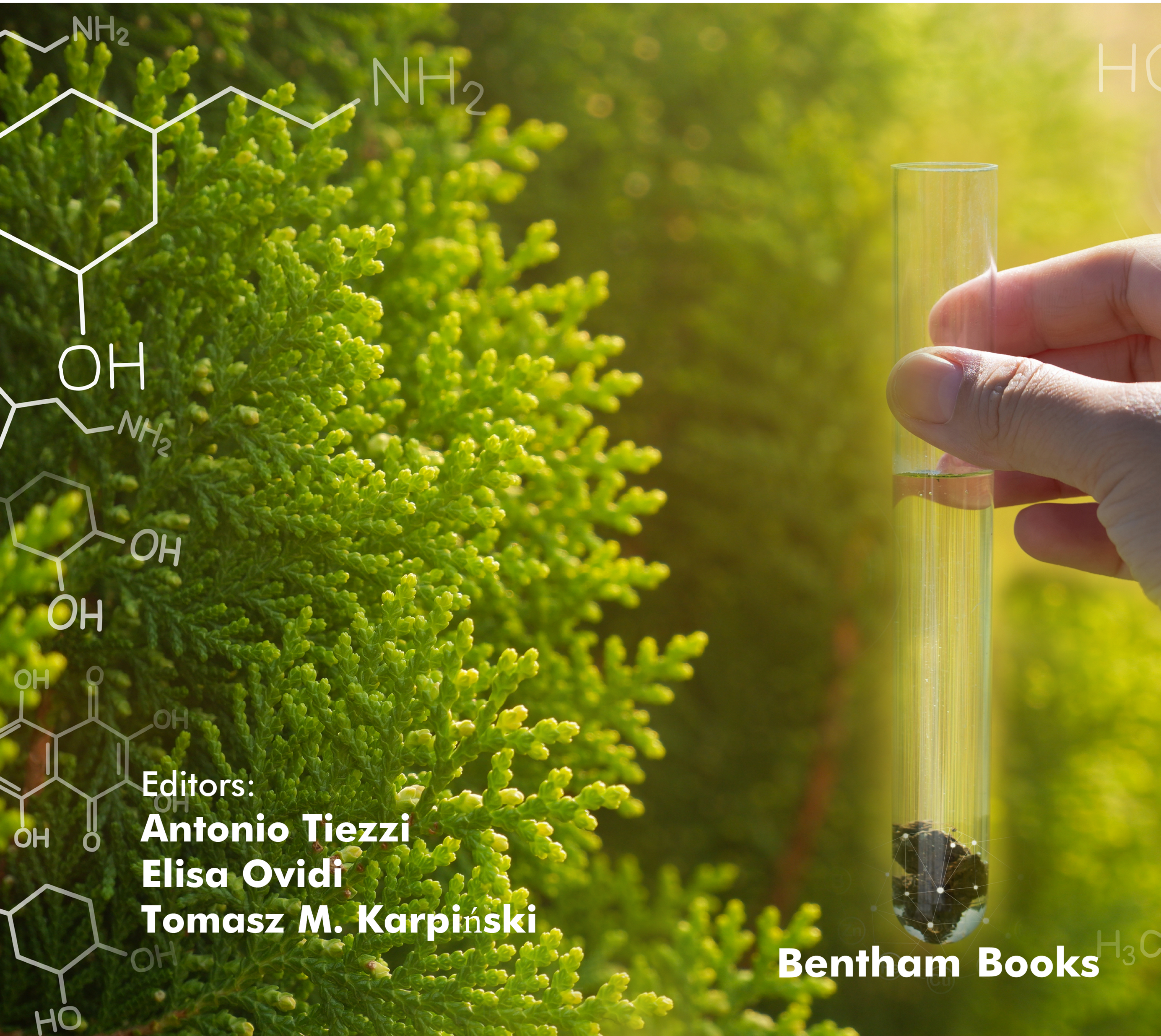
NEW FINDINGS FROM NATURAL SUBSTANCES



Editors:

Antonio Tiezzi

Elisa Ovidi

Tomasz M. Karpiński

Bentham Books

New Findings From Natural Substances

Edited By

Antonio Tiezzi
Department for the Innovation in Biological,
Agrofood and Forestal Systems,
University of Tuscia,
Viterbo, Italy

Elisa Ovidi
Department for the Innovation in Biological,
Agrofood and Forestal Systems,
University of Tuscia,
Viterbo, Italy

Tomasz M. Karpiński
Department of Medical Microbiology,
University of Poznan,
Poznan, Poland

New Findings from Natural Substances

Editors: Antonio Tiezzi, Elisa Ovidi & Tomasz M. Karpiński

ISBN (Online): 978-981-5051-42-1

ISBN (Print): 978-981-5051-43-8

ISBN (Paperback): 978-981-5051-44-5

Published by Bentham Science Publishers Pte. Ltd. Singapore.

