucleolar protein (AgNOR protein) expression is elevated in malignant cells compared to normal cells, the effect of curcumin-associated changes in size (area) and number of silver deposits were also evaluated. Curcumin was shown to induce decreased AgNOR protein pools, mediated possibly by global DNA hypermethylation observed after low concentration of curcumin treatment [31].

Curcumin is an anti-inflammatory agent that is known to have anti-COX-2 activity. One way of preventing and treating cervical cancer is by targeting COX-2. In order to evaluate the effect of curcumin in cervical cancer, the expression of COX-2 and its precursors have been examined by immunohistochemistry. The inhibitory effect of curcumin on cervical cancer cells was determined via 2-dimensional gel electrophoresis, data analysis, and ingenuity pathway analysis. The authours observed no significant differences in the expression of COX-2 in squamous cell carcinoma, and carcinoma *in situ*, although that was not the case in the expression of COX-2 in adenocarcinoma in comparison to normal and squamous cell carcinoma tissues. However, proteins associated with cancer and the cell cycle were shown to be significantly altered in cultured cells [37].

http://dx.doi.org/10.5772/59873

Since cervical cancer is associated with infection with high risk human papillomaviruses (HPVs), the molecular mechanism of curcumin induced apoptosis in HPV-positive cervical cancer HeLa, SiHa and CaSki cells have also been evaluated. Curcumin was shown to cause distinct inhibition of human telomerase reverse transcriptase (hTERT), the catalytic core of telomerase thereby reducing proliferation of cancer cells. Findings in the study revealed that curcumin-mediated apoptosis in these cells may be associated with the up-regulation of pro-apoptotic Bax, AIF, release of cytochrome c and down regulation of anti-apoptotic Bcl-2 and Bcl-xL, accompanied by an increase in caspase-3 and -9 activities. As such, the effect of curcumin as an anti-inflammatory and anti-proliferative agent in these cells was shown to be associated with down regulation of COX-2, iNOS and cyclin D1 at varying extents [54]. Recently, in an attempt to develop a curcumin–based therapy for cervical cancer, the effect of curcumin on four human papillomavirus HPV (+) cervical cancer cell lines and normal fibroblasts has been assessed. Curcumin treatment was shown to selectively eliminate a variety of HPV (+) cervical cancers cells (HeLa, ME-180, SiHa, and SW756), suppress the transforming antigen E6, dramatically inhibits the expression of the pro-cancer protein epidermal growth factor receptor (EGFR), and concomitantly induced p53 levels. In addition, vacurin, a colloidal solution of curcumin which is incorporated in clinically used amphipathic vaginal cream was shown to eliminate apposed HeLa cells while suppressing the expression of EGFR [11].

6. Curcumin and prostate cancer

Emerging evidence suggests that chronic inflammation is a major risk factor for the development and metastatic progression of prostate cancer. In evaluating the effect of curcumin on prostate carcinoma growth, apoptosis and metastasis, curcumin was shown to inhibit the translocation of NFκB to the nucleus through the inhibition of the IκB-kinase (IKKβ), leading to stabilisation of the inhibitor of NFκB, IκBα, in PC-3 prostate carcinoma cells. Inhibition of NFκB activity was demonstrated to reduce expression of CXCL-1 and -2 and abolished the autocrine/paracrine loop that links the two chemokines to NFκB. When used in combination with the synthetic IKKβ inhibitor, SC-541, no additive or synergistic effect was observed while treatment of cells with curcumin and siRNA-based knockdown of CXCL-1 and -2 was shown to induce apoptosis, inhibit proliferation and down-regulate several important metastasis-promoting factors like COX-2, SPARC and EFEMP. In an orthotopic mouse model of hematogenous metastasis, treatment with curcumin inhibited statistically significant formation of lung metastasis. The authours concluded that chronic inflammation can induce a metastasis prone phenotype in prostate cancer cells by maintaining a positive pro-inflammatory and pro-metastatic feedback loop between NFκB and CXCL-1/-2, while reduced metastasis formation *in vivo* can be achieved by the disruption of this feedback loop by curcumin-induced inhibition of the NFκB signalling [23].

On the other hand, protein kinase D1 (PKD1), a multifunctional kinase that is highly expressed in normal prostate and decreased expression levels, has been associated with the progression of prostate cancer. Curcumin has been found to activate PKD1, with subsequent changes in β-catenin signalling by hindering nuclear β-catenin transcription activity and increasing the

levels of membrane β-catenin in prostate cancer cells. Modulation of these cellular events by curcumin is shown to correlate with decreased cell proliferation, colony formation, cell motility and enhanced cell-cell aggregation in prostate cancer cells. It has also been demonstrated that inhibition of cell motility is mediated by decreasing the levels of active cofilin, a downstream target of PKD1. The potent anti-cancer effect of curcumin *in vitro* in the study was shown to correlate well with those in prostate cancer xenograft mouse model, thus highlighting a novel molecular mechanism of curcumin action via the activation of PKD1 in prostate cancer cells [58]. The effect of curcumin in suppressing prostate cancer cell invasion, tumour growth, and metastasis has also been assessed. Curcumin was shown to be capable of suppressing epidermal growth factor (EGF)-stimulated and heregulin-stimulated PC-3 cell invasion, as well as androgen-induced LNCaP cell invasion. Treatment of cells with curcumin was also shown to significantly result in reduced matrix metalloproteinase 9 activities and down-regulation of cellular matriptase, a membrane-anchored serine protease with oncogenic roles in tumour formation and invasion. It was also demonstrated that curcumin inhibits the induction effects of androgens and EGF on matriptase activation, as well as the reduction of the activated levels of matriptase after its overexpression. The reduction of activated matriptase in cells by curcumin was also observed to be partly due to its effect on promoting the shedding of matriptase into an extracellular environment without altering matriptase gene expression. In addition, curcumin was also shown to significantly suppress the invasive ability of prostate cancer cells induced by matriptase overexpression. The data from the study indicate that curcumin exhibits a suppressive effect on prostate cancer cell invasion, tumour growth, and metastasis, in part via the down-regulation of matriptase function [7]. In another study, the anticancer activity of curcumin and genistein combination in human prostate cancer (PC3) cell line with respect to their anti-angiogenic effect has been examined. The combination of both compounds was shown to decrease cell viability, induce apoptosis and cell cycle arrest at G_0 phase in a dose- and time-dependent manner. In order to understand the anti-angiogenic effect of the combination, the authours determined the expression of ARNT and HIF-1α protein levels which were shown to significant decline when compared to the control group and their respective monotherapy-treated groups [1].

The transcriptional activity of the androgen receptor (AR) is modulated by interaction with co-regulators, one of which is β-catenin. The effect of curcumin in inhibiting AR expression has also been elucidated through its role in mediating Wnt/β-catenin signalling pathway with regard to AR/β-catenin interactions. Curcumin induced a significant inhibition of AR expression in a dose-dependent manner as well as its suppression of β-catenin in the nuclear and cytoplasmic extracts and whole cell lysates. Phosphorylation of Akt and glycogen synthase kinase-3β was shown to be attenuated, while phosphorylated β-catenin was increased after curcumin treatment. Cyclin D1 and c-myc, the target genes of the β-catenin/T-cell factor transcriptional complex, were also shown to be decreased; a finding that highlights the effect of curcumin in modulating the Wnt/β-catenin signalling pathway and may thus play a significant role in mediating inhibitory effects on LNCaP prostate cancer cells [10]. The anti-tumour activity of curcumin against androgen-independent prostate cancer cells *in vitro* and the possible mechanism of action have also been investigated. The results showed curcumin to effectively inhibit the proliferation of PC-3 cells *in vitro* with cell cycle arrest at the G_2/M

http://dx.doi.org/10.5772/59873

interphase. The percentage of apoptotic cells was shown to be significantly higher in curcumin-treated groups than in control group and curcumin was shown to selectively inhibit the activities of NF-κB and AP-1 signalling pathways in PC-3 cells significantly [36].

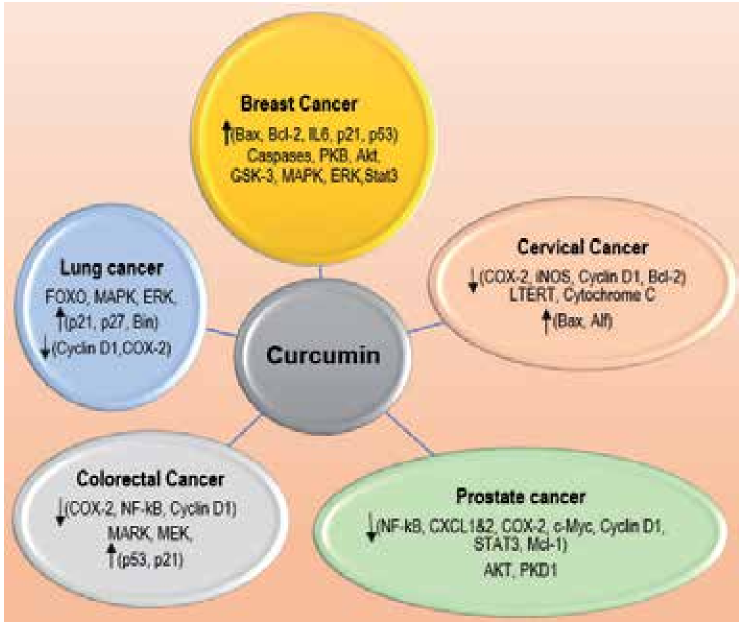


Figure 2. Molecular targets of curcumin and/or its chemically-related analogues and possible mechanisms of action in various types of malignant growths.

The effect of six cyclohexanone analogues of curcumin (Figure 1) has also been investigated for their effects on growth and apoptosis, by evaluating the ability of these compounds to inhibit NF-κB activity in PC-3 human prostate cancer cells. Five out of the six curcumin analogues were shown to have stronger inhibitory effects compared to curcumin on the growth of these cells. They also showed stronger stimulatory effects on apoptosis in PC-3 cells than curcumin, with a more potent inhibition of NF-κB activity than curcumin which correlates well with effects on growth inhibition and apoptosis stimulation in PC-3 cells [62]. Furthermore, the therapeutic potential of a novel poly(lactic-co-glycolic acid)-curcumin nanoparticles (PLGA-Curcumin NPs) for prostate cancer treatment has also been assessed. Findings revealed PLGA-Curcumin NPs to efficiently internalise in prostate cancer cells and release biologically active curcumin in cytosolic compartment of cells for effective therapeutic activity. It was also shown that PLGA-Curcumin NPs can effectively inhibit the proliferation and colony formation ability of prostate cancer cells than free curcumin. PLGA-Curcumin NPs showed superior tumour regression compared to curcumin in xenograft mice. It was also revealed that PLGA-Curcumin NPs inhibit nuclear β-catenin and androgen receptor (AR) expression in cells and in tumour xenograft tissues. Furthermore, it was shown to suppress STAT3 and Akt phosphorylation resulting in apoptosis via inhibition of key anti-apoptotic proteins, Mcl-1, Bcl-xL and caused induction of PARP cleavage [65].).

7. Conclusion

The limiting factor associated with curcumin is its poor solubility in water and likewise when soluble, it is extremely sensitive at physiological pH. However, the potential of curcumin, its derivatives and/or metabolites on cancer cells has been recognised and demonstrated in various cancer cells and its varying mechanisms of action elucidated depending on the tumour cell type. Coupled with this, the pleiotropic property of the curcumin molecule enables it to target the DNA, RNA and enzymes (proteins) within cells thereby eliciting sequential and/or simultaneous therapeutic effects. It is therefore pertinent to strive to refine the properties of curcumin through targeted delivery, tissue distribution and bioavailability in tumour cells in the presence of an adjuvant. These strategies can be achieved through the design and development of nanoparticles, self-assemblies, nanogels, liposome and other formulations that possess characteristics tailored according to specific requirements in order to efficiently harness therapeutic potential of curcumin in the treatment of a variety of malignant growths.

Acknowledgements

This work was made possible by grants from the Medical Research Council (South Africa) awarded to LJM, National Research Foundation, NRF (Thuthuka) awarded to MPM and the University of Limpopo.

Author details

Victor P. Bagla, Matlou P. Mokgotho and Leseilane J. Mampuru*

*Address all correspondence to: Leseilane.mampuru@ul.ac.za

Department of Biochemistry, Microbiology and Biotechnology, School of Molecular and Life Sciences, Faculty of Science and Agriculture, University of Limpopo, Sovenga, South Africa

References

[1] Aditya, N.P., Shim, M., Yang, H., Lee, Y., & Ko, S. (2014). Antiangiogenic effect of combined treatment with curcumin and genistein on human prostate cancer cell line. *Journal of Functional Foods* 8: 204–213.

[2] Bachmeier, B. E., Mohrenz, I.V., Mirisola, V., Schleicher, E., Romeo, F., Höhneke, C., Jochum, M., Nerlich, A. G. & Pfeffer, U. (2008). Curcumin downregulates the inflammatory cytokines CXCL1 and -2 in breast cancer cells via NFkB. *Carcinogenesis* 29(4): 779–789.

[3] Boonrao, M., Yodkeeree, S., Ampasavate, C., Anuchapreeda, S., & Limtrakul, P. (2010). The inhibitory effect of turmeric curcuminoids on matrix metalloproteinase-3 secretion in human invasive breast carcinoma cells. *Archives Pharmacology Research* 33: 989-998.

[4] Chakraborty, G., Jain, S., Kale, S., Raja, R., Kumar, S., Mishra, R., & Kundu, G.C. (2008). Curcumin suppresses breast tumour angiogenesis by abrogating osteopontin-induced VEGF expression. *Molecular Medicine Reports* 1: 641–646.

[5] Chen, B., Zhang, Y., Wang, Y., Rao, J., Jiang, X., & Xu, Z. (2014). Curcumin inhibits proliferation of breast cancer cells through Nrf2-mediated down-regulation of Fen1 expression. *Journal of Steroid Biochemistry and Molecular Biology* 143: 11–18

[6] Chen, Q-Y., Zheng, Y., Jiao, D-M., Chen, F-y., Hu, H-z, Wu, Y-Q, Song, J., Yan, J., Li-Jun Wu., & Lv, G-Y. (2014). Curcumin inhibits lung cancer cell migration and invasion through Rac1-dependent signalling pathway. *Journal of Nutritional Biochemistry* 25: 177–185.

[7] Cheng, T.S., Chen, W.C., Lin, Y.Y., Tsai, C.H., Liao, CI., Shyu, H.Y., Ko, C.J., Tzeng, S.F., Huang, C.Y., Yang, P.C., Hsiao, P.W., & Lee, M.S. (2013). Curcumin-targeting pericellular serine protease matriptase role in suppression of prostate cancer cell invasion, tumor growth, and metastasis. *Cancer Prevention Research* 6(5): 495-505.

[8] Chiu, T.L., & Su, C.C. (2009). Curcumin inhibits proliferation and migration by increasing the Bax-to-Bcl-2 ratio and decreasing NF-kappaBp65 expression in breast cancer MDA-MB-231 cells. *International Journal of Molecular Medicine* 23: 469–475.

[9] Cho, H. S., Park, J. H., & Kim, Y. J. (2007). Epigenomics: Novel aspect of genomic regulation. *Biochemistry and Molecular Biology* 40(2): 151–155.

[10] Choi, H.Y., Lim, J.E., & Hong, J.H. (2010). Curcumin interrupts the interaction between the androgen receptor and Wnt/β-catenin signalling pathway in LNCaP prostate cancer cells. *Prostate Cancer and Prostatic Diseases* 13(4):343-349. doi: 10.1038/pcan.2010.26.

[11] Debata, P.R, Castellanos, M.R., Fata, J.E., Baggett, S., Rajupet, S., Szerszen, A., Begum, S., Mata, A., Murty, V.V., Opitz, L.M., & Banerjee, P. (2013). A novel -based vaginal cream Vacurin selectively eliminates apposed human cervical cancer cells. *Gynaecologic Oncology* 129(1): 145-153.

[12] Fenton, J.I., & McCaskey, S. J. (2013). Curcumin and docosahexaenoic acid block insulin-induced colon carcinoma cell proliferation. *Prostaglandins, Leukotrienes and Essential Fatty Acids* 88(3): 219-226

[13] Hassan, Z. K & Daghestani, M. H. (2012). Curcumin effect on MMPs and TIMPs genes in a breast cancer cell Line. *Asian Pacific Journal of Cancer Prevention* 13: 3259-3264.

[14] Hendrayani, S-F., Al-Khalaf, H.H. & Aboussekhra, A. (2013). Curcumin triggers p16-dependent senescence in active breast cancer-associated fibroblasts and suppresses their paracrine procarcinogenic effects. *Neoplasia* 15(6): 631–640.

[15] Hua, W.F., Fu, Y.S., Liao, Y.J., Xia, W.J., Chen, Y.C., Zeng, YX, Kung, HF., & Xie, D. (2010). Curcumin induces down-regulation of EZH2 expression through the MAPK pathway in MDA-MB-435 human breast cancer cells. *European Journal of Pharmacology* 637:16–21.

[16] Huang, H.C., Lin, CL., & Lin, J.K. (2011). 1, 2, 3, 4, 6-penta-O-galloyl-β-D-glucose, quercetin, curcumin and lycopene induce cell-cycle arrest in MDA-MB-231 and BT474 cells through down-regulation of Skp2 protein. *Journal of Agriculture and Food Chemistry* 59: 6765–6775.

[17] Huang, T., Chen, Z., & Fang, L. (2013). Curcumin inhibits LPS-induced EMT through down-regulation of NF-κB-Snail signalling in breast cancer cells. *Oncology Reports* 29: 117-124.

[18] Hutzen, B., Friedman, L., Sobo, M., Lin, L., Cen, L., De Angelis, S., Yamakoshi, H., Shibata, H., Iwabuchi, Y., & Lin, J. (2009). Curcumin analogue GO-Y030 inhibits STAT3 activity and cell growth in breast and pancreatic carcinomas. *International Journal of Oncology* 35(4): 867-872.

[19] Jiang, M., Huang, O., Zhang, X., Xie, Z., Shen, A., Liu, H., Geng, M., & Shen, K. (2013). Curcumin induces cell death and restores tamoxifen sensitivity in the antiestrogen-resistant breast cancer cell Lines MCF-7/LCC2 and MCF-7/LCC9. *Molecules* 18: 701-720.

[20] Kanga, N., Wang, M-M., Wang, Y-H., Zhang, Z-N., Cao, H-R., Lv, Y.-H., Yang, Y., Fan, P.-H., Qiu, F., & Gaoa, X-M (2014). Tetrahydrocurcumin induces G2/M cell cycle arrest and apoptosis involving p38 MAPK activation in human breast cancer cells. *Food and Chemical Toxicology* 67: 193–200.

[21] Kasinski, L. A., Du, Y., Thomas, S. L., Zhao, J., Sun, S-Y., Khuri, F. R., Wang, C-Yu., Shoji, M., Sun, A., Snyder, J. P., Liotta, D., & Fu, H.(2008). Inhibition of IkB kinase-nuclear factor-κB signalling pathway by 3,5 *Bis*(2-flurobenzylidene)piperidin-4-one (EF24), a novel monoketone analog of curcumin. *Molecular Pharmacology* 74: 654–661.

[22] Khan, S., Shukla, S., Sinha, S., & Meeran, S. M. (2013). Role of adipokines and cytokines in obesity-associated breast cancer: Therapeutic targets. *Cytokine and Growth Factor Reviews* 24: 503–513.

[23] Killian, P.H., Kronski, E., Michalik, K.M., Barbieri, O., Astigiano, S., Sommerhoff, C.P., Pfeffer, U., Nerlich, A.G., & Bachmeier, B.E. (2012). Curcumin inhibits prostate cancer metastasis *in vivo* by targeting the inflammatory cytokines CXCL1 and -2. *Carcinogenesis* 33(12): 2507–2519.

[24] Kim, H.I., Huang, H., Cheepala, S., Huang, S., & Chung, J. (2008). Curcumin inhibition of integrin (α6β4)-dependent breast cancer cell motility and invasion. *Cancer Prevention and Research* 1: 385-391.

[25] Kim, J-M, Noh E-M., Kwon, K-B., Kim, J-S., You, Y-O., Hwang, J-K., Hwang, B-M., Kim, B-S., Lee, S-H., Lee, S. J., Jung, S. H., Youn, H. J., & Lee Y-R. (2012). Curcumin suppresses the TPA-induced invasion through inhibition of PKC-dependent MMP-expression in MCF-7 human breast cancer cells. *Phytomedicine* 19: 1085–1092.

[26] Kim, S-R., Park, H-J., Bae, Y-H., Ahn, S-C., Wee, H-J., Yun, I., Jang, H-O., Bae, M-K., & Bae, S-K. (2012). Curcumin down-regulates visfatin expression and inhibits breast cancer cell invasion. *Endocrinology* 153: 554–563.

[27] Kizhakkayil, J., Thayyullathil, F., Chatboth, S., Hago, A., Patel, M., & Galadari, S. (2010). Modulation of curcumin-induced AKt phosphorylation and apoptosis by PI3K inhibitor in MCF-7 cells. *Biochemical and Biophysical Research Communications* 394(3): 476-481.