First published in 2022.

BENTHAM SCIENCE PUBLISHERS LTD.

End User License Agreement (for non-institutional, personal use)

This is an agreement between you and Bentham Science Publishers Ltd. Please read this License Agreement carefully before using the ebook/echapter/ejournal (**"Work"**). Your use of the Work constitutes your agreement to the terms and conditions set forth in this License Agreement. If you do not agree to these terms and conditions then you should not use the Work.

Bentham Science Publishers agrees to grant you a non-exclusive, non-transferable limited license to use the Work subject to and in accordance with the following terms and conditions. This License Agreement is for non-library, personal use only. For a library / institutional / multi user license in respect of the Work, please contact: permission@benthamscience.net.

Usage Rules:

1. All rights reserved: The Work is the subject of copyright and Bentham Science Publishers either owns the Work (and the copyright in it) or is licensed to distribute the Work. You shall not copy, reproduce, modify, remove, delete, augment, add to, publish, transmit, sell, resell, create derivative works from, or in any way exploit the Work or make the Work available for others to do any of the same, in any form or by any means, in whole or in part, in each case without the prior written permission of Bentham Science Publishers, unless stated otherwise in this License Agreement.
2. You may download a copy of the Work on one occasion to one personal computer (including tablet, laptop, desktop, or other such devices). You may make one back-up copy of the Work to avoid losing it.
3. The unauthorised use or distribution of copyrighted or other proprietary content is illegal and could subject you to liability for substantial money damages. You will be liable for any damage resulting from your misuse of the Work or any violation of this License Agreement, including any infringement by you of copyrights or proprietary rights.

Disclaimer:

Bentham Science Publishers does not guarantee that the information in the Work is error-free, or warrant that it will meet your requirements or that access to the Work will be uninterrupted or error-free. The Work is provided "as is" without warranty of any kind, either express or implied or statutory, including, without limitation, implied warranties of merchantability and fitness for a particular purpose. The entire risk as to the results and performance of the Work is assumed by you. No responsibility is assumed by Bentham Science Publishers, its staff, editors and/or authors for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products instruction, advertisements or ideas contained in the Work.

Limitation of Liability:

In no event will Bentham Science Publishers, its staff, editors and/or authors, be liable for any damages, including, without limitation, special, incidental and/or consequential damages and/or damages for lost data and/or profits arising out of (whether directly or indirectly) the use or inability to use the Work. The entire liability of Bentham Science Publishers shall be limited to the amount actually paid by you for the Work.

General:

1. Any dispute or claim arising out of or in connection with this License Agreement or the Work (including non-contractual disputes or claims) will be governed by and construed in accordance with the laws of Singapore. Each party agrees that the courts of the state of Singapore shall have exclusive jurisdiction to settle any dispute or claim arising out of or in connection with this License Agreement or the Work (including non-contractual disputes or claims).
2. Your rights under this License Agreement will automatically terminate without notice and without the

Bentham Science Publishers Pte. Ltd.
80 Robinson Road #02-00
Singapore 068898
Singapore
Email: subscriptions@benthamscience.net

CONTENTS

FOREWORD

The use of a "phytocomplex" instead of a monomolecular drug is what characterizes modern herbal medicine in the field of medical disciplines. The official definition is given by the World Health Organization (WHO), the European Medicines Agency (EMA) and the Italian Medicines Agency (AIFA) focuses on the use of medicinal plants and their preparations for therapeutic purposes without highlighting any distinction within official medicine.

This concept has always supported the use of medicinal plants for the treatment of numerous diseases. Since prehistoric times, when the casual use of leaves, roots, or seeds had shown useful effects for the healing of wounds, lowering fever or reducing pain, up to Paracelsus who, in the early 1500s, criticized the medical thought of the time and promoted a philosophical vision of nature as the custodian "of the cure for every disease". Paracelsus argued that the healing powers contained in nature had to be found and used, and identified it in plant extracts, containing the "quintessence", the true strength of the medicine capable of healing. With the birth of the scientific thought of Galileo and Newton, a new phase began, where the phytocomplex was gradually replaced by single isolated active ingredients, up to the synthetic pharmaceuticals with the creation of acetylsalicic acid in 1897.

The observation of some iatrogenic reactions and the simultaneous birth of nutraceuticals in 1989, led at the turn of the new millennium to a rediscovery of the preventive and curative power of plant species, first food and then medicinal, much appreciated by an increasing number of "consumers". In turn, this induced the pharmaceutical industry (especially with food supplements) and the whole world of research to focus on the phytochemical and biological characterization of a large number of plant species and phytocomplexes, in full compliance with the original definition of phytotherapy mentioned above.

This has led to an incredible increase in the scientific production that revolves around compounds of natural origin, whose pharmacological aspects have been highlighted, which has at the same time allowed the development of new extraction techniques, on the one hand, and new clinical applications on the other.

The historical passages described above, far from being just a trace of the past, often represent the starting point of a study that finds a basis for the scientific investigation in the traditional uses. In accordance with the principles of modern pharmacology, this has often allowed not only to confirm the therapeutic activity of plants and plant extracts traditionally used in ancient medical treatment but also to expand this use by assigning the spectrum of action of medicaments (phytocomplexes) which are polyfunctional both for their various chemical composition and for the extraordinary biological potential of many natural compounds. An increasing number of studies are aimed at elucidating the activity of isolated compounds, such as flavonoids, alkaloids, or terpenes, but above all phytocomplexes, the joint action of several compounds represents an interesting synergism of action. This means that far from being an interesting but outdated chapter in the history of medicine, phytotherapy is the most widespread therapeutic method in the world at any latitude, so much so that it is part of the strategic program of the World Health Organization "Health for all".

The need to increase the level of scientific development regarding the use of medicinal plants, not only for medical personnel, led to the enormous development of herbal medicine. If we use this term as a keyword on a search engine like Pubmed, we find that almost 6,500 scientific articles are published in indexed journals, and this number becomes triple if the keyword is natural compounds. However, scientific journals often do not have a widespread circulation capable of satisfying the need for dissemination of the great wealth of research produced. In this context, this publication aims to present a path made up of specific examples, which presents the entire (or almost) reference framework on the use of medicinal plants, starting from the path that leads to the traditional use of a plant species such as Heliotropium to have a scientific validation allowing to include it among the most interesting species in the phytochemical field, due to the many traditional uses, but also for the particular phytochemical composition and the potential applications. However, the use of natural compounds is not only limited to phytotherapy. This is the case of essential oils which, depending on their preparation, but above all on the part of the plant used, have different phytocomplexes that lend themselves to different uses, from therapeutic applications (such as in aromatherapy) to food.

Nevertheless, essential oils represent a varied world, made up of many variables and characteristics, both from a phytochemical and a biological point of view. Hence, a lot of research has led essential oils to be evaluated also for more specific therapeutic purposes such as those related to cancer. The model proposed for the application of essential oils to leukemic models is of absolute interest as well as being a replicable model for other cell lines and pharmacological insights.

The individual components of essential oils also proved to be of interest. Rosmarinic acid is presented here, as a compound widely present in many plants and which, due to its phenolic nature, has an interesting spectrum of activity on a wide range of diseases.

Among the biological activities ascribable to many officinal species, the antimicrobial one has particular importance, not only for the validity certified by the wide traditional use but also by numerous studies that have recently led to the characterization of both single active ingredients and phytocomplexes, in which the synergies of action are particularly interesting.

Of course, the study of single compounds (isolated or identified in phytocomplexes) requires important technological insights. An application example is presented here, to demonstrate how new technologies, such as the combination of liquid chromatography (LC) with high resolution mass spectrometry (HR-MS), allow to characterize the important terpenic compounds of the genus *Astragalus*.

The study of the chemical and chemical-physical characteristics of natural compounds requires rapid, effective and, possibly, inexpensive experimental models. This publication presents the nematode *Caenorhabditis elegans* model, useful for the evaluation of the antioxidant and neuroprotective activity of compounds and extracts of plant origin.

Finally, a reference to the bioactive compounds present in foods could not be missing. Nutrition is closely related to maintaining good health, thanks to the presence of active ingredients in many foods. Here, olive oil was considered, as it is the subject of numerous studies focusing on the phytochemical composition (mainly fatty acids and polyphenols) and clinical trials assessing its health benefits. The focus on hydroxytyrosol (HTyr) is representative of one of the most relevant compounds with phytotherapeutic activity.

Overall, therefore, this publication stands out as a real guide towards the scientific validation of the use of plant species, compounds and phytocomplexes in phytotherapy.

Giancarlo Statti
Professor of Pharmaceutical Biology
Department of Pharmacy
Health and Nutritional Sciences
University of Calabria
via Pietro Bucci - 87036 Rende (CS)
Italy

PREFACE

Plants provide food for a large part of the human population and are an important source of carbohydrates, oils and fats, proteins; in addition, wood and fibers and their derivates obtained by human technological findings are widely used as basic components of our life.