[28] Ko, J-C., Tsai, M-S., Weng, S-H., Kuo, Y-H., Chiu, Y-F, Lin, Y-W. (2011). Curcumin enhances the mitomycin C-induced cytotoxicity via down-regulation of MKK1/2–ERK1/2-mediated Rad51 expression in non-small cell lung cancer cells. *Toxicology and Applied Pharmacology* 255(3): 327-338.

[29] Kronski, E., Fiori, M. E., Barbieric, O., Astigiano, S., Mirisola, V., Killian, P H., Bruno, A., Pagani, A., Rovera, F., Pfeffer, U., Sommerhoff, C P., Noonanf, D.M., Nerlich, A. G., Fontana, Laura., & Bachmeier, B.E. (2014). miR181b is induced by the chemopreventive polyphenol curcumin and inhibits breast cancer metastasis via down-regulation of the inflammatory cytokines CXCL1 and -2. *Molecular Oncology* 8(3): 581–595.

[30] Kunnumakkara, A. B., Anand, P., & Aggarwal, B.B. (2008). Curcumin inhibits proliferation, invasion, angiogenesis and metastasis of different cancers through interaction with multiple cell signaling proteins. *Cancer Letters* 269: 199–225.

[31] Lai, H.W., Chien, S.Y., Kuo, S.J., Tseng, L.M., Lin, H.Y., Chi, C.W., & Chen D-R (2012). The potential utility of curcumin in the treatment of HER-2-overexpressed breast cancer: an *in vitro* and *in vivo* comparison study with herceptin. *Evidence Based Complementary and Alternative Medicine* doi:10.1155/2012/486568.

[32] Lev-Ari, S., Starr, A., Katzburg, S., Berkovich, L., Rimmon, A., Ben-Yosef, R., Vexler, A., Ron, I. & Earon, G. (2014). Curcumin induces apoptosis and inhibits growth of orthotopic human non-small cell lung cancer xenografts. *Journal of Nutritional Biochemistry* 25(8): 843-850.

[33] Lewinska, A., Adamczyk, J., Pajak, J., Stoklosa, S., Kubis, B., Pastuszek, P., Slota, E., & Wnuk, M. (2014). Curcumin-mediated decrease in the expression of nucleolar organizer regions in cervical cancer (HeLa) cells. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 771: 43–52.

[34] Lin, Li., Hutzen, B., Ball, S., Foust, E, Sobo, M., Deangelis, S., Pandit, B., Friedman, L., Li, C., Li, P-K., Fuchs, J., and Lin, J. (2009). New curcumin analogues exhibit enhanced growth-suppressive activity and inhibit AKT and signal transducer and activator of transcription 3 phosphorylation in breast and prostate cancer cells. *Cancer Science* 100 (9) 1719–1727.

[35] Lin, S-S., Huang, H-P, Yang, J-S., Wu, J-Y, Hsai, T-C, Lin, C-C., Lin, C-W., Kuo, C-L., Wood, W. G., Chung, J-G (2008). DNA damage and endoplasmic reticulum stress mediated -induced cell cycle arrest and apoptosis in human lung carcinoma A-549 cells through the activation of cascade- and mitochondrial-dependent pathway. *Cancer Letters* 272(1): 77-90.

[36] Liu, H., Zhou, B-H., Qiu, X., Wang, H-S., Zhang, F., Fang, R., Wang, X-F., Cai, S-H., Du, J., & Bu, X-Z. (2012). T63, a new 4-arylidene curcumin analogue, induces cell cycle arrest and apoptosis through activation of the reactive oxygen species–FOXO3a pathway in lung cancer cells. *Free Radical Biology and Medicine* 53 (12) 15: 2204-2217.

[37] Liu, Q., Loo, W.T., Sze, S.C., & Tong, Y. (2009). Curcumin inhibits cell proliferation of MDA-MB-231 and BT-483 breast cancer cells mediated by down-regulation of NFκB, cyclinD and MMP-1 transcription. *Phytomedicine* 16: 916–922.

[38] Liu, S., Wang, Z., Hu, Z., Zeng, X., Li, Y., Su, Y., Zhang, C., & Ye, Z. (2011). Anti-tumor activity of curcumin against androgen-independent prostate cancer cells via inhibition of NF-κB and AP-1 pathway *in vitro*. *Journal of Huazhong University of Science and Technology* 4: 530-534.

[39] Madden, K., Flowers, L, Salani, R., Horowitz, I., Logan, S., Kowalski, K., Xie, J., & Mohammed, S.I. (2009). Proteomics-based approach to elucidate the mechanism of antitumor effect of in cervical cancer. *Prostaglandins, Leukotrienes and Essential Fatty Acids* 80(1): 9-18.

[40] Mo, N., Li, Z.Q., Li, J., & Cao, Y.D. (2012). Inhibits TGF-β1-induced MMP-9 and invasion through ERK and Smad signaling in breast cancer MDAMB- 231 cells. *Asian Pacific Journal of Cancer Prevention* 13 (11): 5709-5714.

[41] Mohankumar, K., Pajaniradje., S., Sridharan, S., Singh, V.K., Ronsard, L., Banerjea, A. C., Benson, C.S.,Coumar, M.S., & Rajagopalan, R. (2014). Mechanism of apoptotic induction in human breast cancer cell, MCF-7, by an analog of curcumin in comparison with curcumin – An in vitro and *in silico* approach. *Chemico-Biological Interactions* 210: 51–63.

[42] Mosieniak, G., Adamowicz, M., Alster, O., Jaskowiak, H., Szczepankiewicz, A.A., Wilczynski, G.M., Ciechomska, I. A. & Sikora, E. (2012). Curcumin induces permanent growth arrest of human colon cancer cells: Link between senescence and autophagy. *Mechanisms of Ageing and Development* 133(6): 444-455.

[43] Nair, H.B., Sung, B., Yadav, V. R., Kannappan, R., Chaturvedi, M.M., & Aggarwal, B.B. (2010). Delivery of anti-inflammatory nutraceuticals by nanoparticles for the prevention and treatment of cancer. *Biochemical Pharmacology* 80(12): 1833–1843.

[44] Narasimhan, M., & Ammanamanchi, S. (2008). Curcumin blocks RON tyrosine kinase-mediated invasion of breast carcinoma cells. *Cancer Research* 68: 5185–5192.

[45] Park, S., Cho, D.H, Andera, L., Suh, N., & Kim, I. (2013). Curcumin enhances TRAIL-induced apoptosis of breast cancer cells by regulating apoptosis-related proteins. *Molecular and Cellular Biochemistry* 383(1-2): 39-48.

[46] Prasad, C.P., Rath, G., Mathur, S., Bhatnagar, D., & Ralhan, R. (2010). Expression analysis of maspin in invasive ductal carcinoma of breast and modulation of its expression by curcumin in breast cancer cell lines. *Chemistry and Biological Interaction* 183: 455–461.

[47] Prasad, C.P., Rath, G., Mathur, S., Bhatnagar, D., & Ralhan, R. (2009). Potent growth suppressive activity of curcumin in human breast cancer cells: Modulation of Wnt/beta-catenin signaling. *Chemico-Biological Interactions* 181(2): 263–271.

[48] Rana, C., Vaish, V., Piplani, H, Nehru, B., Sanyal, S. N. (2012). Nuclear Factor-κB is expressed in early colon cancer and its down-regulation by curcumin and diclofenac is associated with the suppression of proliferation and the induction of apoptosis. *Biomedicine and Preventive Nutrition* 2(4): 228-238.

[49] Ravindran, J., Sahdeo, P., & Aggarwal, B. B. (2009). Curcumin and cancer cells: How many ways can curry kill tumour cells selectively? *American Association of Pharmaceutical Scientists* 11(3): 495-510.

[50] Reuter, S., Eifes, S., Dicato, M., Aggarwal, B. B., & Diederich, M. (2008). Modulation of anti-apoptotic and survival pathways by curcumin as a strategy to induce apoptosis in cancer cells. *Biochemical Pharmacology* 76(1): 340–1351.

[51] Rowe, L.D., Ozbay, T., O'Regan, R.M., & Nahta, R. (2009). Modulation of the BRCA1 protein and induction of apoptosis in triple negative breast cancer cell lines by the polyphenolic compound curcumin. *Breast Cancer* 3: 61–75.

[52] Sandur, S.K., Deorukhkar, A., Pandey, M. K., Pabón, A.M., Shentu, S., Guha, S., Aggarwal, B.B., & Krishnan, S. (2009). Curcumin modulates the radio-sensitivity of colorectal cancer cells by suppressing constitutive and inducible NF-κB activity. *International Journal of Radiation Oncology* 75(2): 534-542.

[53] Sen, G.S., Mohanty, S., Hossain, D.M., Bhattacharyya, S., Banerjee, S., Chakraborty, J., Das, T., & Sa, G. (2011). Curcumin enhances the efficacy of chemotherapy by tailoring p65NFκB-p300 cross-talk in favor of p53-p300 in breast cancer. *Journal of Biological Chemistry* 286: 42232–42247.

[54] Shahar, L-A, Starr, A., Katzburg, S., Berkovich, L., Rimmon, A., Yosef, R. B., Vexler, A., Ron, I., & Earon, G. (2014). Curcumin induces apoptosis and inhibits growth of

orthotopic human non-small cell lung cancer xenografts. *Journal of Nutritional Biochemistry* 25: 843–850.

[55] Shankar, S., Kumar, D., & Srivastava, R. K. (2013). Epigenetic modifications by dietary phytochemicals: Implications for personalized nutrition. *Pharmacology and Therapeutics* 138: 1–17.

[56] Singh, M., & Singh, N. (2009). Molecular mechanism of induced cytotoxicity in human cervical carcinoma cells. *Molecular and Cellular Biochemistry* 325(1-2): 107-119.

[57] Soung, Y.H., & Chung, J. (2011). Curcumin inhibition of the functional interaction between integrin α6β4 and the epidermal growth factor receptor. *Molecular Cancer Therapy* 10: 883–891.

[58] Ströfer, M., Jelkmann, W., & Depping, R. (2011). Curcumin decreases survival of Hep3B liver and MCF-7 breast cancer cells. The role of HIF. *Strahlenther Onkologie* 187: 393–400.

[59] Sun, S-H., Huang, H-C., Huang, C., & Lin, J-K. (2012). Cycle arrest and apoptosis in MDA-MB-231/Her2 cells induced by curcumin. *European Journal of Pharmacology* 690: 22–30.

[60] Sundram, V., Chauhan, S.C., Ebeling, M., & Jaggi, M. (2012). Curcumin attenuates β-catenin signalling in prostate cancer cells through activation of protein kinase D1. P*LoS One* 7(4):e35368. doi: 10.1371/journal.pone.0035368. Epub 2012 Apr 16.

[61] Thomas, S.L., Zhong, D., Zhou, W., Malik, S., Liotta, D., Snyder, J.P., Hamel, E., & Giannakakou, P. (2008). EF24, a novel analogue, disrupts the microtubule cytoskeleton and inhibits HIF-1. *Cell Cycle* 7(15): 2409-2417.

[62] Vinod, B.S., Antony, J., Nair, H.H., Puliyappadamba, V.T., Saikia, M., Narayanan, S. S., Bevin, A. & Anto, R. J. (2013). Mechanistic evaluation of the signalling events regulating curcumin-mediated chemosensitisation of breast cancer cells to 5-fluorouracil. *Cell Death and Disease* 4: e505; doi:10.1038/cddis.2013.26.

[63] Wang, X., Zhang, Y., Zhang, X., Tian, W., Feng, W., & Chen, T. (2012). The curcumin analogue hydrazinocurcumin exhibits potent suppressive activity on carcinogenicity of breast cancer cells via STAT3 inhibition. *International Journal of Oncology* 40: 1189-1195.

[64] Wei, X., Du,Z., Cui, XX, Verano, M, Mo, R.G., Tang, Z.K., Conney, A.H., Zheng, X and Zhang, K (2012). Effects of cyclohexanone analogues of curcumin on growth, apoptosis and NF-κB activity in PC-3 human prostate cancer cells. *Oncology Letters* 4: 279-284.

[65] Wright, L. E., Frye, J.B., Lukefahr, A.L., Timmermann, B.N., Mohammad, K.S., Guise, T.A., & Funk, J.L. (2013). Curcuminoids block TGF-β signalling in human breast cancer cells and limit osteolysis in a murine model of breast cancer bone metastasis. *Journal of Natural Products* 76(3): 316–321.

[66] Xu, Y., Zhang, J., Han, J., Pan, X., Cao, Y., Guo, H., Pan, Y., An, Y., & Li, X. (2012). Curcumin inhibits tumor proliferation induced by neutrophil elastase through the upregulation of α1-antitrypsin in lung cancer. *Molecular Oncology* 6(4): 405-417.

[67] Yallapu, M.M., Khan, S., Maher, D.M., Ebeling, M.C., Sundram, V., Chauhan, N., Ganju, A., Balakrishna, S., Gupta, B.K., Zafar, N., Jaggi, M., & Chauhan, S.C. (2014). Anti-cancer activity of loaded nanoparticles in prostate cancer. *Biomaterials* 35(30): 8635-8648.

[68] Yang, J., Cao, Y., Sun, J., & Zhang, Y. (2010). Curcumin reduces the expression of Bcl-2 by up-regulating miR-15a and miR-16 in MCF-7 cells. *Medical Oncology* 27: 1114–1118.

[69] Ye, M-X., Zhao, Y-L., Li, Y., Miao, Q., Li, Z-K, Ren, X-L., Song, L-Q., Yin, H., & Zhang, J. (2012). Curcumin reverses cisplatin resistance and promotes human lung adenocarcinoma A549/DDP cell apoptosis through HIF-1α and caspase-3 mechanisms. *Phytomedicine* 19(8–9): 779-787.

[70] Yodkeeree, S., Ampasavate, C., Sung, B., Aggarwal, B., & Limtrakul, P. (2010). Demethoxycurcumin suppresses migration and invasion of MDA-MB-231 human breast cancer cell line. *European Journal of Pharmacology* 627: 8–15.

[71] Yoon, M.J., Kim, E.H., Lim, J.H, Kwon, T.K., & Choi, K.S (2010). Superoxide anion and proteasomal dysfunction contribute to curcumin-induced paraptosis of malignant breast cancer cells. Free Radical Biology and Medicine 48: 713–726.

Chapter 12

Lead Compounds from Cucurbitaceae for the Treatment of Cancer

Marcos Soto-Hernández, Jorge Cadena Iñiguez,
Lourdes C. Arévalo-Galarza, Edelmiro Santiago-Osorio,
Itzen Aguiñiga -Sánchez and Lucero del Mar Ruíz-Posadas

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/60048

1. Introduction

1.1. Phytochemicals in the cucurbitaceae

The seeds of several species are a rich source of proteins, lipids, unsaturated fatty acids (table 1), phytosterols, vitamin E, and some minerals such as Mn, Zn, Cu, and carotenoids. The flesh of the fruits also contains some important compounds, such as flavonoids, that show antioxidant activity.

Other phytochemicals in the cucurbits are scarce, but polysaccharides is important to mention. These compounds, bound to proteins, are often considered as key active compounds in some species particularly with regards to diabetes. Alkaloids and saponins have been described in *Momordica* and *Sechium*, and also polyamines and galactolipids. In *Sechium*, the presence of proteins with ribosome-inhibiting activities has been described.

Crop	Water (%)	Calories (kcal)	Protein (g)	Fat (g)	Carbohydrate (g)	Fiber (g)
Melon						
cantaloupe	90	35	0.9	0.3	8.4	0.8
Caaba	92	26	0.9	0.1	6.2	0.8
Honeydew	90	35	0.5	0.1	9.2	0.6

© 2015 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Crop	Water (%)	Calories (kcal)	Protein (g)	Fat (g)	Carbohydrate (g)	Fiber (g)
Winter squash						
Acorn	80	40	0.8	0.1	10.4	1.5
Butternut	86	45	1.0	0.1	11.7	
Hubbard	88	40	2.0	0.5	8.7	
Spaghetti	92	31	0.6	0.6	6.9	
Summer squash	94	20	1.2	0.2	404	1.9
Cucumber	96	13	0.7	0.1	2.8	0.8
Watermelon	92	32	0.6	0.4	7.2	0.5
Bitter gourd (momordica)	94	17	1.0	0.2	3.7	2.8
Wax gourd (Benincasa)	96	13	0.4	0.2	3.0	2.9
Luffa gourd (Luffa)	94	20	1.2	0.2	4.4	
Calabash gourd (Lagenaria)	96	14	0.6	0.02	3.4	
Chayote (Sechium)	94	19	0.8	0.1	4.5	1.7
Pumpkin and squash, seeds	7	541	24.5	45.9	17.8	3.9
Watermelon, seeds	5	557	28.3	47.4	15.3	

Data obtained from the US Department of Agriculture, Agricultural Research Service (2001) USDA Nutrient Database for Standard Reference, Release 14. Nutrient Data Laboratory Home (www.nat.usda.gov/fnic/foodcomp).

Table 1. Nutrient composition of Cucurbits in 100-g edible raw portion

2. Cucurbitacins

The cucurbitacins, as characteristic compounds of many species of the cucurbits, are tetracyclic triterpenes arising from a rearrangement of the protostane cation. They are unsaturated and polyfunctional oxygenated compounds and occur most often as glycosides. They are particularly toxic substances, the bitterness and cytotoxicity being the contributing factors for this toxicity [1].

They are divided into twelve groups, from cucurbitacin A to cucurbitacin T. The cucurbitacin I, B, D, E, and L the most used *in vivo* and *in vitro* studies and differ by the acetylation of groups OH or by the presence of double bond (Figure 1) that increase the lipophilic and toxic properties [2, 3].

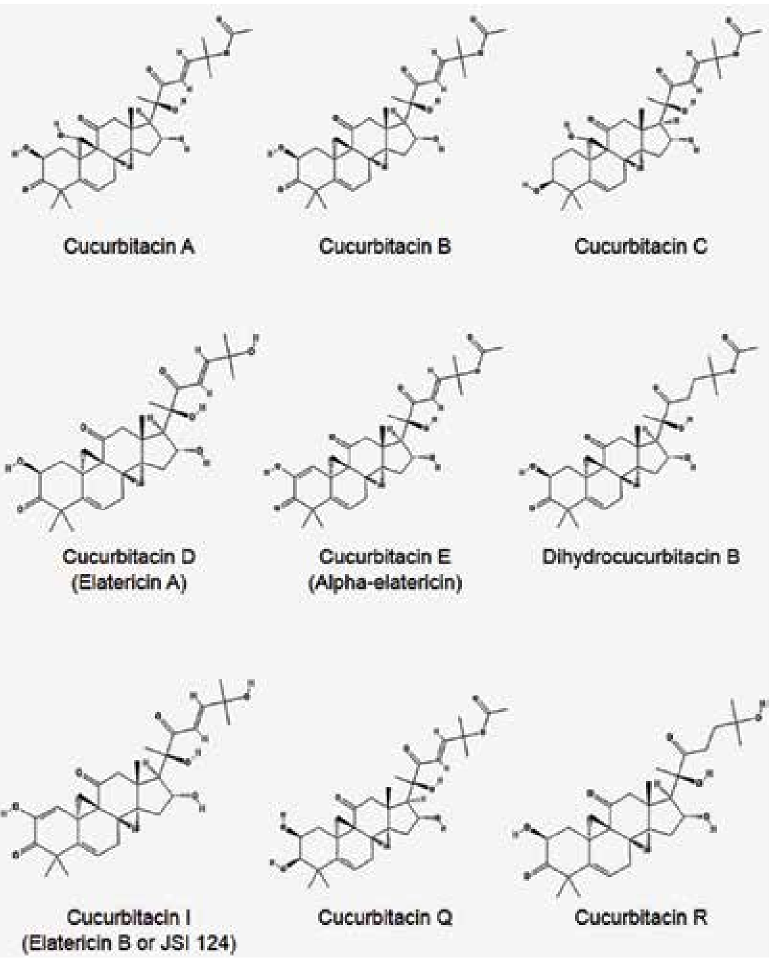


Figure 1. The chemical structure of major cucurbitacins

3. Physical properties and solubility of cucurbitacins

Some of the cucurbitacins are crystalline solids, but others are gums or semisolids and their structure contribute to their scarce solubility in water and this factor is a challenge in pursuing their biological activity. There are some studies [4, 5, 6] that tried to search how to deliver the active principle without this problem, such as the use of polymeric micelles for nanoscale drug delivery. The amphiphilic block copolymers has the capacity to accommodate several types of molecules for drug delivery and the poly(ethylene oxide)-block-poly(ε-caprolactone) called PEO-b-PCL, which is a biocompatible copolymer successfully used for the solubilization of compounds of poor solubility in water, is suitable for solubilization and controlled delivery of cucurbitacin I and B [6].

4. Bioactivity of cucurbitacins

Besides their cytotoxic and anticancer activity, they also show other pharmacological effects *in vivo* as in *in vitro*, for example, as purgative, anti-inflammatory, cough, flu, fever, or anti-fertility. They also show other functions as plant growth regulators and antifeeding agents in insects. A combination of cucurbitacins B + E glucosides showed a notorious antioxidant activity and it is believed that their beneficial properties are due to their ability to interact with reactive oxygen species [10]. It is know that nitric oxide has various functions, but its uncontrolled production can be toxic in many pathological conditions, such as the inflammatory tissue damaged, and in this connection some members of the *Cucurbitaceae* family have been tested as potential inhibitory agents for nitric oxide production [11]. From *Hemsleya pengxianensis*, Dihydrocurbitacin F-25-O-acetate was isolated as one of the main components and it was shown that this compound plays a main role as an anti-infection agent [12].

It was shown that at the molecular level, the cucurbitacins have a role in the inhibition of the JAK/STAT3 pathway that is a major contributor in oncogenesis and five cucurbitacins were tested, A, B, E, I, and Q, and found that the last one inhibit the activation of STAT3 but not JAK2, instead cucurbitacin A inhibit JAK2 but not STAT3 and the cucurbitacins B,E, and I inhibit the activation of both. Furthermore, cucurbitacin Q but not A induces apoptosis and inhibits human tumor growth in mice [13]. To validate these interesting results, another model was described in the Sézay syndrome(Sz), which is an aggressive lymphoma/leukemia of the skin, that used cucurbitacin I to inhibit the JAK/STA3 pathway [16] and it was demonstrated that inhibition of STAT3 using cucurbitacin I induced apoptosis of the cells and it was mentioned that this inhibitor of STAT3 is a potent therapeutic agent for Sz.

Cucurbitacin B and R isolated from *Cayaponia tayuya* induced dramatic changes in the cytoskeleton, inhibiting proliferation and induced apoptosis of isogenic colon cancer cell lines HCT116 and Hke-3 [14]. The cucurbitacin glucosides extracted from *Citrillus colocynthis* leaves effects on human breast cancer cell growth and it is suggested that this kind of glucosides exhibit pleitropic effects on cells causing both cell cycle arrest and apoptosis [13].

Also, the cucurbitacin E has demonstrated some interesting results because it has emerged in one empirical screening strategy to define agents with potent growth inhibitory activity. It was demonstrated that one early effect of this cucurbitacin is the rearrangement of the actin and the vimentin cytoskeleton. The growth inhibitory actions of a series of cucurbitacins correlate with these effects and that the actin and vermectin cytoskeleton are potential targets for this kind of compounds [17].

In another model with cucurbitacin B isolated from *Trichosanthes cucurmerina*, it was demonstrated that this compound has a cytotoxic effect on breast cancer cell lines SKBR-3 and MCF-7 and that growth inhibition was attributed to G2/M phase arrest and apoptosis and also that the cucurbitacin B mediates its effect by inhibiting the translocation of β-catenin and galectin-3 proteins to the nucleus rather than by disrupting the β-catenin/galectin-3/TCF-4 complex formation. This means that cucurbitacin B targeting of the Wnt signaling pathway could be an innovative approach for breast cancer treatment [19].

It was observed that when an antitumor agent, such as doxorubicin (DOX), is combined with some natural products such as capsaicin derivatives, gingerol, ferulic acid, or cucurbitacin E, it has a notorious effect on tumor cells. The first ones did not change DOX permeability in the tumor cells but instead cucurbitacin E significantly promoted DOX influx into the tumour cells and maintained its levels in the tumour cells [18].

In another model, the induction of cancer cell-specific apoptosis via activation of TRAIL (tumour necrosis factor-related apoptosis-inducing ligand) signaling has become an important focus of cancer research and in this sense it was found that cucurbitacins B and D were among the sensitizers of cancer cells to TRAIL-mediated apoptosis in a high-throughput screen [32]. It was found that sensitization by these cucurbitacins is rapid and persistent, making them potentially useful reagents for developing increased understanding of the sequence of molecular events that can lead to TRAIL sensitization and subsequent apoptosis.

The model of proteasome is another approach to understand the molecular mechanism of chemotherapeutic agents. It was found that the proteasome is an abundant catalytic complex present in both nucleus and cytoplasm of eukaryotic cells. Proteasome-mediated degradation plays an essential role in the regulation of most intracellular proteins such as NF-κB and recently proteasome inhibitors have been used as a new anticancer therapy. In this sense it was found that cucurbitacin D induced apoptosis through suppression of proteasome activity both in vivo and in vitro, making this compound a promising candidate for clinical applications in the treatment of T-cell leukemia [20].

5. Response of cancer cells to cucurbitacin exposure

Like other plant-derived substances, cucurbitacins induce toxicity in different cancer cell lines with several morphological and physiological changes (Table 2). Drastic changes in cell shape, such as rounding, swelling, pinocytic blebbing, submembranous inclusions, and blisters, are observed within a couple of hours. Some of the morphological changes could be explained by the dysregulation of cytoskeleton homeostasis by cucurbitacins. Duncan et al. reported a dramatic increase in F-actin to G-actin ratio and abnormal reorganization of the vimentin network by cucurbitacin E in human prostate cancer cell lines [17, 28]. Studies with cucurbitacin B also showed the aggregation of F-actin in various human cancer cell lines [29, 30, 31], implying the disruption of the dissociation process of F-actin by an unknown mechanism. However, unlike vinca alkaloids and taxanes, there is no clear evidence that cucurbitacin affects the microtubule network.

Multinucleation is another common morphological change that was consistently reported in human cancer cell cultures exposed to cucurbitacin for more than 24 h. According to Duncan et al., multinucleation implies that cucurbitacin blocks cytokinesis, but not karyokinesis [17]. This is in conjunction with the observation that actin (which is involved in cytokinesis) is disrupted, whereas microtubules (which are involved in karyonesis) are not.

Multinucleation can result from the disruption of the cell cycle. Many reports showed that cucurbitacins induced cell cycle arrest, mostly in the G2/M phase [31, 32, 33, 39], but S-phase

arrest in HL-60 and U937 human leukaemia cell lines was also reported [11]. G2/M arrest happens in the early period of cucurbitacin exposure and results in apoptotic death of the tumor cells [39]. Tannin-Spitz et al. showed that G2/M arrest occurred in breast cancer cell lines (MCF-7 and MDA-MB-231) exposed to cucurbitacin B/E glucosides by the inhibition of cyclin-dependent kinase (cdk) p34 ^{CDC2} and cyclin B1, both in expression level and activation status [33]. G2/M has shown arrest by upregulation of cdk inhibitor p21^{WAFI}, and by downregulation of cyclin A and cyclin E in pancreatic cancer cell lines (Panc-1 and MiaPaCa-2) exposed to cucurbitacin B [32].

6. Cucurbitacins and their molecular mechanism of action

By what molecular mechanism do cucurbitacins achieve cell cycle arrest, apoptosis, and growth suppression of cancer cells? There are several oncogenic signaling pathways that are commonly involved in cancer cell proliferation and survival. The JAK-STAT pathway, the Akt-PKB pathway, and the MAPK pathway are important in cancer cells and are also targets of the cucurbitacin family.

The JAK-STAT pathway induces Janus-kinases (JACKs) and signal transducers and activators of transcription (STATs), and regulates cytokine and growth factor signals (Figure 2). In many cancer cells, constitutive activation of STAT3 and STAT5 has been known to play important roles in tumorigenesis [34]. After the initial finding by Blaskovich et al. that cucurbitacin I (JSI-124) is a dual inhibitor of STAT3 and JACK [16], many studies confirmed that cucurbitacin I is a powerful JAK-STAT inhibitor by blocking the tyrosine phosphorylation of STAT3 and JAK2 in various human cancers [33, 35, 36, 37, 38, 39]. However, cucurbitacin I did not affect other oncogenic signaling pathways, such as the Akt-PKB or MAPK/ERK pathways [35].

Furthermore, it has been discovered that cucurbitacin B inhibits the tyrosine phosphorylation of STAT3, STAT5, and JAK2 in pancreatic cancer cell lines (Panc-1 and MiaPaCa-2) *in vitro* and in Panc-1 xenografts *in vivo* [32]. Inhibition of the JACK-STAT pathway affected various downstream targets involved in progrowth signaling (e.g., c-myc, cyclins, surviving) and apoptosis (e.g., p53, Bcl-xL, Bcl-2) [34, 36]. Therefore G2/M arrest and apoptosis in pancreatic cancer cells exposed to cucurbitacin B could be explained as a result of inhibition of the JAK-STAT pathway and was confirmed by the downregulation of p21^{WAFI}, cyclin A, and cyclin E, and upregulation of Bcl-xL. Like cucurbitacin I, cucurbitacin B did not inhibit other progrowth signaling pathways, such as the Akt/PKB and MAPK/ERK pathways in pancreatic cancer cells [32]. Interestingly, Sun et al. found that cucurbitacin A, which only differs with cucurbitacin B by its C-11 hydroxyl group, lost its activity as a STAT3 inhibitor while maintaining its activity as a JACK2 inhibitor [36], showing that the inhibition of STAT3 and JAK2 may follow different molecular mechanisms.

The anti-cancer mechanism of cucurbitacin in breast cancer cells is still not clear. Tannin-Spitz et al. exposed breast cancer cell lines (MCF-7 and MDA-MB-231) to cucurbitacin B/E glucosides and found that cucurbitacins increased the tyrosine phosphorylation of STAT3, unlike other cancers [33]. Considering the activated JAK-STAT pathway in breast cancer [34], this result

was contradictory. The authors hypothesized that concurrent inactivation of PBK in a cell type-specific manner might explain this unique regulation, which requires further research.

Considering the important role of STAT3 during inflammation [40], it is not surprising that part of the anti-cancer activity of cucurbitacins is linked to their anti-inflammatory activity. Chronic inflammation can make individuals predisposed to many types of cancer [40]. This seems to affect both cancer cells and normal macrophages through different mechanisms. In cancer cells, cucurbitacins work as STAT3 inhibitors, and make cells more susceptible to the attack of reactive oxygen species (ROS) and free radicals during inflammation [43]. In normal macrophages, however, cucurbitacins work as inhibitors of the IKK/NF-κB pathway rather than inhibitors of STAT3 [42, 43]. Inhibition of the IKK/NF-kB pathway by cucurbitacins results in the inhibition of key inflammatory enzymes, such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), whose overproduction contributes to tumorigenesis [41, 42, 43]. However, it is still not clear how cucurbitacins can selectively choose their target pathway depending on cell type.

Interestingly, when Escandell et al. used two human colon cancer cell lines without activated STAT3 (HCT116 and Hke-3), 23,24-dihydrocucurbitacin B and cucurbitacin R still suppressed tumour growth at a significant level [44]. Furthermore, the presence of active kRas in HCT116 cells showed more protection from apoptosis than Hke-3 cells, which do not have active kRas [25]. Since kRas is upstream of ERK, the result implies the effect of cucurbitacins on the MAPK pathway.

Indeed, the MAPK signaling cascade was another target that cucurbitacins acted upon. It has been shown that cucurbitacin B affects the MAPK pathway in glioblastoma (GBM) multiforme cells *in vitro* [31]. Cucurbitacin B-treated GBM cells showed an increased level of phosphorylated p38, phosphorylated JNK,and phosphorylated c-Jun, as well as a decreased level of phosphorylated ERK at the same time. Upregulation of JNK may induce apoptosis in GBM cells, and downregulation of p38 and ERK may block cytokine signaling. Chan et al. also showed that both phosphotyrosine-STAT3 and phospho-ERK were suppressed in the K562 leukemia cell line [45]. Increased JNK phosphorylation by cucurbitacin D in the Hep3B human hepatocellular carcinoma cell line was also reported [46].

The Akt-PKB pathway mediates signals from receptor tyrosine kinases (RTHKs) and integrins. Currently, no cucurbitacins are known to inhibit the phosphorylation of Akt. Although Tannin-Spintz et al. showed the downregulation of PKB in breast cancer cell lines [33], further research is required to confirm the effect of cucurbitacin on the Akt-PKB pathway.

7. Synergistic effect of cucurbitacins with chemotherapeutic agents

Despite its excellent anti-cancer activity, clinical use of cucurbitacin has challenges to overcome, such as low therapeutic index and nonspecific toxicity. One of the solutions to these problems would be the use of cucurbitacins in combination, not only to enhance the efficacy of the treatment, but also to avoid the build-up of resistance in cancer cells. Moreover, some

drug combinations show strong synergism that helps to achieve the same therapeutic effect with a lower dose, and hence less toxicity. Encouragingly, some reports have shown that cucurbitacins show a synergistic effect with chemotherapeutic agents that are already established in the treatment of human cancers.

Saduka et al. showed that cucurbitacin E promotes cellular accumulation of doxorubicin, both by facilitating influx to and by preventing efflux from the tumour cells, implying synergistic effects of the two drugs [47]. Another study by Ramalhete et al. using cucurbitacin derivatives from *Momordica balsamica* confirmed statically the synergistic effect of some cucurbitacin derivatives and doxorubicin using the fractional inhibitory concentration index (FIX) [48]. Recently, the synergistic effect of cucurbitacin B with gemcitabine in pancreatic cancer was discovered [32]. Using an isobologram and combination index (CI) method, it was shown that in a certain concentration range, cucurbitacin B and gemcitabine showed a CI value less than 0.9, which showed synergism between two drugs *in vitro*.

Strikingly, cucurbitacin B induced no apparent toxicity *in vivo* [32]. As a single agent at high dose (1.0 mg/kg), cucurbitacin B induced a slight body weight loss after the first treatment, but no further toxicity was observed during the seven weeks of treatment. Any signs of toxicity after treatment were searched for using various immunohistochemistry stainings on major organs such as the liver, spleen, and kidney, as well as a blood and serum chemistry test. However, no signs of toxicity by cucurbitacin B were found. Considering the high dose of cucurbitacin B near LD_{50} value (1.1 mg/kg) [26], the result was remarkable.

As Raikhlin-Eisenkraft et al. pointed out, many factors can affect the toxicity of cucurbitacins [25]. The bioreactivity of a compound can vary greatly depending on the presence of other compounds and the microenvironment *in vivo*. The discrepancy between the results and previous case reports can be partly explained by the extent of purity of cucurbitacins. Rapid advances in purification technology over the decades may have eliminated the other impurities coeluted with cucurbitacins from the plant source, which may be the true cause of toxicity in the past. Changes in the type of solvents for elution and dilution can also play a role. Defective immune system in athymic nude mice can be another possibility. For this reason, it was suggested that cucurbitacin toxicity in humans needs to be re-studied and should not be the reason to rule it out as a potential anti-cancer drug.

Cucurbitacin	**Plant Source**	**Effectiveness on cancer cell lines**
Cucurbitacin A	*Trichosanthes cucumerina* (snake gourd)	Lung: A549 cell lines
Cucurbitacin B	*Trichosanthes cucumerina* (snakegourd) *Cucurbita andreana* (buttercup squash). *Wibrandia ebracteata.*	Leukemia and lymphoma: HL60, U937, THP1, NB4, K562, BALL1, Reh, RCH, LY4, Daudi, D901, SP49, Jeko1 and NCEB1. Hepatocellular: Hep-2. Breast: SKBR2, MCF-7, T47D and MDA-B435. Lung: A549, SK

Cucurbitacin	Plant Source	Effectiveness on cancer cell lines
	(no common name) *Luffa operculata* (Sponge Cucumber)	LU1 and NCI-H460. Colon: COCA-2 and HCT-116. Brain: SF-268. Pancreatic cancer cell lines.
Cucurbitan glucosides	*Citrullus colocynthis* (Bitter cucumber)	Breast: ER^{+}MCF-7 and ER´MDA- MB231.
Cucurbitacin E & its glucoside (Elaterin)	*Bacopa monnieri* (Water hyssop) *Cucurbita andreana* (winter Squash) *Citrullus colocynthis*. (Bitter cucumber)	Ovarian sarcoma: M5076. Colon: HCT-116 Breast: MCF-7 and ZR-75-1. Lung: NCI-H460. Brain: SF-268. Prostate: PC-3 Hepatocellular: HepG2
Cucurbitacin D (Elatericin A)	*Trichosanthes kirilowii* (Chinese Cucumber) *Cucurbita andreana* (Winter Squash)	Hepatocellular: Hep-2. Leukemia and lymphoma: HL60, U937, THP, BALL1, Reh, RCH, LY4, Daudi, MD901, SP49, Jeko 1 and NCEB1. Breast: MCF-7 Colon: HCT-116. Lung: NCI-H460. Brain: SF-268
Dihydrocucurbitacin B	*Wibrandia ebracteata* (no common name) *Trichosantes kirilowii* (Chinese Cucumber) *Cayaponia tayuya* (Tayuya)	Leukemia. Hepatocellular Hep-2. Breast: Bcap37 Hela, SW620, SMMC-7721, K562 and MCF-7. Colon: HCT116 and Hke3.
Cucurbitacin I & its glucoside (Elatericin B) (JSI 124)	*Momordica balsamina L* (Balsam pear). *Cayaponia tayuya.* (Tayuya) *Cucurbita andreana.* (Winter Squash) *Citrulus colocynthis* (Bitter cucumber)	Colon HCT-116. Breast: MCF-7, MDA-MB-231, MDA-MB-468, and Panc-1. Lung: NCI-H460. Brain: SF-268. Gliboblastomamultiforme: Y251 and A172. Hepatocellular: Hep-G2
Cucurbitacin Q	*Cayaponia tayuya.* (Tayuya)	Lung: A549 Human and murine cancers: A549, Mda-MB-435 and v-SRV/NIH 3T3
Cucurbitacin R	*Cayaponia tayuya* (Tayuya)	Colon: HCT 116 and Hke-3

Table 2. Cucurbitacin compounds from different plant species and their bioactivity on cancer cells.

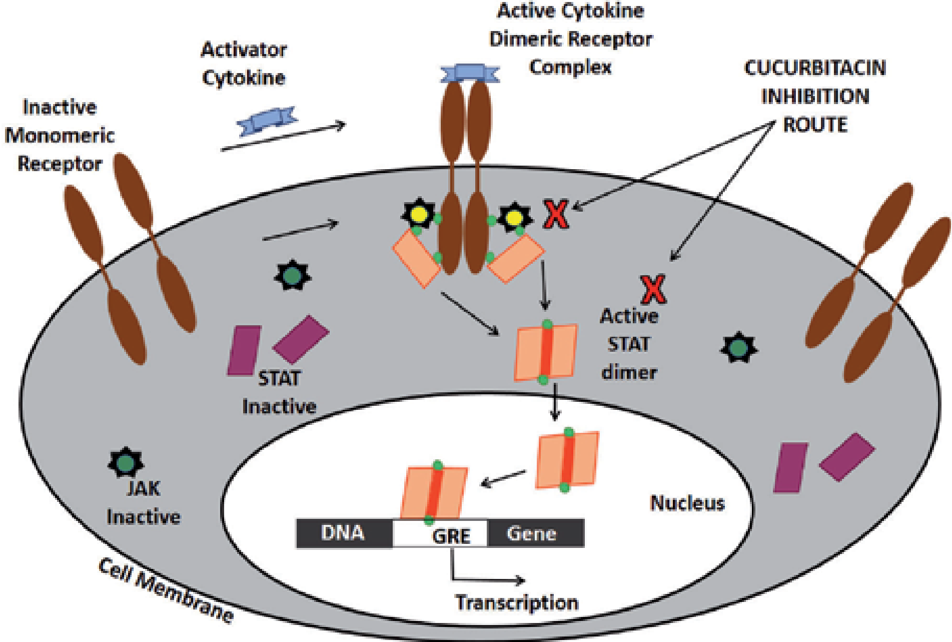


Figure 2. Mechanism of activation of Janus kinases and signal transducer and activator transcription (JAK/STAT) pathway. Upon activator cytokine binding to its receptor on cell surface (e.g.,IL-6 receptor), JAK/STAT pathway is activated (left) leading to sequential cell response. Inhibition signalling process by cucurbitacins is indicate on JAK or STAT STAT signalling.

8. Conclusions

This review has highlighted the interest or importance of some phytochemicals present in members of the *Cucurbitaceae* family, a group of plants used for a long time for many purposes such as ornamental, food, and medicinal. It is in this last area where their phytochemicals constituents have been pointed out as responsible for their medicinal properties. Flavonoids, carotenoids, sterols, oils, vitamins, and minerals are some of these phytochemicals, but is the terpenoid fraction known as cucurbitacins that has received special attention. This is because such compound has been associated as a potential cytotoxic compounds. More than ten years ago, a large number of cucurbitacins have been isolated and the use or applications for the treatment of different types of cancer was described. The review also described details about the mechanisms of how these phytochemicals act at the cellular level, but it seems that they are not specifically bound protein targets forming thioethers bonds through a Michael-type addition. This would allow these compounds to be conjugated with a broad array of potential proteins targets, many of which would be inhibited or disrupted as a consequence. As a result, their value as chemical biology probes is still limited and must be confirmed by independent means. The different findings of the reports reviewed also indicated that the binding mode of

cucurbitacins to protein targets means that optimization for selectivity would be unlikely to work, which would make it very difficult to reduce toxicities or improve the therapeutic window for future clinical applications. However, alternative ways that their potential therapeutic utility could be improved in the future is through targeted delivery to tumour cells, for example, through antibody-conjugation or incorporation in nanoparticles.

Author details

Marcos Soto-Hernández[1*], Jorge Cadena Iñiguez[2], Lourdes C. Arévalo-Galarza[3], Edelmiro Santiago-Osorio[4], Itzen Aguiñiga -Sánchez[4] and Lucero del Mar Ruíz-Posadas[1]

*Address all correspondence to: msoto@colpos.mx

1 Colegio de Postgraduados Campus Montecillo, Botany Department Texcoco, Estado de México, México

2 Colegio de Postgraduados Campus San Luis Potosí SLP, México

3 Colegio de Postgraduados Campus Montecillo, Postharvest Physiology Department, Texcoco, Edo. de México, México

4 Facultad de Estudios Superiores Zaragoza, National Autonomos University of Mexico, Mexico City, Mexico

References

[1] Chen X, Bao J, Guo J, Ding Q, Lu J, Huang M, Wang Y. Biological activities and potential molecular targets of cucurbitacins: A focus on cancer. Anticancer Drugs. 2012; 23: 777-87.

[2] Chen JC, Chiu MN, Nie RL, Cordell GA, Qiu SX. Cucurbitacins and cucurbitane glycosides: Structures and biological activities. Nat Prod Rep. 2005; 22: 386-99.

[3] Bartalis J, Halaweish FT. Relationship between cucurbitacins reversed-phase high performance liquid chromatography hydrophobicity index and basal cytotoxicity on HepG2 cells. J Chromatogr B. 2005; 818: 159-66.

[4] Aliabadi HM, Lavasanifar A. Polymeric micelles for drug delivery. Expert Op Drug Deliv. 2006; 3: 139-62.

[5] Kwon GK, Forrest ML. Amphiphilic block copolymer micelles for nanoscale drug delivery. Drug Dev Res. 2006; 67: 15-22.

[6] Molavi O, Ma Z, Mahmud A, Alshamsan A, Samuel J, Lai R, Kwon GS, Lavasanifar A. Polymeric micelles for the solubilization and delivery of STAT3 inhibitor cucurbitacins in solid tumors. Int J Pharmaceutics. 2008; 347: 118-27.

[7] Clericuzio M, Mella M, Vita-Finzi P, Zema M, Vidari G. Cucurbitane triterpenoids from *Leucopaxillus gentianeus.* J Nat Prod. 2004; 67: 1823-28.

[8] Recio MC, Prieto M, Bonucelli, Orci C, Manez S, Giner RM, Cerda-Nicolas M, Rios JL. Anti-inflammation activity of two cucurbitacins isolated from *Cayaponia tayuya* roots. Planta Medica. 2004; 70: 414-20.

[9] Escandell JM, Recio MC, Manez S, Giner RM, Cerdá-Nicolás JM, Gil-Benso R, Ríos JL. Dihydrocucurbitacin B inhibits delayed-type hypersensitivity reaction by suppressing lymphocyte proliferation. J Pharmacol Exp Ther. 2007; 322: 1261-68.

[10] Tannin-Spitz T, Bergman M, Grossman, S. Cucurbitacinglucosides: Antioxidant and free-radical activities. Biochem Biophys Res Comm. 2007; 364: 181-86.

[11] Park CS, Lim H, Han KJ, Baek SH, Sohn HO, Lee DW. Inhibition of nitric oxide generation by 23,24-dihydrocurubitacin D in mouse peritoneal macrophages. J Pharmacol Exp Ther. 2004; 309: 705-10.

[12] Sun J, Blaskovich MA, Jove R, Livingston SK, Coppola D, Sebti SM. Cucurbitacin Q: A selective STAT3 activation inhibitor with potent antitumor. Oncogene. 2005; 24: 3236-45.

[13] Tehila TS, Shlomo G, Sara D, Hugo EG, Margalit B. Growth inhibitory activity of cucurbitacin glycosides isolated from *Citrillus colocynthis* on human breast cancer cells. Biochem Pharmacol. 2007; 73: 56-67.

[14] Witkowsky A, Woynarowska B, Konopa J. Inhibition of the biosynthesis of deoxyribonucleic acid, ribonucleic acid and protein in HeLa S3 cells by cucurbitacins, glucocorticoid-like cytotoxic triterpenes. Biochem Pharmacol. 1984; 33: 995-1004.

[15] Scandell JM, Kaler P, Recio MC, Sasazuke T, Shirasawa S, Augenlicht L, Rios JL, Klampfer L. Activated K-Ras protect colon cancer cells from cucurbitacin induced apoptosis: The role of p53 and p21. Biochem Pharmacol. 2008; 76: 198-207.

[16] van Kester MS, Luiting JJ, von dem Borne PA, Willemze R, Tensen CP, Vermeer MH. Cucurbitacin I inhibits STAT3 and induces apoptosis in Sezary cells. J Invest Dermatol. 2008; 128: 1691-95.

[17] Duncan KL, Duncan MD, Alley MC, Sausville EA. Cucurbitacin E-induced disruption of the actin and vimentin cytoskeleton in prostate carcinoma cells. Biochem Pharmacol. 1996; 52: 1553-60.

[18] Duncan MD, Duncan KL. Cucurbitacin E targets proliferating endothelia. J Surg Res. 1997; 69: 55-60.

[19] Dakeng S, Duangmano S, Jiratchariyakul W, U-Pratya Y, Bögler O, Patmasiriwat P. Inhibition of Wnt signaling by cucurbitacin B in breast cancer: Reduction of Wnt-as-

sociated proteins and reduced translocation of galectin-3-mediated β-catenin to the nucleus. J Cell Biochem. 2012; 113: 49-60.

[20] Ding N, Yamashista U, Matsuoka H, Sugiura T, Tsukada J, Noguchi J, Yoshida Y. Apoptosis induction through proteasome inhibitory activity of cucurbitacin D in human T-cell leukemia. Cancer. 2011; 117: 2735-46.

[21] Cragg GM, Newman DJ. Plants as source of anti-cancer agents. In Ethnopharmacology. Elisabetsky E, Etkin NL (Eds.) Eols pubs 2006, Oxford, UK.

[22] Pezzuto JM. Plant-derived anticancer agents. Biochem Pharmacol. 1997; 53: 121-33.

[23] Geissman TA. New substances of plant origin. Ann Rev Pharmacol. 1964; 4: 305-16.

[24] Chen JC, Chiu MH, Nie RL, Cordell GA, Qiu SX. Cucurbitacins and cucurbitane glycosides: Structure and biological activities. Nat Prod Rep. 2005; 22: 386-99.

[25] Raikhlin-Eisenfraft B, Bentur Y. *Ecballium elaterium* (squirting cucumber) remedy or poison? Clin Toxicol. 2000; 38: 305-08.

[26] Metcalf RL. Coevolutionary adaptations of rootworm beetles (Coleoptera: Chrysomelidae) to cucurbitacins. J Chem Ecol. 1986; 12: 1109-24.

[27] Shoemaker RH. The NCI60 human tumour cell line anticancer drug screen. Nat Rev Cancer. 2006; 10: 813-23.

[28] Musza LL, Speight P, McElhiney S, Barrow CJ, Gillum AM, Cooper R, Killar LM. Cucurbitacins, cell adhesion inhibitors from *Conobea scopariodes*. J Nat Prod. 1994; 57: 1498-1502.

[29] Haritunians T, Gueller S, Zhang L, Badr R, Yin D, Xing H, Fung MC, Koeffler HP. Cucurbitacin B induces differentiation, cell cycle arrest and actin cytoskeletal alterations in myeloid leukemia cells. Leuk Res. 2008; 32: 1366-73.

[30] Wakimoto N, Yin D, O′Kelly J, Haritunians T, Karlan B, Said J, Xing H, Koeffler HP. Cucurbitacin B has a potent antiproliferative effect on breast cancer cells *in vitro* and *in vivo.* Cancer Sci. 2008; 99: 793-97.

[31] Yin D, Wakimoto N, Xing H, Lu D, Huynh T, Wang X, Black KL, Koeffler HP. Cucurbitacin B markedly inhibits growth and rapidly affects the cytoskeleton in glioblastoma multiforme. Int J Cancer. 2008; 123: 1364-75.

[32] Thoennissen NH, Iwanski GB, Doan NB, Okamoto R, Lin P, Abbassi S, Song HJ, Yin D, Toh M, Xie WD, Said JW, Koeffler HP. Cucurbitacin B induces apoptosis by inhibition of the JAK/STAT pathway and potentiates antiproliferative effects of gemcitabine on pancreatic cancer cells. Cancer Res. 2009; 69: 5876-84.

[33] Tannin-Spitz T, Grossman S, Dovrat S, Gottlieb HE, Bergman M. Growth inhibitory activity of cucurbitacin glucosides isolated from *Citrillus colocynthis* on human breast cancer cells. Biochem Pharmacol. 2007; 73: 56-67.

[34] Yu H, Jove R. The STATS of cancer: new molecular targets come of age. Nat Rev Cancer. 2004; 4: 97-105.

[35] Blaskovich MA, Sun J, Cantor A, Turkson J, Jove R, Sebti SM. Discovery of JSI-124 (cucurbitacin I), a selective Janus Kinase/signal transducer and activator of transcription 3 signaling pathway inhibitor with potent antitumor activity against human and murine cancer cell lines in mice. Cancer Res. 2003; 63: 1270-79.

[36] Sun J, Blaskovich MA, Jove R, Livingston SK, Coppola D, SebtiSM Cucurbitacin Q: A selective STAT3 activation inhibitor with potent antitumor activity. Oncogene. 2003; 24: 3226-45.

[37] Su Y, Li G, Zhang X, Gu J, Zhang C, Tian Z, Zhang J. JSI-124 inhibits glioblastoma multiforme cell proliferation through G(2)/M cell cycle arrest and apoptosis augment. Cancer Biol Ther. 2008; 7: 1243-49.

[38] van Kesten MS, Out-Luiting JJ, von dem Borne PA, Willemze R, Tensen CP, Vermeer MH. Cucurbitacin I inhibits STAT3 and induces apoptosis in Sézary cells. J Invest Dermatol. 2008; 128: 1691-95.

[39] Shi X, Franko B, Frantz C, Amin HM, Lai R. JSI-124 (cucurbitacin I) inhibits Janus Kinase-3/signal transducer and activator of transcription-3 signalling, dowregulates nucleophosmin-anaplastic lymphoma kinase (ALK), and induces apoptosis in ALK-positive anaplastic large cell lymphoma cells. Br J Haematol. 2006; 135: 26-32.

[40] Lin, W, Karin M. A cytokine-mediated link between innate immunity, inflammation, and cancer. J Clin Invest. 2007; 117: 1175-83.

[41] Jayaprakasam B, Seeram NP, Nair MG. Anticancer and anti-inflammatory activities of cucurbitacins from *Cucurbita andreana*. Cancer Lett. 2003; 189: 11-16.

[42] Park CS, Lim KJ, Baek SH, Sohn HO, Lee DW, Yun HY, Baek KJ, Lee HS, Kwon NS. Inhibition of nitric oxide generation by 23,24-dihydrocucurbitacin D in mouse peritoneal macrophages. J Pharmacol Exp Ther. 2004; 309: 705-10.

[43] Escandell, JM, Recio MC, Máñez S, Giner RM, Cerdá-Nicolás M, Ríos JL Cucurbitacin R reduces the inflammation and bone damage associated with adjuvant arthritis in lewis rats by suppression of tumor necrosis factor-alpha in T-lymphocytes and macrophages. J Pharmacol Exp Ther. 2007; 320: 581-90.

[44] Escandell JM, Kaler, P, Recio MC, Sasazuki T, Shirasawa S, Augenlicht L, Ríos JL, Klampfer L. Activated kRas protects colon cancer cells from cucurbitacin-induced apoptosis: The role of p53 and p21. Biochem Pharmacol. 2008; 76: 198-207.

[45] Chan KT, Li K, Liu SL, Chu KH, Toh M, Xie WD. Cucurbitacin B inhibits STAT3 and the Raf/MEK/ERK pathway in leukemia cell line K 562. Cancer Lett. 2009; 289:46-52.

[46] Takahashi N, Yoshida Y, Sugiura T, Matsuno K, Fujino A, Yamashita U. Cucurbitacin D isolated from *Trichosanthes kirilowii* induces apoptosis in human hepatocellular carcinoma cells *in vitro*. Int Immunopharmacol. 2009; 9: 508-13.

[47] Sadzuka Y, Hatakeyema H, Daimon T, Sonobe T. Screening of biochemical modulator by tumor cell permeability of doxorubicin. Int J Pharmacol. 2008; 354: 63-69.

[48] Ramalhete C, Molnár J, Mulhovo S, Rosário VE, Ferreira MJ. New Potent P-glycoprotein modulators with the cucurbitane scaffold and their synergistic interactions with doxorubicin on resistant cancer cells. Bioorg Med Chem. 2009; 17: 6942-51.

Chapter 13

Green Tea Catechins for Prostate Cancer Chemoprevention

Ganna Chornokur and Nagi B. Kumar

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/59872

1. Introduction

1.1. Prostate cancer: Opportunity for prevention

Prostate cancer (PCa) is the most commonly diagnosed cancer and second most common cause of cancer deaths in American men. The American Cancer Society estimates that there will be 233,000 new cases of prostate cancer in the United States (US) in 2014, and 29,480 men will die from this disease. [1] The initiation and progression of PCa involves a complex series of events. During progression, genetic changes and loss of cellular control are observed as cell phenotypes change from normal to dysplasia (prostatic intraepithelial neoplasia or PIN), severe dysplasia (high-grade PIN or HGPIN), atypical small acinar proliferation (ASAP) or focal glandular prostatic atypia (FGA), [2, 3] clinically localized and finally to metastatic disease. [2, 4 - 7] The frequency of HGPIN as well as latent PCa is evenly distributed, suggesting that external factors such as diet, physical activity, and other lifestyle factors are important in the transformation from these early stages into more aggressive, clinical cancer. [2, 4 - 9] Although early screening and detection has been used historically as strategies for PCa prevention, these recommendations have been a subject of much debate in recent years. Although screening using serum prostate-specific antigen (PSA) has not been shown to significantly reduce either PCa-specific or overall mortality, it has been linked to the substantial overtreatment of clinically insignificant, potentially indolent tumors. [10 - 12] With questions about value of prostate-specific antigen (PSA)-based screening, [10 - 12] few prevention options are available for asymptomatic men who are at high risk for PCa. To address the urgent need for alternative preventive strategies, hormonal agents including the 5-alpha-reductase inhibitors finasteride and dutasteride, which block the conversion of testosterone to dihydrotestosterone, have been evaluated for PCA chemoprevention. [13 - 15] Although a reduction in PCa incidence of 23%–

© 2015 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

25% was observed with these agents, [13, 15] concerns about higher incidence of aggressive cancers and toxicities have limited their clinical adoption, establishing the need to identify alternative chemoprevention agents [14] with a more favorable safety profile. These features of prostate cancer, namely, high prevalence, long latency, significant mortality and morbidity, availability of HGPIN and ASAP as intermediate predictive stages of progression, and urgent need for alternative strategies for prevention other than screening, provide the most promise and best opportunity for evaluating agents for chemoprevention.

2. Cancer chemoprevention

Chemoprevention refers to the inhibition of preinvasive and invasive cancer and its progression or treatments of identifiable precancers. [16, 17] Successful chemopreventive interventions require a thorough evidence-based understanding of the mechanism and hallmarks of carcinogenesis. Novel technologies, methods, and approaches in genomics, metabolomics, and proteomics have enabled this field of research. In order for chemopreventive efforts to be successful, it is imperative to employ these innovative methods and approaches to develop pharmacologic agents (including botanicals/biologicals) to reverse or halt the process of carcinogenesis. Chemopreventive agents include antipromotion and antiprogression compounds that could prevent or stop the survival, growth, and dissemination/metastasis of cells that are already committed to become malignant. [16, 17]

3. Botanicals as agents for cancer chemoprevention

Although several targeted "smart" drugs have emerged over the past decade, it is clear that diseases like cancer have an etiology based on perturbations of multiple signaling pathways. Thus, targeting multiple pathways may represent a more effective approach to cancer control. [18 - 20] In addition, the monotargeted "smart" drugs are associated with high cost and produce numerous side effects. These drawbacks of monotargeted drugs underscore the importance for the development of multitargeted, innocuous, inexpensive, and readily available botanicals for the prevention of cancer. [20] Botanicals have been shown to influence multiple biochemical and molecular cascades that inhibit mutagenesis and proliferation, induce apoptosis, and suppress the formation and growth of human cancers, modulating several hallmarks of carcinogenesis, with a significantly superior safety profile than most agents evaluated to date. Multiple botanicals have been identified and appear promising for PCa chemoprevention. [31]

The objective of this chapter is to review and summarize the most current literature focusing on GTC for prostate cancer chemoprevention based on evidence from laboratory, *in vitro*, and *in vivo* studies and clinical chemoprevention trials.

http://dx.doi.org/10.5772/59872

4. Green tea catechins and prostate cancer chemoprevention

4.1. Epidemiological evidence of the role of GTC and prostate cancer prevention

Several reviews summarizing epidemiological evidence have suggested a protective effect of tea consumption against human cancers of the breast, cervix, colon and rectum, gallbladder, liver, lung, nasopharynx, pancreas, prostate, stomach, ovary, and uterus. [32 - 35] Twenty percent of green tea is consumed in Asian countries such as China, Japan, and Korea, where populations drinking green tea consistently demonstrate lower PCa risks. Epidemiological studies, however, have been mixed, potentially due to confounding factors such as variations in tea consumption(salted, hot), dietary, tobacco and alcohol use, genetic differences, and recall bias. [36, 50] The risk for PCa increases in Asians who migrate to the US if original dietary habits are abandoned. There is epidemiological evidence that Asian men consuming green tea have lower PCa risk, potentially due to exposure over their lifetime that prevents transformation or progression to later stages of prostate carcinogenesis. The conflicting epidemiological results have been attributed to confounding factors that include consumption of salted or very hot tea, geographical location, use of tobacco and alcohol, other dietary differences, and lack of standardization of quantities and compositions of the tea products consumed. In a case–control study conducted in China during 2001–2002, prostate cancer risk declined with increasing frequency, duration, and quantity of green tea consumption [OR = 0.28 (95% CI = 0.17−0.47)], showing a dose–response relationship. [39] Four to five times more men die of CaP in the US than that in Japan. [36] It also appears that the onset of CaP occurs later in life and/or CaPs grow more slowly in Japanese populations compared to Western populations, which may be attributed to the consumption of GTC and soy products. Thus, the potential preventive properties of GTP in prostate cancer, as demonstrated by evidence from epidemiological studies although limited, appear promising.

4.2. Preclinical evidence of the role of GTC and prostate cancer prevention

Several published preclinical studies using green tea, green tea leaves, green tea extracts, GTP mixtures, green tea catechin (GTC) mixtures, and the individual catechins have demonstrated chemopreventive efficacy in several cancers. [51 - 57] Using the TRAMP mice model, Gupta et al. [51] were able to demonstrate that oral infusion of GTP extract at a human achievable dose (equivalent to six cups of green tea per day) significantly inhibits CaP development and increases survival in these mice. When 0.1% GTP (weight/volume) was provided as the sole source of drinking water to transgenic adenocarcinoma mouse prostate (TRAMP) mice 8–32 weeks of age, the result was (i) substantial reduction in the tumor incidence, tumor burden, and delay in metastasis as assessed by magnetic resonance imaging (MRI); (ii) substantial reduction in prostate (by 64%) and genitourinary (GU) (by 72%) weight; (iii) substantially decreased serum insulin-like growth factor-I (IGF-I) and restored to normal insulin-like growth factor binding protein-3 levels (IGFBP-3); and (iv) marked decrease in the expression of proliferating cell nuclear antigen (PCNA) in the prostate compared with control (water-fed) TRAMP mice. Furthermore, GTP consumption caused significant apoptosis of CaP cells, which possibly resulted in reduced dissemination of cancer cells, thereby causing the inhibition of

CaP development, progression, and metastasis of CaP to distant organ sites. However, in another similar animal model, epigallocatechin gallate (EGCG) only slightly reduced these levels. [52] These observations may be attributed to the pharmacokinetic properties of EGCG, which has relatively low oral bioavailability. [52] These inconsistencies could also be explained by differential doses, different methods of infusion, duration of intervention, and inadequate timing of castration necessary to observe changes in markers of progression and the antioxidant property of EGCG. Oral administration of GTPs (*vs.* pure EGCG) at 500 mg/kg/day in drinking water to TRAMP mice is expected to cause a higher systemic exposure compared to gavage and may explain the protective effects observed by Gupta et al. [51] compared with Suttie et al. [52] Earlier preclinical trials [54, 57, 107] have observed decreasing effectiveness of GTC with advancing stage of PCa using a TRAMP mouse model. We investigated the safety and efficacy of PolyE administered with the goal to reduce the progression of CaP in TRAMP mice which is an established model of CaP. [56] In this study, 119 TRAMP and 119 C57BL/6J mice were treated orally with one of three doses of PolyE (200, 500, and 1000 mg/kg/day) in drinking water ad libitum, replicating human achievable doses. Safety and efficacy assessments in our study were performed when mice were 12, 22, and 32 weeks old. The number and the size of tumors in the PolyE group were significantly decreased compared with the control group (water-fed). In water-fed 32-week-old control TRAMP mice, prostate carcinoma metastasis to distant sites was observed in 100% of mice (8/8), compared with 13% of mice (2/16) treated with high-dose PolyE during the same period. Furthermore, PolyE treatment significantly inhibited metastasis in TRAMP mice in a dose-dependent manner ($P = 0.0003$). Long-term (32 weeks) treatment with PolyE was safe and well tolerated with no evidence of toxicity in C57BL/6J mice. PolyE similar to other GTC formulations has been observed to be effective chemopreventive agents in preventing the progression of prostate cancer in TRAMP mice with no toxicity in these mouse models. Our findings provide additional preclinical evidence for the safety and chemopreventive effect of PolyE in preventing metastatic progression of prostate cancer. [56] Preclinical studies, including our recent work with PolyE, have consistently demonstrated chemopreventive efficacy in PCa, significantly delaying primary tumor incidence and tumor burden, with reduction in markers of proliferation and potent and selective *in vitro* and *in vivo* proapoptotic activity on PCa cells.

4.3. Potential mechanism by which GTC modulates prostate carcinogenesis:

A body of research evidence indicates that tea and tea compounds reduce the growth of several human cancer cell lines *in vitro*, including stomach, lung, prostate, colon, leukemia, oral tumor, liver, breast, and cervix, as well as HPV-immortalized cervical epithelial cells. Among the constituents of GTCs, laboratory studies have identified EGCG as the most potent chemopreventive agent that affects a number of molecular processes including induction of apoptosis and inhibition of tumor growth and angiogenesis with no significant effect on benign controls. [58 - 62] EGCG has been reported to affect several cancer-related proteins, including p27, Bcl-2 or Bcr-Abl oncoproteins, Bax, matrix metalloproteinases (MMP-2 and MMP-9), [63] androgen receptor, EGF receptor, activator protein 1 (AP1), and some cell cycle regulators. [64 - 66]

In their recent review of signaling pathways that are affected by green tea polyphenols, Khan et al. [67] reported that various pathways mediated via mitogen-activated protein kinases (MAPK) signaling were affected, including extracellular-signal-related kinases (ERK1/2), c-Jun N-terminal kinases/stress-activated protein kinases (JNK1/2/3 or SAPKs), p38 MAPK, ERK3/4, ERK5, and ERK7/8. Ultimately, MMP-2 and MMP-9 expressions were affected by EGCG via inhibition of phosphorylation of ERK1/2 and p38 pathways. Since MMP-9 and MMP-2 are known biomarkers of invasion and metastasis, the inhibition of their expression leads to inhibition of prostate cancer spread. Insulin-like growth factors (IGF) exert multiple effects on glucose, fat, and protein metabolism and play important roles in regulating cell proliferation, differentiation, apoptosis, and transformation. PI3K/Akt and Ras/MAPK pathways can be activated via IGF-signaling, supporting cell proliferation. GTCs are known to suppress cellular signaling via the IGF-axis, thus affecting cell proliferation. Cyclooxygenase (COX) is a rate-limiting enzyme in the prostaglandin biosynthesis. COX-2 can be activated via NF-κB signaling pathway, leading to inflammation and cell proliferation. EGCG was shown to inhibit COX-2 expression at both the mRNA and the protein levels in the prostate, thus slowing down the cancer growth. Finally, EGCG have a notable effect on cell cycle arrest. Gupta et al. [68] have earlier reported that treatment of CaP cell lines LNCaP and DU145 with EGCG caused cell cycle disruptions and reduced proliferation of cells due to down-regulation of various cyclins as well as altered binding to CDKs. Treatment of human CaP cell lines LNCaP and PC-3 with GTP caused the inhibition of class I HDAC activity, leading to cell cycle arrest and induction of apoptosis.

Yang et al. [69] have recently reported that phenolic groups of EGCG can directly bind to multiple molecules due to their hydrogen bonds. The molecules of interest include B-cell CLL/lymphoma 2 protein (BCL2), which has antiapoptotic properties, leading to induction of apoptosis. Some other EGCG binding targets include glucose-regulated protein 78 kDa, vimentin, IGF-1R, and peptidylcis/trans isomerase. Via this mechanism, EGCG can modulate the formation of reactive oxygen species (ROS) as well as affect other molecular pathways described above.

Rizzi et al. [70] reported that Poly E induced cell cycle arrest at the G_0/G_1 checkpoint for PNT1a prostate cell lines (modeling early stage disease) and G_2/M for PC3 cells (modeling late stage disease). The authors showed that in the model of an early stage disease, autophagy was the first mechanism to activate in response to endoplasmic reticulum stress (ERS). After that stage, which lasted approximately 12 h, activation of caspases occurred, indicating anoikis cell death. In the model of an aggressive CaP, Poly E induced severe ERS which eventually led to GADD153/CHOP activated Puma, a BH3-only protein, committing cells to necroptosis, a programmed caspase-independent mechanism of cell death. These results may aid in the identification of novel targets and strategies aimed at sensitizing apoptosis-resistant cells to alternative death pathways.

Hagen et al. [71] reported that EGCG induced apoptosis in PC3 cells, via the caspase 9-dependent mechanism. Furthermore, EGCG, both alone and in combination with cisplatin, promoted the expression of the proapoptotic splice isoform of caspase 9, and it modifies the

alternative splicing of caspase 9, favoring the proapoptotic isoform. The latter finding suggests that EGCG may affect the alternative splicing of cancer-associated genes *in vivo*.

Connors et al. [72] have previously proposed a mechanistic model according to which GTCs antitumor action in prostate cancer acts through proteasome. GTC-induced inhibition of chymotrypsin-like activity of the proteasome results in the accumulation proteasome targets p21, p27, Bax, and IκBα. The accumulation of cell cycle regulators p21 and p27 result in G1 cell cycle arrest, whereas the accumulation of the proapoptotic protein, Bax, contributes to cell apoptosis. The oncogenic transcription factor, NFκB, is down-regulated, presumably by the elevation of IκBα, its intrinsic inhibitor. This results in the reduced expression of NFκB target genes, antiapoptotic, Bcl-xL and Bcl-2, cell cycle regulators, cyclin D and cyclin E, and metastasis-related genes, VEGF, angiopoietin 1/2, MMPs, and uPA. Reductions in cyclins D and E, Bcl-2, and Bcl-xL further drive the processes of cell cycle arrest, decreased cell proliferation, and apoptosis, respectively. Additionally, the reduction in metastasis-related genes inhibits tumor cell invasion and metastasis.

Based on their studies of GTP in cell culture systems, Adhami et al. [63] were able to demonstrate that EGCG in GTP induces apoptosis, cell growth inhibition, and cyclin kinase inhibitor WAF-1/p21-mediated cell cycle dysregulation.Using cDNA microarrays, they also observed that the EGCG treatment of LNCaP cells results in the induction of genes that functionally exhibit growth-inhibitory effects and repression of genes that belong to the G-protein signaling network. When oral feeding of GTP was the sole source of drinking fluid for TRAMP mice, a significant inhibition of VEGF, MMP-2, and MMP-9 was demonstrated, suggesting antimetastatic and antiangiogenic properties of GTP that may affect CaP progression. [63] Bettuzzi et al. have validated these findings, showing that oral administration of GTP to TRAMP mice reduced CaP onset from 100% to 20% [94] mediated by induction of clusterin (CLU) expression, which is potently up-regulated during prostate gland involution [95] but down-regulated in human CaP specimens [73, 74] and exerts antiproliferative [75] and proapoptotic activity [76 - 79] in PNT1a and PC-3 cells.

EGCG potently and selectively inhibits the proteasome activity in intact human CaP cells and consequently accumulates IkBα and p27 proteins, leading to growth arrest (Figure 1). [59 - 61] ECCG has been shown to decrease NF-κB DNA binding and abrogates NF-κB activation by DNA-damaging agents. Our group also reported that EGCG was able to inhibit NF-κB activation through stabilization of IκBα. [58 - 61] NF-κB is a eukaryotic transcription factor involved in the regulation of COX-2 and many other genes. [79] The constitutive activation of NF-κB has been reported in many tumors, [80, 81] associating it with progression of epithelial cells, including prostate, toward malignancy. This inhibition of proteasome activity by EGCG occurred at or near physiological concentrations *in vitro* (IC50 = 0.1–0.2 mM) and *in vivo* (1–10 mM) at the concentrations found in the serum of green tea drinkers. [59 - 61] The ubiquitin/proteasome system plays an important role in the degradation of cellular proteins. This proteolytic system includes two distinct steps: ubiquitination and degradation. [82, 83] The 26S proteasome is a high molecular weight (2.4MDa), ATP-dependent, multiprotease complex. [84 - 86] This complex, which exists in both the cytoplasm and nucleus, is composed of a catalytic subunit (20S) and regulatory subunits. [87] The 20S catalytic subunit contains at least

three enzymatic activities, chymotrypsin-like (prefers Tyr or Phe at the P1 site), trypsin-like (prefers Lys or Arg), and glutamyl peptidyl hydrolytic-like (prefers Asp or Glu). [88] The inhibition of only the chymotrypsin-like activity has been tightly associated with apoptosis induction. Most substrates that are degraded by the proteasome are first covalently modified with ubiquitin (a 76 amino acid polypeptide). [82] Many substrates of the proteasome are proteins that are involved in the regulation of cell cycle progression and apoptosis. [86] For example, the proteasome degrades regulators of cyclin-dependent kinases (CDK) such as cyclins and the CDK inhibitors p21waf and p27kip as well as the CDK phosphatases CDC25 A, B, and C. The proteasome also degrades IκBα, an important inhibitor of the tumor survival factor NF-κB. Many physical (i.e., radiation), chemical (cancer chemotherapeutic agents), viral, and biological (cytokines and growth factors) agents induce phosphorylation, ubiquitination, and subsequent degradation of IκBα by the proteasome, freeing up NF-κB to translocate to the nucleus and to modulate genes involved in proliferation, invasion, and tumor survival. [89, 90] For example, NF-κB up-regulates the antiapoptotic protein Bcl2 and down-regulates the proapoptotic protease caspase 8. Therefore, by inhibiting the proteasome, IκBα will accumulate, which will inhibit NF-κB from promoting tumor survival. The proteasome is also responsible for degrading the tumor suppressor p53. Many tumor cells inactivate p53 by overexpressing an E3 ligase called mdm2, which binds p53 and ubiquitinates it for degradation by the proteasome. [82, 83] In human tumors, which overexpress mdm2, the inhibition of the proteasome is predicted to induce tumor cell apoptosis by accumulating p53. CEP1612, a dipeptidyl proteasome inhibitor, was able to rapidly induce apoptosis in all the human cancer cell lines tested, including breast, prostate, leukemia, lung, bone, brain, and head and neck, but not in human normal fibroblasts and normal breast cells. [85, 91] They also reported that proteasome inhibition was sufficient to overcome apoptotic protection by Bcl-2 or Bcr-Abl oncoprotein. [91] Recently, it has been reported that proteasome inhibition accumulates Bax (but not Bcl-2) protein in mitochondria, resulting in increased ratio of Bax/Bcl-2, associated with cytochrome *c* release and apoptosis induction. [92] It has also been reported that during TNF-α-induced apoptosis, Bcl-2, but not Bax, protein is degraded through ubiquitin/proteasome-dependent pathway, [93] which also increased the Bax/Bcl-2 ratio. Therefore, selectively degrading one or more Bcl-2 family proteins by the proteasome should change the ratio of pro- to antiapoptotic proteins, which might contribute to the apoptotic commitment.

In summary, there is increasing experimental evidence that GTCs slow down prostate carcinogenesis via an umbrella of mechanisms and cellular pathways that work in concert to affect multiple hallmarks of cancer. The main pathways include proteasome inhibition, cell cycle arrest, inhibition of cell proliferation, induction of apoptosis, suppression of growth and invasion, and inhibition of metastasis. Further research should elucidate the exact sequence of cellular events induced by GTCs that lead to suppression of prostate malignancy.

4.4. Clinical experience: green tea polyphenols and prostate cancer

Several phase I studies, sponsored by the National Cancer Institute's Division of Cancer Prevention (NCI, DCP) and others, comparing the pharmacokinetics and safety of oral green tea, polyphenon E, and EGCG, have been completed and published. [96 - 99, 100] By conduct-

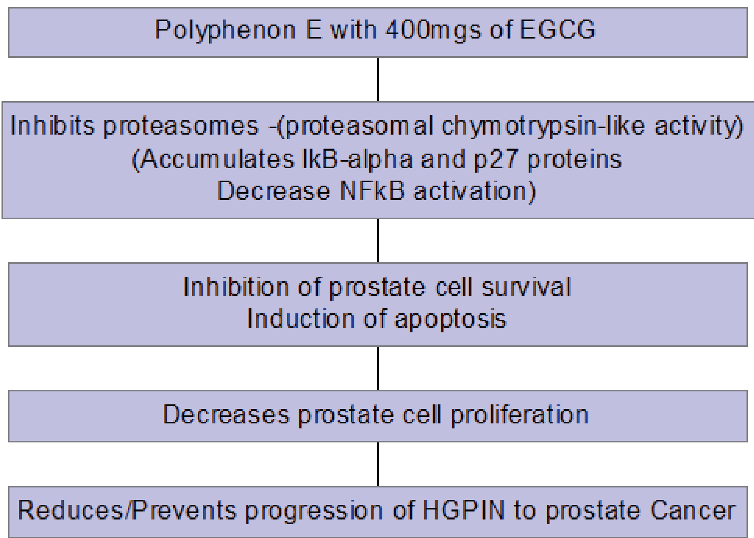


Figure 1. Modeling relative to the primary mechanism of Polyphenon E in Prostate Cancer

ing a phase I trial of oral green tea extract in adult patients with solid tumors, Pisters et al. [98] reported that a safe dose of green tea extract was equivalent to 7–8 Japanese cups (120 ml) of green tea three times daily for 6 months. They concluded that the side effects (neurological and gastrointestinal effects) of the green tea extract preparation were caffeine related, and not from EGCG. The average cup of green tea contains 10–50 mg of caffeine; therefore, overconsumption may have a stimulatory effect in some individuals. Thus, although green tea is practical, nontoxic, and has the potential to be developed for chemoprevention, one has to consume 8–10 cups to obtain these benefits and the side effects due to caffeine are a concern.

In a single-dose study sponsored by NCI, DCP, each subject received two single doses containing 200, 400, 600, or 800 mg EGCG provided by each of two different formulations (polyphenon E or a purified EGCG preparation) separated by a 2-week washout period. [96] Plasma EGCG levels increased with dose and formulation had no effect on EGCG pharmacokinetics. Although little EGCG circulated in conjugated form, EGC and EC were highly conjugated. In a completed multidose study, healthy subjects with sun-sensitive skin received 800 mg EGCG alone or as polyphenon E in one or divided daily doses for 4 weeks. [101] Adverse effects were predominantly mild gastrointestinal complaints. Plasma levels of EGCG were significantly higher in the 400 mg qd versus 400 mg bid dose group for both formulations. Plasma antioxidant levels and UV light-induced minimum erythema dose (MED) were unaffected by any treatment. In the third completed phase I study, the effect of fasting on pharmacokinetics was examined in healthy adults taking 400, 800, or 1200 mg EGCG as polyphenon E. Plasma levels of free EGCG were dramatically higher when taking polyphenon E in the fasting state compared to the fed state for all three dose levels; the average C_{max} increased more than 3.6-fold, and the average AUC increased more than 2.3-fold when taking polyphenon E on an empty stomach. In a study to test if the oral bioavailability of green tea

http://dx.doi.org/10.5772/59872

catechins can be enhanced when consumed in the absence of food, Chow et al. [97] observed that greater bioavailability of free catechins can be achieved by taking the polyphenon E capsules on an empty stomach after an overnight fast thus optimizing the biological effects of tea catechins. Recent studies including individual case reports have indicated several grade 1–2 AEs, including case studies of liver toxicities and rectal bleeding (personal communication from DCP), all of which indicate the need for continued monitoring of safety in well-designed clinical trials.

Bettuzzi et al. [102 - 103] completed a randomized clinical trial to assess safety and efficacy of GTCs for chemoprevention of CaP in men diagnosed with HGPIN. [73] Purity and content of GTCs preparations were assessed by HPLC (EGC 5.5%, EC 12.24%, EGCG 51.88%, ECG 6.12%, total GTCs 75.7%, caffeine <1%). After 1-year intervention with GTC tablets containing 600 mg EGCG/day, only one tumor was diagnosed among the 30 GTC-treated men (incidence: about 3%), while nine cancers were found among the 30 placebo-treated men (incidence: 30%). After 9 months of treatment, subjects taking GTC had a 17% decrease in total PSA values; otherwise, PSA values did not change significantly between the two arms of the study, probably because of high individual differences. No significant side effects were documented. As a secondary observation, they found that the administration of GTCs at this dose was also effective at reducing LUTS. However, other studies intervening in men with localized prostate cancer in the presurgical phase (from biopsy to prostatectomy) with GTC failed to observe chemopreventive benefit [104 - 106] observed in trials targeting earlier stages of prostate carcinogenesis (HGPIN). These early trials indicate that GTC may not exert a meaningful effect once diagnosed with prostate cancer and, consistent with earlier preclinical trials, [54, 104] have observed decreasing effectiveness of GTC with advancing stage of PCa using a TRAMP mouse model. In summary, evidence from phase I/II studies has demonstrated bioavailability and tolerance to GTC at doses ranging from 200 to 1200 mg per day and for a duration of 1 year with observation of chemoprevention effects in the early stages (HGPIN) of prostate carcinogenesis and not in the later stages. With few options available for the chemoprevention of early precursors of PCa, GTC shows promise to be further evaluated as chemoprevention agents targeting high-risk cohorts with HGPIN.

5. Conclusions and future directions

GTCs are promising agents for the chemoprevention of prostate cancer. Early observations indicate that GTCs at human achievable doses are most effective against early stage prostate carcinogenesis in preclinical and clinical trials and are attractive chemopreventive agents with a favorable safety profile. There is an urgent need to continue to identify chemopreventive agents to reduce the burden of cancer. However, it is just as critical to learn from the experience of past chemoprevention trials [108 - 115] that underscore the need for systematically characterizing these agents for cancer chemoprevention and defining their efficacy, safety, and mechanism of action using preclinical and early-phase work before undertaking phase III trials and ultimately translating the findings to clinical practice.

Author details

Ganna Chornokur and Nagi B. Kumar*

*Address all correspondence to: nagi.kumar@moffitt.org

Department of Cancer Epidemiology, Moffitt Cancer Center and Research Institute, Tampa, FL, USA

References

[1] American Cancer Society. http://www.cancer.org/Cancer/ProstateCancer/DetailedGuide/prostate-cancer-key-statistics

[2] Epstein JI, Herawi M. Prostate needle biopsies containing prostatic intraepithelial neoplasia or atypical foci suspicious for carcinoma: implications for patient care. J Urol. 2006;175(3 Pt 1):820–34.

[3] Iczkowski KA. Current prostate biopsy interpretation: criteria for cancer, atypical small acinar proliferation, high-grade prostatic intraepithelial neoplasia, and use of immunostains. Arch Pathol Lab Med. 2006;130(6):835–43.

[4] Bostwick DG, Burke HB, Djakiew D, et al. Human prostate cancer risk factors. Cancer. 2004;101(10 Suppl):2371–490.

[5] Bostwick DG, Qian J. High-grade prostatic intraepithelial neoplasia. Mod Pathol. 2004;17(3):360–79.

[6] Kelloff GJ, Lieberman R, Steele VE, et al. Chemoprevention of prostate cancer: conceptsand strategies. Eur Urol. 1999;35(5–6):342–50.

[7] Mohamed MA, Greif PA, Diamond J, et al. Epigenetic events, remodelling enzymes and their relationship to chromatin organization in prostatic intraepithelial neoplasia and prostatic adenocarcinoma. BJU Int. 2007;99(4):908–15.

[8] Burzon D, Kahnoski RJ, Bennett JK, Barnette KG, Steiner MS. Men with high-grade prostatic intraepithelial neoplasia (HGPIN) remain at high risk for prostate cancer regardless of whether HGPIN is detected on subsequent biopsies. J Urol. 2005;173(4 Suppl):Abst No. 673.

[9] Lieberman R. Prostate cancer chemoprevention: strategies for designing efficient clinical trials. Urology. 2001;57(4 Suppl 1):224–9.

[10] Andriole GL, Crawford ED, Grubb RL, 3rd, et al. Mortality results from a randomized prostate-cancer screening trial. N Engl J Med. 2009;360(13):1310–9.

[11] Ilic D, O'Connor D, Green S, Wilt TJ. Screening for prostate cancer: an updated Cochrane systematic review. BJU Int. 2011;107(6):882–91.

[12] Moyer VA, Force USPST. Screening for prostate cancer: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med. 2012;157(2):120–34.

[13] Andriole GL, Bostwick DG, Brawley OW, et al. Effect of dutasteride on the risk of prostate cancer. N Engl J Med. 2010;362(13):1192–202.

[14] Hamilton RJ, Kahwati LC, Kinsinger LS. Knowledge and use of finasteride for the prevention of prostate cancer. Cancer Epidemiol Biomarkers Prev. 2010;19:2164–71.

[15] Thompson IM, Goodman PJ, Tangen CM, et al. The influence of finasteride on the development of prostate cancer. N Engl J Med. 2003;349(3):215–24.

[16] Murtola TJ, Kujala PM, Tammela TL. High-grade prostate cancer and biochemical recurrence after radical prostatectomy among men using 5α-reductase inhibitors and alpha-blockers. Prostate. 2013 Jun;73(9):923–31.

[17] Kelloff GJ, Lippman SM, Dannenberg AJ, et al. Progress in chemoprevention drug development: the promise of molecular biomarkers for prevention of intraepithelial neoplasia and cancer--a plan to move forward. Clin Cancer Res. 2006;12(12):3661–97.

[18] Sung B, Prasad S, Yadav VR, Aggarwal BB. Cancer cell signaling pathways targeted by spice-derived nutraceutials. Nutr Cancer. 2012;64:173–197.

[19] Gupta SC, Prasad S, Kim JH, Patchva S, Webb LJ, Priyadarsini IK, Aggarwal BB. Multitargeting by curcumin as revealed by molecular interaction studies. Nat Prod Rep. 2011;28:1937–55.

[20] Kannappan R, Gupta SC, Kim JH, Aggarwal BB. Tocotrienols fights cancer by targeting multiple cell signaling pathways. Genes Nutr. 2012;7:43–52.

[21] Aravindaram K, Yang NS. Anti-inflammatory plant natural products for cancer therapy. Planta Med. 2010;76(11):1103–17.

[22] Clinton SK. Lycopene: chemistry, biology, and implications for human health and disease. Nutr Rev. 1998;56(2 Pt 1):35–51.

[23] Giovannucci E, Rimm EB, Liu Y, Stampfer MJ, Willett WC. A prospective study of tomato products, lycopene, and prostate cancer risk. J Natl Cancer Inst. 2002;94(5):391–8.

[24] Kakarala M, Brenner DE, Korkaya H, et al. Targeting breast stem cells with the cancer preventive compounds curcumin and piperine. Breast Cancer Res Treat. 2010;122(3):777–85.

[25] Kumar NB, Krischer JP, Allen K, et al. A phase II randomized, placebo-controlled clinical trial of purified isoflavones in modulating steroid hormones in men diagnosed with localized prostate cancer. Nutr Cancer. 2007;59(2):163–8.

[26] Lippman SM, Klein EA, Goodman PJ, et al. Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). JAMA. 2009;301(1):39–51.

[27] Minich DM, Bland JS. A review of the clinical efficacy and safety of cruciferous vegetable phytochemicals. Nutr Rev. 2007;65(6 Pt 1):259–67.

[28] Omenn GS, Goodman GE, Thornquist MD, et al. Risk factors for lung cancer and for intervention effects in CARET, the Beta-Carotene and Retinol Efficacy Trial. J Natl Cancer Inst. 1996;88(21):1550–9.

[29] Scott EN, Gescher AJ, Steward WP, Brown K. Development of dietary phytochemical chemopreventive agents: biomarkers and choice of dose for early clinical trials. Cancer Prev Res (Phila). 2009;2(6):525–30.

[30] Kumar NB, Dhurandhar M, Aggarwal NT, et al. Proceedings of the Indo-U.S. bilateral workshop on accelerating botanicals/biologics agent development research for cancer chemoprevention, treatment, and survival. Cancer Med. 2013;2(1):108–15.

[31] Kumar N, Chornokur G. Molecular targeted therapies using botanicals for prostate cancer chemoprevention. Transl Med (Sunnyvale). 2012;Suppl 2:005.

[32] Kelloff GJ, Crowell JA, Hawk ET, et al. Clinical development plan: tea extracts, green tea polyphenols, epigallocatechin gallate. J Cell Biochem. 1996;26(Suppl):236–57.

[33] Bushman JL. Green tea and cancer in humans: a review of the literature. Nutr Cancer. 1998;31(3):151–9.

[34] Higdon JV, Frei B. Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. Crit Rev Food Sci Nutr. 2003;43(1):89–143.

[35] Ahn WS, Yoo J, Huh SW, et al. Protective effects of green tea extracts (polyphenon E and EGCG) on human cervical lesions. Eur J Cancer Prev. 2003;12(5):383–90.

[36] Jian L, Xie LP, Lee AH, Binns CW. Protective effect of green tea against prostate cancer: a case–control study in southeast China. Int J Cancer. 2004;108(1):130–5.

[37] Lee J, Demissie K, Lu SE, Rhoads GG. Cancer incidence among Korean-American immigrants in the United States and native Koreans in South Korea. Cancer Control. 2007;14(1):78–85.

[38] Maskarinec G, Noh JJ. The effect of migration on cancer incidence among Japanese in Hawaii. Ethn Dis. 2004;14(3):431–9.

[39] Nelson WG. Agents in development for prostate cancer prevention. Expert Opin Investig Drugs. 2004;13(12):1541–54.

[40] Wu AH, Yu MC, Tseng CC, Hankin J, Pike MC. Green tea and risk of breast cancer in Asian Americans. Int J Cancer. 2003;106(4):574–9.

[41] Borrelli F, Capasso R, Russo A, Ernst E. Systematic review: green tea and gastrointestinal cancer risk. Aliment Pharmacol Ther. 2004;19(5):497–510.

[42] Ito K. Prostate cancer in Asian men. Nat Rev Urol. 2014;11(4):197–212.

[43] Kelloff GJ, Lippman SM, Dannenberg AJ, et al. Progress in chemoprevention drug development: the promise of molecular biomarkers for prevention of intraepithelial neoplasia and cancer—a plan to move forward. AACR Task Force on Cancer Prevention. Clin Cancer Res. 2006 Jun 15;12(12):3661–97. Review.

[44] Liang W, Binns CW, Jian L, Lee AH. Does the consumption of green tea reduce the risk of lung cancer among smokers? Evid Based Complement Alternat Med. 2007;4(1):17–22. 17342237.

[45] Lin YW, Hu ZH, Wang X, Mao QQ, Qin J, Zheng XY, Xie LP. Tea consumption and prostate cancer: an updated meta-analysis. World J Surg Oncol. 2014;12:38.

[46] Sun CL, Yuan JM, Koh WP, Yu MC. Green tea, black tea and breast cancer risk: a meta-analysis of epidemiological studies. Carcinogenesis. 2006;27(7):1310–5.

[47] Woolcott CG, King WD, Marrett LD. Coffee and tea consumption and cancers of the bladder, colon and rectum. Eur J Cancer Prev. 2002;11(2):137–45.

[48] Yuan JM. Cancer prevention by green tea: evidence from epidemiologic studies. Am J Clin Nutr. 2013;98(6 Suppl):1676S–81S.

[49] Montague JA, Butler LM, Wu AH, et al. Green and black tea intake in relation to prostate cancer risk among Singapore Chinese. Cancer Causes Control. 2012;23(10): 1635–41.

[50] Ben-Shlomo Y, Evans S, Ibrahim F, et al. The risk of prostate cancer amongst black men in the United Kingdom: the PROCESS cohort study. Eur Urol. 2008;53(1):99–105.

[51] Gupta S, Hastak K, Ahmad N, Lewin JS, Mukhtar H. Inhibition of prostate carcinogenesis in TRAMP mice by oral infusion of green tea polyphenols. Proc Natl Acad Sci U S A. 2001;98(18):10350–5.

[52] Suttie A, Nyska A, Haseman JK, Moser GJ, Hackett TR, Goldsworthy TL. A grading scheme for the assessment of proliferative lesions of the mouse prostate in the TRAMP model. Toxicol Pathol. 2003;31(1):31–8.

[53] Gupta S, Ahmad N, Mohan RR, Husain MM, Mukhtar H.Prostate cancer chemoprevention by green tea: in vitro and in vivo inhibition of testosterone-mediated induction of ornithine decarboxylase. Cancer Res. 1999 May 1;59(9):2115–20.

[54] Harper CE, Patel BB, Wang J, Eltoum IA, Lamartiniere CA. Epigallocatechin-3-gallate suppresses early stage, but not late stage prostate cancer in TRAMP mice: mechanisms of action. Prostate. 2007;67(14):1576–89.

[55] Khan N, Adhami VM, Mukhtar H. Review: green tea polyphenols in chemoprevention of prostate cancer: preclinical and clinical studies. Nutr Cancer. 2009;61(6):836–41.

[56] Kim S, Ku J-L. Metformin induces apoptotic cell death and inhibits CD133+ cancer stem cells in 5-Fu-resistance colorectal cancer cells. AACR Annual Meeting 2014; 4/6/14; San Diego, CA: AACR; 2014.

[57] Siddiqui IA, Zaman N, Aziz MH, et al. Inhibition of CWR22Rnu1 tumor growth and PSA secretion in athymic nude mice by green and black teas. Carcinogenesis. 2006;27(4):833–9.

[58] Nam S, Smith DM, Dou QP. Ester bond-containing tea polyphenols potently inhibit proteasome activity in vitro and in vivo. J Biol Chem. 2001;276(16):13322–30.

[59] Le Y, Cui Y, Iribarren P, Ying G, Wang JM. Manipulating chemoattractant and receptor genes. In Vivo. 2002;16(1):1–23.

[60] Kazi A, Wang Z, Kumar N, Falsetti SC, Chan TH, Dou QP. Structure–activity relationships of synthetic analogs of (–)-epigallocatechin-3-gallate as proteasome inhibitors. Anticancer Res. 2004;24(2B):943–54.

[61] Smith DM, Wang Z, Kazi A, Li LH, Chan TH, Dou QP. Synthetic analogs of green tea polyphenols as proteasome inhibitors. Mol Med. 2002;8(7):382–92.

[62] Kazi A, Daniel KG, Smith DM, Kumar NB, Dou QP. Inhibition of the proteasome activity, a novel mechanism associated with the tumor cell apoptosis-inducing ability of genistein. Biochem Pharmacol. 2003;66(6):965–76.

[63] Adhami VM, Ahmad N, Mukhtar H. Molecular targets for green tea in prostate cancer prevention. J Nutr. 2003;133(7 Suppl):2417S-24S.

[64] Liang YC, Lin-shiau SY, Chen CF, Lin JK. Suppression of extracellular signals and cell proliferation through EGF receptor binding by (–)-epigallocatechin gallate in human A431 epidermoid carcinoma cells. J Cell Biochem. 1997;67(1):55–65.

[65] Chung JY, Huang C, Meng X, Dong Z, Yang CS. Inhibition of activator protein 1 activity and cell growth by purified green tea and black tea polyphenols in H-ras-transformed cells: structure–activity relationship and mechanisms involved. Cancer Res. 1999;59:4610–7.

[66] Liang YC, Lin-Shiau SY, Chen CF, Lin JK. Inhibition of cyclin-dependent kinases 2 and 4 activities as well as induction of Cdk inhibitors p21 and p27 during growth arrest of human breast carcinoma cells by (–)-epigallocatechin-3-gallate. J Cell Biochem. 1999;75(1):1–12.

[67] Khan N, Mukhtar H. Modulation of signaling pathways in prostate cancer by green tea polyphenols. Biochem Pharmacol. 2013;85(5):667–72.

[68] Gupta S, Hussain T, Mukhtar H. Molecular pathway for (–)-epigallocatechin-3-gallate-induced cell cycle arrest and apoptosis of human prostate carcinoma cells. Arch Biochem Biophys. 2003;410:177–85.

[69] Yang CS, Wang H. Cancer therapy combination: green tea and a phosphodiesterase 5 inhibitor? Clin Invest. 2013;123(2):556–58.

[70] Rizzi F, Naponelli V, Silva A, et al. Polyphenon E®, a standardized green tea extract, induces endoplasmic reticulum stress, leading to death of immortalized PNT1a cells by anoikis and tumorigenic PC3 by necroptosis. Carcinogenesis. 2014;35(4):828–39.

[71] Hagen RM, Chedea VS, Mintoff CP, Bowler E, Morse HR, Ladomery MR. Epigallocatechin-3-gallate promotes apoptosis and expression of the caspase 9a splice variant in PC3 prostate cancer cells. Int J Oncology. 2013.

[72] Connors SK, Chornokur G, Kumar NB. New insights into the mechanisms of green tea catechins in the chemoprevention of prostate cancer. Nutr Cancer. 2012;64(1):4–22.

[73] Saverio b, Pierpaola D, Serenella A, Cesare C, Bruno M, Auro T, Arnaldo C. Tumor progression in accompanied by significant changes in the levels of expression of polyamine metabolism regulatory genes and clusterin (sulfated glycoprotein 2) in human prostate cancer specimens. Cancer Res. 2000;60:23–30.

[74] Scaltriti M, Brausi M, Amorosi A, et al. Clusterin (SGP-2, ApoJ) expression is down-regulated in low- and high-grade human prostate cancer. Int J Cancer. 2004;108(1): 23–30.

[75] Bettuzzi S, Scorcioni F, Astancolle S, Davalli P, Scaltriti M, Corti A. Clusterin (SGP-2) transient overexpression decreases proliferation rate of SV40-immortalized human prostate epithelial cells by slowing down cell cycle progression. Oncogene. 2002;21(27):4328–34.

[76] Grassilli E, Bettuzzi S, Monti D, Ingletti MC, Franceschi C, Corti A. Studies on the relationship between cell proliferation and cell death: opposite patterns of SGP-2 and ornithine decarboxylase mRNA accumulation in PHA-stimulated human lymphocytes. Biochem Biophys Res Commun. 1991;180(1):59–63.

[77] July LV, Akbari M, Zellweger T, Jones EC, Goldenberg SL, Gleave ME. Clusterin expression is significantly enhanced in prostate cancer cells following androgen withdrawal therapy. Prostate. 2002;50(3):179–88.

[78] Pucci S, Bonanno E, Pichiorri F, Angeloni C, Spagnoli LG. Modulation of different clusterin isoforms in human colon tumorigenesis. Oncogene. 2004;23(13):2298–304.

[79] Surh YJ, Chun KS, Cha HH, Han SS, Keum YS, Park KK, Lee SS. Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-kappa B activation. Mutat Res. 2001;480–481:243–68.

[80] Garcia R, Bowman TL, Niu G, et al. Constitutive activation of Stat3 by the Src and JAK tyrosine kinases participates in growth regulation of human breast carcinoma cells. Oncogene. 2001;20(20):2499–513.

[81] Segev DL, Hoshiya Y, Stephen AE, et al. Mullerian inhibiting substance regulates NFkappaB signaling and growth of mammary epithelial cells in vivo. J Biol Chem. 2001;276(29):26799–806.

[82] Drexler HC. Activation of the cell death program by inhibition of proteasome function. Proc Natl Acad Sci U S A. 1997;94(3):855–60.

[83] Jentsch S. Ubiquitin-dependent protein degradation: a cellular perspective. Trends Cell Biol. 1992;2(4):98–103.

[84] Hochstrasser M. Ubiquitin, proteasomes, and the regulation of intracellular protein degradation. Curr Opin Cell Biol. 1995;7(2):215–23.

[85] Dou QP, McGuire TF, Peng Y, An B. Proteasome inhibition leads to significant reduction of Bcr-Abl expression and subsequent induction of apoptosis in K562 human chronic myelogenous leukemia cells. J Pharmacol Exp Ther. 1999;289(2):781–90.

[86] Adams J, Palombella VJ, Sausville EA, et al. Proteasome inhibitors: a novel class of potent and effective antitumor agents. Cancer Res. 1999;59(11):2615–22.

[87] Voges D, Zwickl P, Baumeister W. The 26S proteasome: a molecular machine designed for controlled proteolysis. Annu Rev Biochem. 1999;68:1015–68.

[88] Groll M, Heinemeyer W, Jager S, Ullrich T, Bochtler M, Wolf DH, Huber R. The catalytic sites of 20S proteasomes and their role in subunit maturation: a mutational and crystallographic study. Proc Natl Acad Sci U S A. 1999;96(20):10976–83.

[89] Chen S, Fribley A, Wang CY. Potentiation of tumor necrosis factor-mediated apoptosis of oral squamous cell carcinoma cells by adenovirus-mediated gene transfer of NF-kappaB inhibitor. J Dent Res. 2002;81(2):98–102.

[90] Ghosh S, Karin M. Missing pieces in the NF-kappaB puzzle. Cell. 2002;109 Suppl:S81–96.

[91] An B, Goldfarb RH, Siman R, Dou QP. Novel dipeptidyl proteasome inhibitors overcome Bcl-2 protective function and selectively accumulate the cyclin-dependent kinase inhibitor p27 and induce apoptosis in transformed, but not normal, human fibroblasts. Cell Death Differ. 1998;5(12):1062–75.

[92] Li B, Dou QP. Bax degradation by the ubiquitin/proteasome-dependent pathway: involvement in tumor survival and progression. Proc Natl Acad Sci U S A. 2000;97(8):3850–5.

[93] Dimmeler S, Breitschopf K, Haendeler J, Zeiher AM. Dephosphorylation targets Bcl-2 for ubiquitin-dependent degradation: a link between the apoptosome and the proteasome pathway. J Exp Med. 1999;189(11):1815–22.

[94] Caporali A, Davalli P, Astancolle S, D'Arca D, Brausi M, Bettuzzi S, Corti A. The chemopreventive action of catechins in the TRAMP mouse model of prostate carcinogenesis is accompanied by clusterin over-expression. Carcinogenesis. 2004;25(11): 2217–24.

[95] Leskov KS, Klokov DY, Li J, Kinsella TJ, Boothman DA. Synthesis and functional analyses of nuclear clusterin, a cell death protein. J Biol Chem. 2003;278(13):11590–600.

[96] Chow HH, Cai Y, Alberts DS, et al. Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and polyphenon E. Cancer Epidemiol Biomarkers Prev. 2001;10(1):53–8.

[97] Chow HH, Hakim IA, Vining DR, et al. Effects of repeated green tea catechin administration on human cytochrome P450 activity. Cancer Epidemiol Biomarkers Prev. 2006;15(12):2473–6.

[98] Pisters KM, Newman RA, Coldman B, et al. Phase I trial of oral green tea extract in adult patients with solid tumors. J Clin Oncol. 2001;19(6):1830–8.

[99] Chow HH, Cai Y, Hakim IA, et al. Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and polyphenon E in healthy individuals. Clin Cancer Res. 2003;9(9):3312–9.

[100] Ullmann U, Haller J, Decourt JD, Girault J, Spitzer V, Weber P. Plasma-kinetic characteristics of purified and isolated green tea catechin epigallocatechin gallate (EGCG) after 10 days repeated dosing in healthy volunteers. Int J Vitam Nutr Res. 2004;74(4): 269–78.

[101] Ullmann U, Haller J, Decourt JP, et al. A single ascending dose study of epigallocatechin gallate in healthy volunteers. J Int Med Res. 2003;31(2):88–101.

[102] Bettuzzi S, Brausi M, Rizzi F, Castagnetti G, Peracchia G, Corti A. Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia: a preliminary report from a one-year proof-of-principle study. Cancer Res. 2006;66(2):1234–40.

[103] Brausi M, Rizzi F, Bettuzzi S. Chemoprevention of human prostate cancer by green tea catechins: two years later. A follow-up update. Eur Urol. 2008;54(2):472–3.

[104] Jatoi A, Ellison N, Burch PA, et al. A phase II trial of green tea in the treatment of patients with androgen independent metastatic prostate carcinoma. Cancer. 2003;97(6):1442–6.

[105] McLarty J, Bigelow RL, Smith M, Elmajian D, Ankem M, Cardelli JA. Tea polyphenols decrease serum levels of prostate-specific antigen, hepatocyte growth factor, and vascular endothelial growth factor in prostate cancer patients and inhibit production of hepatocyte growth factor and vascular endothelial growth factor in vitro. Cancer Prev Res (Phila). 2009;2(7):673–82.

[106] Nguyen MM, Ahmann FR, Nagle RB, et al. Randomized, double-blind, placebo-controlled trial of polyphenon E in prostate cancer patients before prostatectomy: evaluation of potential chemopreventive activities. Cancer Prev Res (Phila). 2012;5(2):290–8.

[107] Adhami VM, Siddiqui IA, Sarfaraz S, Khwaja SI, Hafeez BB, Ahmad N, Mukhtar H. Effective prostate cancer chemopreventive intervention with green tea polyphenols in the TRAMP model depends on the stage of the disease. Clin Cancer Res. 2009;15(6):1947–53.

[108] Albanes D, Heinonen OP, Taylor PR, et al. Alpha-tocopherol and beta-carotene supplements and lung cancer incidence in the alpha-tocopherol, beta-carotene cancer prevention study: effects of base-line characteristics and study compliance. J Natl Cancer Inst. 1996 Nov 6;88(21):1560–70.

[109] Omenn GS. Chemoprevention of lung cancers: lessons from CARET, the beta-carotene and retinol efficacy trial, and prospects for the future. Eur J Cancer Prev. 2007;16(3):184–91.

[110] Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J Natl Cancer Inst. 1998;90(18):1371–88.

[111] Vogel VG, Costantino JP, Wickerham DL, et al. Effects of tamoxifen vs raloxifene on the risk of developing invasive breast cancer and other disease outcomes: the NSABP Study of Tamoxifen and Raloxifene (STAR) P-2 trial. JAMA.2006 Jun 21;295(23):2727–41. Epub 2006 Jun 5.

[112] Bertagnolli MM, Eagle CJ, Zauber AG, et al. Celecoxib for the prevention of sporadic colorectal adenomas. N Engl J Med. 2006 Aug 31;355(9):873–84.

[113] Steinbach G, Lynch PM, Phillips RK, et al. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med. 2000 Jun 29;342(26): 1946–52.

[114] Baron JA, Cole BF, Sandler RS, et al. A randomized trial of aspirin to prevent colorectal adenomas. N Engl J Med. 2003 Mar 6;348(10):891–9.

[115] Zell JA, Pelot D, Chen WP, McLaren CE, Gerner EW, Meyskens FL. Risk of cardiovascular events in a randomized placebo-controlled, double-blind trial of difluoromethylornithine plus sulindac for the prevention of sporadic colorectal adenomas. Cancer Prev Res (Phila). 2009 Mar;2(3):209–12.

Chapter 14

Eleutherine Plicata – Quinones and Antioxidant Activity

Luiz Claudio da Silva Malheiros,
João Carlos Palazzo de Mello and
Wagner Luiz Ramos Barbosa

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/59865

1. Introduction

Iridaceae is a family of petalloid monocots with 77 genera and more than 1,630 species, which, despite worldwide distribution, has a remarkable concentration in the southern continents. This family has its major center of irradiation in Southern Africa and in Brazil, it is represented by 14 genera and 110 species [1].

Eleutherine plicata Herb. is an Iridaceae popularly known in the Amazonian region as marupazinho, marupari, palmerinha, and marupá-pirranga, where it occurs in the form of a clump, with red bulbs like an onion (Figure 1). Its leaves are entire, pleated, and simple; the flowers are colored in white to pink; and the red bulbs are widely used in Brazilian folk phytotherapy, especially in the Amazonian region [2].

The ethnoguided surveys conducted by Barbosa et al. (2009) [3] in the city of Igarapé-Miri and by Jardim et al. [4] in Santa Barbara, both in the Brazilian State Pará, revealed the use of Marupazinho (*Eleutherine plicata* Herb.) to treat diarrhea caused by amoeba and that the people prepare a tea from the bulb of the plant, which is drunk before the meal.

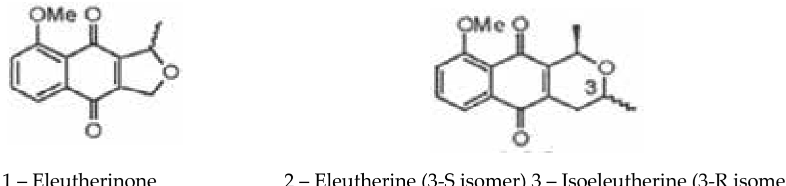
The aqueous extract (AE) of the dried bulbs contains isoeleutherine, which appears as a peak of 99.14% purity in the LC-DAD chromatographic analyses. A buffered aqueous solution containing 2.5 mg of dried AE/mL showed antiamoebic activity, a complete inhibition of the *Entamoeba histolytica* trophozoites growth, in 24 hours [5]. It is remarkable that this solution is less concentrated than the tea (about 3.5 mg/mL), both prepared according to popular knowledge, but normalized according to the Brazilian Pharmacopoeia [6], being indeed sufficient to promote the total annihilation of the tested trophozoites.

© 2015 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Figure 1. *Eleutherine plicata* Herb. (herbaceousborneo.blogspot.com)

The experimental characterization of the antiamoebic activity of the decoction prepared with bulbs of *E. plicata* contributes to validate its alleged popular use. The detection of isoeleutherine in the analyzed decoction can explain partially the reported antiamoebic activity, which can be attributed to the pro-oxidant activity of the substance [7].

Eleutherine bulbosa (Mill.) Urb., an accepted name for *E. plicata*, according to Tropicos®[1], is also used by traditional Zulu healers as anti-diarrheal [8] and yields eleutherinone (1), eleutherine (2), and isoleutherine (3), three quinones that show strong antifungal activity [9] Figure 2). Quinones show various pharmacological activities including bactericidal, fungicidal, and antiprotozoal, noting that they cause oxidative stress by inducing the endogenous formation of reactive oxygen species [7]. Quinones are oxygenated aromatic derivatives, characterized by the presence of a diketocyclohexa-1,4-diene residue (para-quinones) or alternatively by a diketocyclohexa-1,3-diene grouping (ortho-quinones) [10].



1 – Eleutherinone 2 – Eleutherine (3-S isomer) 3 – Isoeleutherine (3-R isomer)

Figure 2. Chemical constituents isolated from *E. bulbosa* (Mill.) Urb.

Naphthoquinones act under enzymatic influence, accepting an electron to form semiquinone anion radical under catalysis of reduced nicotinamide-adenine dinucleotide 3'-phosphate

---

1 Tropicos.org. Missouri Botanical Garden. 28 Oct 2014 <http://www.tropicos.org>

(NADPH) cytochrome-P-450-reductase, NADPH cytochrome-b5-reductase, and NADPH ubiquinone-oxidoreductase. The semiquinone anion radical then reduces molecular oxygen to superoxide anion radical ($[O_2]^{\cdot-}$), which in the presence of superoxide dismutase is converted into H_2O_2. $[O_2]^{\cdot-}$ under catalysis of transition metals (Fenton Reaction) or reacting with H_2O_2 (Haber-Weiss Reaction) generates $[HO]^{\cdot}$ inside the cell. Although H_2O_2 is not a free radical, it's a very reactive substance and can also promote the oxidation of certain biomolecules [7].

2. Materials and methods

2.1. Materials and equipments

2.1.1. Reagents

Acetone, ethyl acetate, acetonitrile, chloroform, deuterated chloroform, ethanol, hexane, potassium hydroxide, methanol, dimethylsulfoxide (DMSO), silica gel for thin layer chromatography, and silica gel for column chromatography.

2.1.2. Equipments

Stainless steel knives mill (Tecnal®, model TCL-650); ultraviolet (UV) visible (VIS) spectrophotometer spectrum SP 2000; UV chamber 254 and 365nm; analytical balance GEHAKA BK 600; high-performance liquid chromatography system LaChrom7000 Merck-HITACHI® with diode array detector (DAD) and Agilent LiChrospher100 (250 mm × 4.6 mm) column; nuclear magnetic resonance (NMR) spectrometer Plus 300 MHz Variant.

2.2. Methods

2.2.1. Collection and identification of botanical material

The plant material purchased at the Ver-O-Peso Market, Belem, Pará State, Brazil was collected in October 2007 in the same region. The botanical identification occurred by comparison of the prepared exsiccata (Figure 3) to a voucher deposited at the Herbarium of the Emilio Goeldi Museum registered under the number 10543[2].

2.2.2. Processing of plant material and extraction

Approximately 5 kg of fresh bulbs were sliced and after washing, aeration and selection, dehydrated at room temperature for 2 days. Drying was completed under forced hot air circulation at about 40° C. The dried material was ground in a Wiley knives mill to yield 1.20 kg of herbal drug.

2 The authors acknowledge Prof.Dr.Mario Augusto Gonçalves Jardim by the characterization of the plant material.

Figure 3. Voucher specimen of *E. plicata* Herb. prepared for botanical identification.

An ethanol extract (EE) was obtained by successive macerations, using 500 g of the herbal drug and Ethanol 96º GL, until total drug exhaustion. Thereafter, the solvent was removed under reduced pressure in a rotary evaporator.

2.2.3. Fractionation of ethanol extract

About 30 g EE were suspended in 500 mL of methanol/water (1:1) and partitioned with Hexane (4 × 100mL) – HF (Hexane Fraction); Chloroform (5 × 100mL) – CF (Chloroform Fraction) and Ethyl Acetate (5 × 100mL) – EAF (Ethyl Acetate Fraction), leaving a residual hydromethanolic solution – RF. Solvents were removed under reduced pressure in rotary evaporator, for water, a lyophilizer was employed.

2.2.4. Phytochemical screening

Chemical tests were performed on EE, HF, CF, EAF, and RF based on the Guide for Phytochemical Analysis of Plant Extracts [11] in order to verify the presence of 18 classes of secondary metabolites.

2.2.5. Thin Layer Chromatography (TLC) analyses

TLC analyses aiming to corroborate the phytochemical results on EE, HF, CF, EAF, and RF were performed employing silica gel as stationary phase and as eluents hexane/acetone (80:20), chloroform/methanol (90:10), chloroform/methanol/water (70:25:05), chloroform/acetone (99:03) and (99:01), thus the corresponding chromatographic profiles were defined. The obtained chromatograms were observed under visible and ultraviolet light at 254 nm and 365 nm and then sprayed with KOH 10% in methanol to make possible the detection of quinones.

http://dx.doi.org/10.5772/59865

The chromatographic profiles were obtained by determining the retention factor (Rf) and describing the color of each chemical constituent observed as a zone in the chromatograms.

2.2.6. Isolation of substances

Using 2.0 g of lyophilized CF, the first chromatographic separation (A) was performed on a 32–63 μm silica gel column (40 × 2.0 cm) as stationary phase, and chloroform/acetone (99.5:0.5) as mobile phase. The fractions were pooled according their TLC profiles, providing the following samples: A1 = 83 mg (corresponding to the substance **1**); A2 = 380 mg; A3 = 71mg; and A4 = 156 mg.

Sample A2 was chromatographed on a 32–63 μm silica gel column (35 × 1.5 cm), eluted with chloroform/acetone (99.5:0.5) and monitored by TLC, yielding the following fractions: B1 = 175 mg, B2 = 12 mg, B3= 53 mg, and B4 = 74 mg.

Using the same stationary phase in a 20 × 1.5 cm column and chloroform/acetone (99.3:0.7) as eluent, B1 was separated by column chromatography, yielding the following fractions after TLC monitoring: C1 = 8 mg, C2 = 28 mg, C3 = 22 mg, C4=69 mg, and C5 = 15 mg. About 65 mg of C4 were subjected to preparative TLC using normal phase silica gel on a standard chromatoplate (20 × 20 cm), eluted with chloroform/acetone (99:01) to obtain **2**.

2.2.7. Characterization of 1 and 2

About 20 mg of **1** and **2** were dissolved in deuterated chloroform ($CDCl_3$) to be analyzed in a Variant brand Plus NMR Spectrometer. The characterization of these substances was achieved by comparison of their ^{1}H- (300 MHz) and ^{13}C- NMR (75MHz) spectra to those obtained from the same naphthoquinones isolated from other species of *Eleutherine* and reported in the scientific literature.

2.2.8. LC profile of EE, CF, 1, and 2

The LC-DAD profile was recorded using a LaChrom 7000 Merck HITACHI® chromatograph hyphenated to a DAD equipped with a LiChrospher100 Agilent column (250 × 4.6 mm). The mobile phase consisted of ultrapure water and acetonitrile (ACN) as described in Table 1 and was pumped at 1 mL/min. The oven temperature was 26°C (± 1°C) and the detection occurred between 200 nm and 500 nm, this method was adapted from Paramapojn et al. (2008) [12].

Time (min.)	H_2O	ACN
00	85	15
10	70	30
20	50	50
30	20	80

Table 1. Gradient composition (in %) of the eluent used to analyze EE, CF, **1,** and **2**.

Aliquots of 50 μL were applied at the following concentrations: **1**, 500 μg/mL; **2,** 1 mg/mL; CF, 2,500 μg/mL; and crude EE, 1 mL.

2.2.9. Antioxidant activity of EE, 1, and 2

The antioxidant capacity of EE, Ep1, and Ep2 was evaluated using the free radical 2,2-diphenyl-1-picrylhydrazyl ($[DPPH]^{\cdot+}$) and as reference the substance butylhydroxytoluene (BHT). The reaction was accompanied by color change and the activity is monitored by the decrease in absorbance of the mixture at 517 nm relative to the solvent as blank [13].

3. Results and discussion

3.1. Botanical identification

The characterization of an exsiccata containing herborized plant material of *Eleutherine plicata* Herb. confirmed the identity of the investigated herbal drug. According to Tropicos®, *Eleutherine bulbosa* is an accepted name for *E. plicata*.

3.2. Extraction and fractionation

The ethanol extract, EE, weighed 63 g, from which *circa* 30g provided four fractions by solid/liquid partition: HF = 2.103 g, 7%; CF = 3,224 g, 11%; EAF = 5,551 g, 18%; and RF = 12.494 g, 41%. The process generates a loss in mass of about 25%, partially due to the solubility of the constituents of the extract in the employed solvents and the partition coefficient of them; and to the evaporation of volatile substances inherent to the methods used to obtain and concentrate the fractions.

3.3. Phytochemical screening

The positive results of the phytochemical approach of EE and fractions considering the presence of 18 classes of secondary metabolites are shown in Table 2.

The metabolites were detected in fractions according to the polarity of the solvents used in the fractionation, such as steroids and triterpenes, azulenes, anthraquinones and naphthoquinones in HF and CF, which are solvents and metabolites of low polarity. Moreover, saponins, tannins and phenols, and coumarin derivatives present in EAF and RF show middle to high polarities. Reducing sugars, detected only in EE, are metabolites of very high polarity, which in liquid-liquid partition do not migrate to organic layer. saponin, coumarin derivatives, and tannins and phenols were not detected in EE, probably due to their concentration in the crude extract or the occurrence of interference on the reagent used.

3.4. Thin Layer Chromatography (TLC) analyses

TLC analyses are used to define the chromatographic profile of extracts and fractions, thereby contributing to the quality control of herbal drugs and their derivatives. In present case,

METABOLIC CLASSES	SAMPLES				
	EE	HF	CF	EAF	RF
Steroids and Triterpenes	+	+	+	-	-
Azulenes	+	+	+	+	+
Reducing Sugars	+	-	-	-	-
Anthraquinone	+	+	+	-	-
Naphthoquinone	+	+	+	-	-
Saponin	-	-	-	+	+
Phenols and Tannins	-	-	-	+	+
Coumarin Derivatives	-	-	-	+	+

Table 2. Metabolic classes detected in derivatives of *E. plicata*.

different chromatographic systems were tested to obtain the chromatographic profile of EE, HF, and CF, wherein normal phase silica gel and chloroform/acetone (99:1) produced chromatograms with very good resolution.

Figure 4A shows three colored yellow zones with Rfs 0.25, 0.31, and 0.44, respectively, present in EE as well in the fractions HF and CF, and pink colored areas with Rf 0.5 and in 0.62 HF and CF. When the same chromatogram is observed under UV light at 254 nm (Figure 4B), three absorption zones in EE and fractions can be observed with Rfs 0.25, 0.31 and 0.44, respectively, and in HF, one bluish zone by Rf 0.62.

This chromatogram also shows, in EE and fractions, brown colored areas with Rfs 0.25, 0.31, and 0.44, respectively, indicating the presence of naphthoquinones after treatment with KOH 10% in methanol (Figure 4C), which is a reagent to detect quinones [14]. Additionally, three rose colored spots with Rfs 0.5, 0.62, and 0.87, respectively, can be seen in HF.

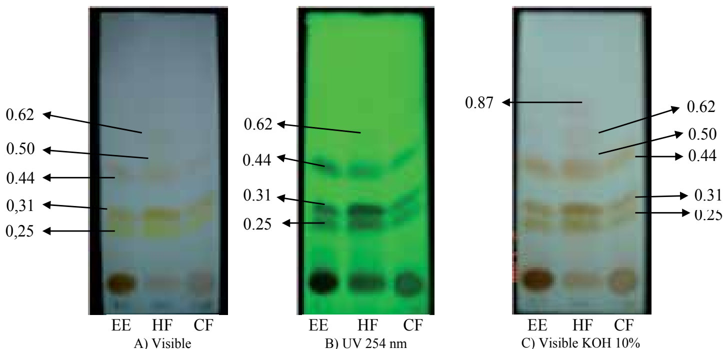


Figure 4. Chromatograms on silica gel eluted with chloroform/acetone 99:1; A – observed under visible light; B – under UV 254nm; C – visible light, after KOH 10%.

3.5. Isolation of major chemical constituents

Sample A1 (83 mg) appears as an isolated chemical substance when analyzed by TLC; it was named **1** and can be observed in HF fraction with Rf 0.62 (Figure 4A, B, C). From C-4, a sample reacting like a naphthoquinone could be purified by preparative TLC yielding 51 mg of a substance that was named **2** that in the TLC analyses showed Rf 0.44 and can also be observed in Figure 4A, B, C.

3.6. Structural characterization of isolated substances

3.6.1. Isoeleutherol

The ^{1}H-NMR spectral data of **1** listed in Table 3 shows characteristic signals of aromatic hydrogen at positions C-4, C-6, C-7, and C-8. The aromatic hydrogen H-4 appears as a singlet (δ = 7.860 ppm), H-6 hydrogen (δ = 7.545 ppm) appears coupled with H-7 (δ = 7.399 ppm) making a doublet. Hydrogen H-7 (δ = 7.399 ppm) couples with H-6 (δ = 7,545 ppm) and H-8 (δ = 6.940ppm), appearing as an overlaid double doublet or false triplet. H-8 hydrogen (δ = 6.940 ppm) appears coupled with H-7 (δ = 7.399) as a doublet.

The signal observed as a singlet (δ = 4.108 ppm) refers to the hydrogen atoms of the methoxy group attached to the aromatic ring. There is also a signal of a methyl group (δ = 1.736 ppm), which is attached to C-1 of the furan ring, coupling with H-1 (δ = 5.718ppm), thus appearing as a doublet.

The hydrogen at C-1 (δ = 5.718ppm) couples with the hydrogen atoms of the methyl group at the same position, generating a quartet. Finally, a phenolic hydrogen appears as a singlet at δ = 9.644.

HYDROGEN	Ep1 δ(ppm)	* ISOELEUTHEROL δ(ppm)	MULTIPLICITY	J (Hz)
1-ME	1.736	1.73	d	6.5
8-OME	4.108	4.11	s	
H-1	5.718	5.70	dd	6.5
H-5	6.927	6.93	d	7.7
H-6	7.399	7.39	t	7.7
H-7	7.545	7.54	t	7.7
H-4	7.863	7.84	s	
9-OH	9.644	9.63	s	

*From: HARA et al., 1997[15]

Table 3. Data of ^{1}H-NMR analysis of Ep1 compared to authentic isoeleutherol.

The ^{13}C-NMR spectral data of **1** listed in Table 4 shows the presence of 14 carbon atoms. The signals with δ = 19.34 and δ = 56.77 are characteristic of methyl carbon atoms of C-11 and C-10, respectively, and the signal at δ = 76.80 is characteristic of carbon C-1. The signals at δ = 126.79, δ = 123.82, δ = 116.67, and δ = 106.44 correspond to the carbons C-4, C-5, C-6 and C-7, respectively. The carbon C-3 of furan ring that is double bonded to an oxygen atom shows a signal at δ = 170.69 and the resonance of non-substituted aromatic carbon atoms such as C-4a and C-8a appears at δ = 137.37 and δ = 117.66. The resonance at δ = 156.42 refers to C-8 where a methoxy group is attached, while the signal at δ = 149.20 corresponds to C-9 bonded to the hydroxyl group. Finally, the carbon atoms C-3a, C-9a of the furan residue condensed with an aromatic ring resonate at δ = 126.04 and δ = 128.07, respectively.

CARBON/ POSITION	Ep1 δ(ppm)	* ISOELEUTHEROL δ(ppm)
C-1	76.80	76.64
C-3	170.69	170.55
C-3a	126.04	125.92
C-4	126.79	126.61
C-4a	137.37	137.24
C-5	123.82	123.67
C-6	116.67	116.50
C-7	106.44	106.33
C-8	156.42	156.61
C-8a	117.66	117.54
C-9	149.35	149.20
C-9a	128.07	127.94
C-10	56.57	56.43
C-11	19.35	19.18

*From: HARA et al., 1997[15]

Table 4. Data of ^{13}C-NMR analysis of Ep1 compared to authentic isoeleutherol.

The very close correspondence of the ^{1}H- and ^{13}C-NMR spectral data of **1** to those found in the literature (Tables 3 and 4) allows to infer that the isolated substance is isoeleutherol (Figure 5), which has been isolated from *Eleutherine americana* Merr. et Heyne by Hara et al. (1997) [15] being this the first report of its occurrence in *E. plicata* Herb.

Hara et al. (1997) [15] found that isoeleutherol did not inhibit the enzyme topoisomerase II DNA dependent, but significantly hinders the HIV replication in H9 lymphocytes.

Figure 5. Chemical structure of isoeleutherol.

Since isoeleutherol seems to be very stable and the major chemical constituent in EE, it could be used as a chemical marker of *E. plicata* and its derivatives.

3.6.2. Isoeleutherine

Substance **2** was analyzed by ^{1}H-NMR and its structure was characterized by comparison of the obtained spectral data to those reported in the literature. Table 5 shows the resonance values of H-6, H-7, and H-8 from the aromatic ring δ = 7.73, δ = 7.64, and δ = 7.27, respectively, with identical coupling constant $J_{6H\text{-}7H}$=$J_{7H\text{-}8H}$=6.7Hz. The signal of H-6 appears as a doublet since it couples with H-7, which in turn appears as a false triplet due the coupling with H-6 and H-8.

The hydrogen atoms of the methyl group attached to C-1 appear as a doublet at δ = 1:54 ($J_{H1\text{-}CH3}$=6.7Hz) and that one bonded to C-3 at δ = 1:33 ($J_{H3\text{-}CH3}$=6.1Hz).

HYDROGÊN	Ep2 δ(ppm)	* ISOELEUTHERINE δ(ppm)	MULTIP.	J (Hz)
1-H	5.00	5.01	q	6.7
1-ME	1.53	1.53	d	6.7
3-H	3.95	3.96	m	
3-ME	1.33	1.34	d	6.1
4-αH	2.69	2.68	dd	3.5–19.0
4-βH	2.23	2.23	dd	11.0–19.0
6-H	7.73	7.74	d	6.7
7-H	7.64	7.64	t	6.7
8-H	7.27	7.27	d	6.7
9-OME	4.00	4.00	s	

*From: HARA et al., 1997[15]

Table 5. Data of ^{1}H-NMR analysis of Ep2 compared to authentic isoeleutherine.

The spectral data listed in Table 5 when compared with that from the literature permit to deduce that **2** corresponds to isoeleutherine (Figure 6), a naphthoquinone already isolated from *Eleutherine bulbosa* Mill [9] and from *E. americana* Merr. by Hara et al. (1997) [15]. This is the first report of the occurrence of isoeleutherine in *E. plicata*.

Figure 6. Chemical structure of isoeleutherine.

3.7. Chromatographic profile by LC-DAD

The LC-DAD profile of EE and CF was obtained using the method developed by Paramapojn et al. (2008) [12] with modifications and the best chromatograms were registered at 250 nm. EE profile shows two peaks of high intensity with retention time – Rt = 18.93 min and 20.83 min, with areas of 45,854,675 and 60,180,902 and a purity of 99.97% and 99.72%, respectively (Figure 7).

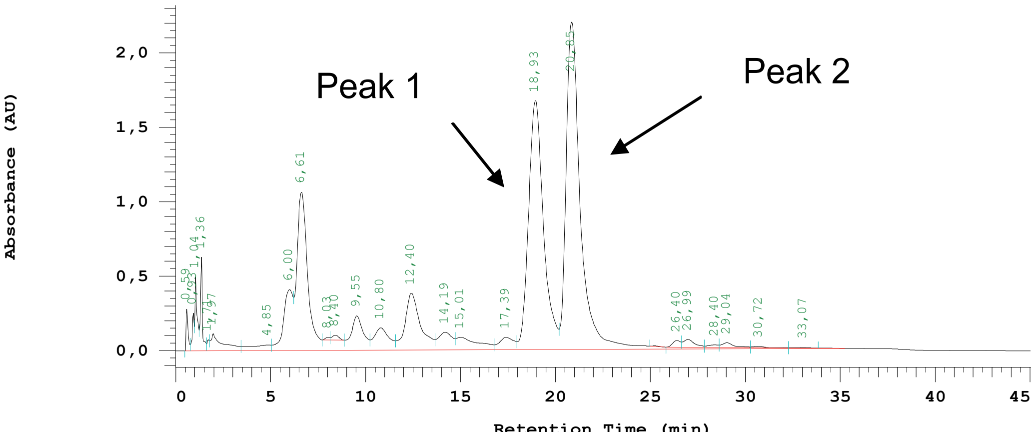


Figure 7. LC-DAD profile of EE registered at 250 nm.

CF chromatogram shows two peaks with high intensity at 19.12 min (Peak 1) and 21.18 min (Peak 2), with areas of 7,813,739 and 1,900,571 and purity of 98.29% and 99.75%, respectively (Figure 8).

The isolated isoeleutherol was also analyzed by LC-DAD under the same conditions as EE and CF, generating a peak at 21.71 min with 3,641,711 area and purity of 99.92% (Figure 9).

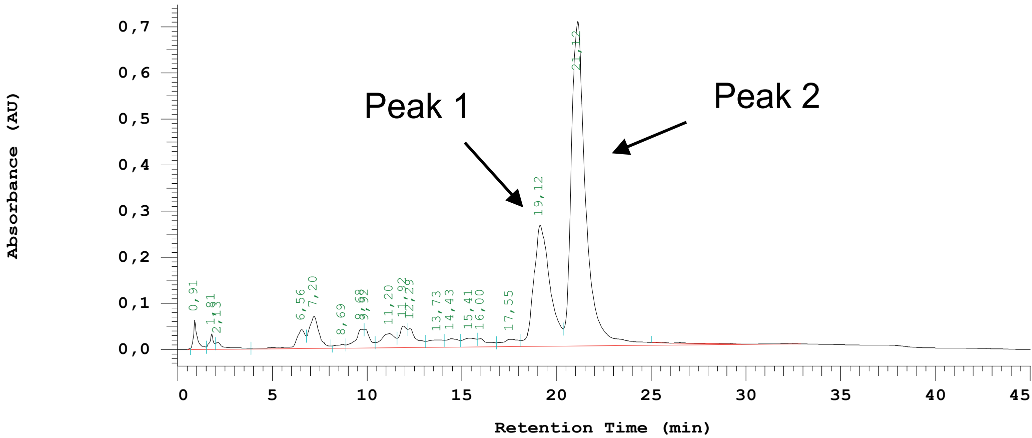


Figure 8. LC-DAD profile of the chloroform fraction registered at 250 nm.

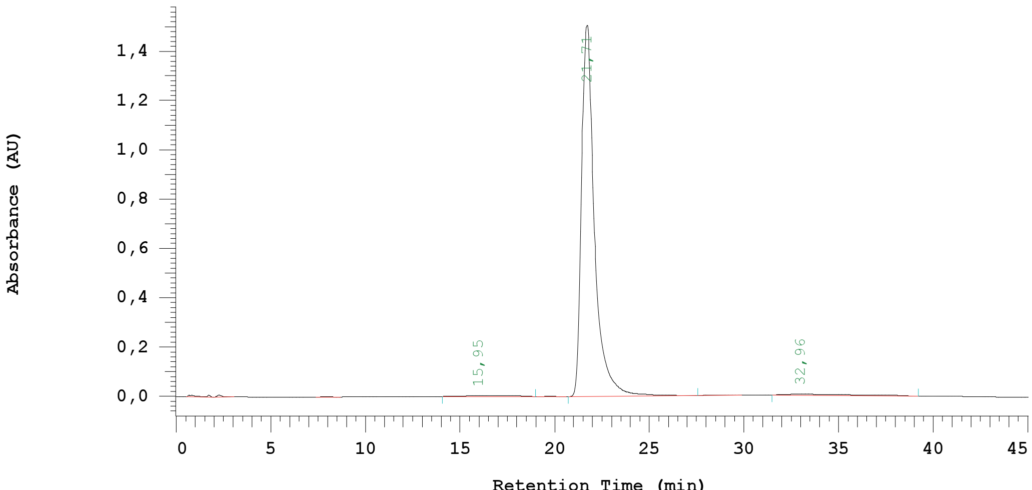


Figure 9. HPLC chromatogram of isoeleutherol at a wavelength of 250 nm.

The same procedure was adopted to analyze the obtained isoeleutherine, producing the LC-DAD profile, showed in Figure 10, where a peak at 18.13min with area 24,727,851 and purity of 99.14% is registered.

The reverse survey feature in the library of the chromatograph shows correlations of 97.40% and 99.89% between the UV spectrum of the peak 01 in EE (Figure 7) and in CF (Figure 8) and that of isoeleutherine. Similarly, the peak 02 in EE (Figure 7) and in CF (Figure 8) showed a correlation of 99.84% and 99.98%, respectively, between the UV spectra of both the peaks and that of isoeleutherol.

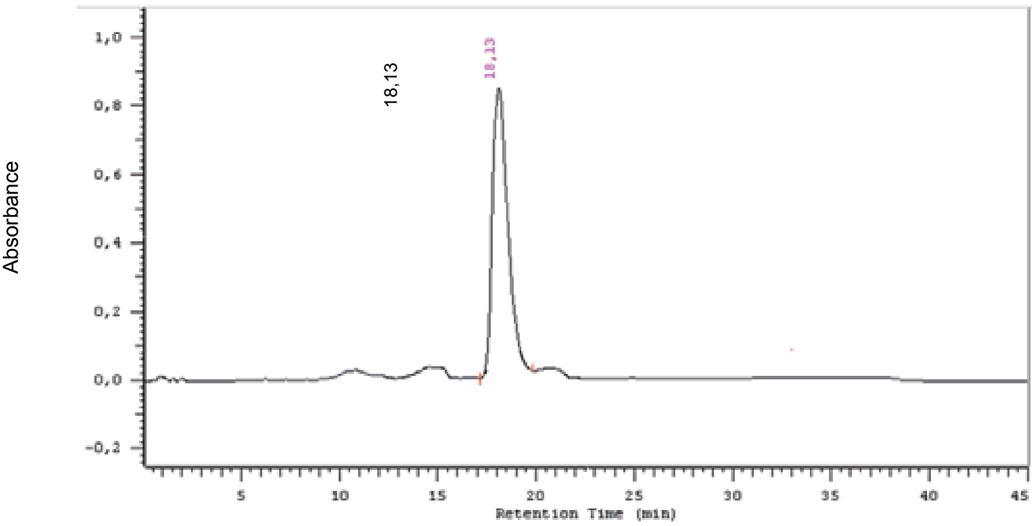


Figure 10. LC-DAD profile of isoeleutherine registered at 250 nm.

3.8. Antioxidant activity of EE, isoeleutherol, and isoeleutherine

Figure 11 and Table 6 show the evaluation of the antioxidant activity of EE, isoeleutherol, and isoeleutherine on DPPH in comparison to the results obtained for BHT used as standard. The antioxidant activity of isoeleutherol (Ep1) appears in concentrations up from 4 μg/mL; for EE, 5 μg/mL; and isoeleutherine (Ep2), 6 μg/mL; while BHT showed activity in concentrations above 1 μg/mL.

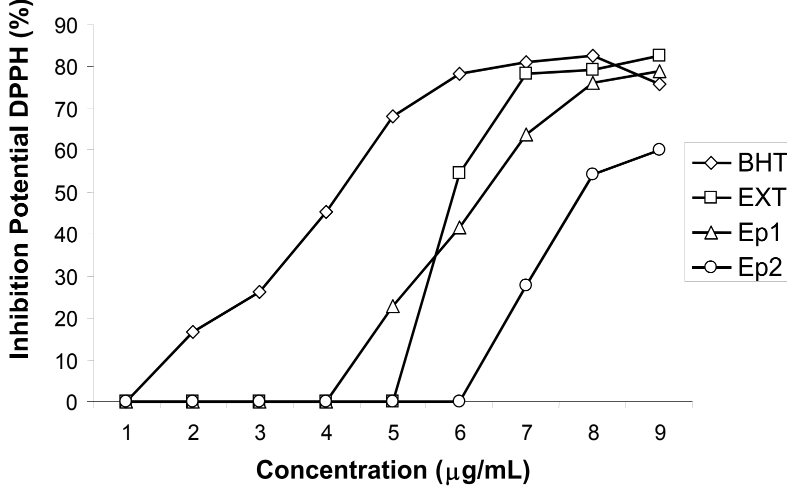


Figure 11. Evaluation of antioxidant activity of EE, and isoeleuterol, and isoeleutherine against DPPH.

Among the tested samples, isoeleutherol showed the best antioxidant activity considering its Inhibition Concentration value (Table 6), followed by EE and isoeleutherine. However, when the IC_{50} values of isoeleutherol and BHT are compared, stay clear that the antioxidant activity of isoeleutherol is about 5× lower.

SAMPLE	IC_{50}
BHT	17.83
EE	94.72
ISOELEUTHEROL	84.63
ISOELEUTHERINE	281.04

Table 6. Determination of IC_{50} of EE, isoeleutherol, isoeleutherine and BHT.

The antioxidant activity of isoeleutherol, higher than that observed for EE and isoelutherine, may be attributed to the hydroxyl group at C-9; this phenolic residue may act as free radical scavenger and sometimes as chelating agent of metal ion with effective action, mainly in preventing lipid oxidation, acting both on the initiation step as on the propagation step of this oxidative process.

Table 6 shows that EE has a higher IC_{50} than isoeleutherol, but lower than that of isoeleutherine. This fact can find explanation in the presence of tannins in EE, which are polyphenols that have adequate chemical structure for the capture of free radicals, contributing to an effective antioxidant capacity of the sample. It is noteworthy to mention that the presence of naphthoquinones in EE may antagonize the activity of tannins since these secondary metabolites can induce oxidative stress or show pro-oxidant capacity, leading EE to present a very low antioxidant activity. Indeed, isoeleutherine presents a negligible antioxidant activity as expected from its structural characteristics, but its occurrence in aqueous extract may be a reason for the antiamoebic activity detected in a previous work [5] and described for *E. bulbosa*, a synonym of *E. plicata* according to TROPICOS®[3], 30 years before today [16].

4. Conclusions

Isoeleutherol and isoeleutherine described before in other *Eleutherine* species were isolated from the chloroform fraction of EE and characterized by ^{1}H- and ^{13}C-NMR. Their HPLC analyses allow confirmation that both are present in the herbal drug, dried bulbs of *E. plicata*. Isoeleutherol seems to be the major chemical constituent in EE and thus can be indicated as a chemical marker for the quality control of this plant species and its derivatives. The occurrence of isoeleutherine in aqueous antiamoebic extract indicates this substance to be used in the quality control of the extract and derivatives, mainly if the substance can be linked to the

3 tropicos.org an information system of Missouri Botanical Garden's electronic databases, Access: 21 Sep. 2014.

reported activity. The isolation and characterization of isoeleutherol and isoeleutherine, which show well-described pharmacological activities, justify the potential of *E. plicata* Herb. to originate a phytomedicine to face neglected diseases, such as Amoeba infection, and probably explain the reason why this plant finds a wide popular use in Amazonian countries.

Author details

Luiz Claudio da Silva Malheiros[1], João Carlos Palazzo de Mello[2] and Wagner Luiz Ramos Barbosa[1*]

*Address all correspondence to: barbosa@ufpa.br

1 Faculty of Pharmaceutical Sciences, Health Sciences Institute, Pará Federal University; Belém, Pará, Brazil

2 Maringá State University, Health Sciences Center, Department of Pharmacy and Pharmacology; Maringá, Paraná, Brazil

References

[1] Reeves, G.; Chase, M.W.; Goldblatt, P.; Rudall, P.; Fay, M.F.; Cox, A.V.; Lejeune, B.; Chies, T.S. Molecular systemics of Iridaceae: Evidence from four plastid DNA regions. American Journal of Botany, 88, 11, 2074-2087, 2001. Available in: www.botanicus.org./item/31753999990077 Accessed: 12 Sep. 2008.

[2] Lorenzi, H; Matos, F.J.A. Plantas medicinais do Brasil – Nativas e Exóticas. São Paulo: Instituto Plantarium de Estudo da Flora Ltda. 2002. 233-234.

[3] Barbosa, W.L.R.; Pinto, L.N. Levantamento etnofarmacêutico da fitoterapia tradicional de Igarapé-Miri. In: VII Reunião da Sociedade Brasileira para o Progresso da Ciência. Manaus-AM, 2001. CD-ROOM-VII SBPC. Manaus: AM, 2001.

[4] Jardim, M.A.G.; Ferreira, M.R.; Leão, R.B.A. Levantamento de plantas de uso terapêutico no município de Santa Bárbara do Pará, estado do Pará, Brasil. Revista Brasileira de Farmácia. São Paulo. 88, 1, 21-25, 2007.

[5] Nascimento, M.S.; Vieira, J.M.S.; Malheiros, L.C.S.; Silva Júnior, J.O.C.; Rodrigues, L.C.S.; Barbosa, W.L.R. Characterisation of Isoeleutherine in aqueous extract of *Eleutherine plicata* Herb, Iridaceae, active against *Entamoeba hystolitica*/ *Entamoeba dispar* in-vitro. International Journal of Pharmaceutical Sciences and Research. 3, 4, 1096-1100, 2012. Available in www.ijpsr.com.

[6] Brasil.Farmacopeia Brasileira 5th Edition, Brasília, Brasil, 2010. Available in www.anvisa.gov.br/hotsite/cd_farmacopeia/index.htm Accessed: 21 Sep. 2014.

[7] Silva, M.N.; Ferreira, V.F.; Souza, M.C.B.V. Um panorama atual da química e da farmacologia de naftoquinonas, com ênfase na β-lapachona e derivados. Química Nova. 26, 3. São Paulo. May/June, 2003.

[8] Lin, J.; Puckree, T.; Mvelase, T.P. Anti-diarrhoeal evaluation of some medicinal plants used by Zulu traditional healer. Journal of Ethnopharmacology. 79, 1, 53, 2002. Available in: www.elsevier.com/locate/jethpharm Accessed: 20 Mar. 2008.

[9] Alves, T.M.A.; Kloos, H.; Zani, C.L. Eleutherinone, a novel fungitox naphthoquinone from *Eleutherine bulbosa* (Iridaceae). Mem. Inst. Oswaldo Cruz, Rio de Janeiro, 98, 5, 2003. Available in: http;//www.scielo.br/scielo.php Accessed: 13 Oct. 2007.

[10] Bruneton, J. Farmacognosia. Fitoquímica e plantas medicinales. 2nd Ed. Zaragoza: ACRIBIA S.A. 2001, 405-420.

[11] Barbosa, W.L.R. Manual para análise fitoquímica e cromatográfica de extratos vegetais. Belém: UFPA, 2001. Available in http://www2.ufpa.br/rcientifica/didaticos_cientificos/pdf_textos/abord_fitoquimica.pdf Accessed: 18 Nov. 2014.

[12] Paramapojn, S.; Ganzera, M.; Gritsanapan, W.; Stuppner, H. Analysis of naphthoquinone derivatives in the Asian medicinal plant *Eleutherine americana* by RP-HPLC and LC-MS. Journal of Pharmaceutical and Biomedical Analysis, 47, 990-993, 2008. Available in: www.elsevier.com/locate/jpba Accessed: 15 Aug. 2008.

[13] Blois, M.S. Antioxidant determinations by the use of a stable free radical. Nature, 181, 1199-1200, 1958. Available in: www.nature.com/nature/journal/v181/n4617/abs/1811199a0.htm Accessed: 20 Aug. 2008.

[14] Wagner, H; Bladt, S. Plant drug analysis: A thin layer chromatography atlas. 2nd Ed. Springer, 275-279, 2001.

[15] Hara, H.; Maruyama, N.; Yamashita, S.; Hayashi, Y.; Lee, K.; Bastow, K.F.; Marumoto, R.; Imakura, M. Elecanacin, a novel new naphthoquinone from the bulb of *Eleutherine americana* Merr. Et Heyne. Chemical Pharmaceutical Bulletin, 45, 10, 1714-1716, 1997. Available in: www.journalarchive.jst.go.jp Accessed: 12 Sep. 2008.

[16] Vickers, W. T. & Plowman, T. *Useful plants* of the Siona and *Secoya*. *Indians of Eastern Ecuador*. Fieldiana. 15:1-63. 1984. Available online on Scholar Google. Access: 21 Sep. 2014.

Edited by A. Venket Rao and Leticia G. Rao

Global dietary recommendations emphasize the consumption of plant-based foods for the prevention and management of chronic diseases. Plants contain many biologically active compounds referred to as phytochemicals or functional ingredients. These compounds play an important role in human health. Prior to establishing the safety and health benefits of these compounds, they must first be isolated, purified, and their physico-chemical properties established. Once identified, their mechanisms of actions are studied. The chapters are arranged in the order from isolation, purification and identification to in vivo and clinical studies, there by covering not only the analytical procedures used but also their nutraceutical and therapeutic properties.

Photo by ajijchan / iStock

IntechOpen