The plant uses come from the past. Since ancient times, humans were able to colonize continents and live in regions having different environmental conditions in which different species of plants were present. Humans learned to use them as foods, tools to survive in the environment and treatment for ailments and health problems with the consequence that different cultures of interaction and use of plants developed and contributed to established practices of traditional medicine. The pharmaceutical properties of plants interested scientists and the progress of science occurred over time, with the consequence that a large number of molecules were identified and characterized. Such molecules, known also as Natural Compounds, were named Secondary Metabolites, mainly present in plant cells and tissues, and playing fundamental roles in plant reproduction and defense from predators and competitors in the environment. With the improvement of science and technology, a large number of plant derived pharmaceuticals have been developed and are presently in the market. Nowadays, a detailed investigation of biodiversity and a careful evaluation and validation of the practices of traditional medicine are associated with an intense research activity in progress in many countries for discovering new plant bioactive molecules potentially useful for new pharmaceuticals for humans, domesticated animals and agriculture.

The study of Natural Substances is an exciting way to learn biology and investigate bioactive molecules for possible applications in different fields. This book, the first of a series of three books, provides a collection of chapters on different plant molecules both in terms of their biological functions in the plant body and applications, especially at the pharmaceutical level, and aims to stimulate further interest in the search for new findings from plants. The chapters are written by leading scientists in their respective fields of interest and provide an up-to date review and future perspectives on each topic. Throughout this book and the other books which will consist of the series, students, teachers, health care practitioners and researchers interested in the study of secondary metabolites will get a useful background for their respective interests.

We wish to thank our colleagues from different universities and countries who encouraged us in the organization of this book and Prof. Giancarlo Statti for the foreword. A special thank also to the Bentham staff, who supported and accompanied us along with the development of the book.

Antonio Tiezzi
University of Tuscia, Viterbo, Italy

Elisa Ovidi
University of Tuscia, Viterbo, Italy

Tomasz M. Karpiński
University of Poznan, Poznan, Poland

List of Contributors

Antonio Tiezzi	Department for the Innovation in Biological, Agrofood and Forestal Systems, University of Tuscia, Viterbo, Italy
César Donoso-Fierro	Laboratorio de Química de Productos Naturales, Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepción, Chile
Claudia Pérez	Laboratorio de Química de Productos Naturales, Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepción, Chile Universidad de Concepción, Unidad de Desarrollo Tecnológico, UDT
Claudio Rojas-Leyton	Laboratorio de Química de Productos Naturales, Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepción, Chile
Diana De Santis	Department for the Innovation in Biological, Agrofood and Forestal Systems, University of Tuscia, 01100 Viterbo, Italy
Dilfuza Egamberdieva	Leibniz Centre for Agricultural Landscape Research (ZALF), Germany Faculty of Biology, National University of Uzbekistan, Tashkent, Uzbekistan
Dilfuza Jabborova	Leibniz Centre for Agricultural Landscape Research (ZALF), Germany Institute of Genetics and Plant Experimental Biology, Academy of Sciences of Uzbekistan, Uzbekistan
Elisa Ovidi	Department for the Innovation in Biological, Agrofood and Forestal Systems, University of Tuscia, Viterbo, Italy
Fabio Mastrogiovanni	Department for the Innovation in Biological, Agrofood and Forestal Systems, University of Tuscia, Viterbo, Italy
Giovanni Turchetti	Department for the Innovation in Biological, Agrofood and Forestal Systems, University of Tuscia, Viterbo, Italy
Hicham Harnafi	Laboratory of Bioresources, Biotechnologies, Ethnopharmacology and Health, Faculty of Sciences, University Mohamed I, 60 000, Oujda, Morocco
Hidayat Hussain	Department of Bioorganic Chemistry, Leibniz Institute of Plant Biochemistry, Halle (Saale), Germany
Ilham Touiss	Laboratory of Bioresources, Biotechnologies, Ethnopharmacology and Health, Faculty of Sciences, University Mohamed I, 60 000, Oujda, Morocco
José Becerra	Laboratorio de Química de Productos Naturales, Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepción, Chile Universidad de Concepción, Unidad de Desarrollo Tecnológico, UDT, Biobío 4191996, Chile
Mahmoud A. Elfaky	Department of Natural Products and Alternative Medicine, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, KSA
Mariana Roxo	Institute of Pharmacy and Molecular Biotechnology, Heidelberg University, Heidelberg, Germany

Michael Wink	Institute of Pharmacy and Molecular Biotechnology, Heidelberg University, Heidelberg, Germany
Mohamed L. Ashour	Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo 11566, Egypt
Nilufar Z. Mamadalieva	Institute of the Chemistry of Plant Substances of the Academy Sciences of Uzbekistan, Tashkent, Uzbekistan Department of Bioorganic Chemistry, Leibniz Institute of Plant Biochemistry, Halle (Saale), Germany
Roberta Bernini	Department of Agriculture and Forest Sciences, University of Tuscia, Viterbo, Italy
Stefania Garzoli	Department of Drug Chemistry and Technology, Sapienza University, Rome, Italy
Tomasz M. Karpiński	Department of Medical Microbiology, University of Poznan, Poznan, Poland
Valentina Laghezza Masci	Department for the Innovation in Biological, Agrofood and Forestal Systems, University of Tuscia, Viterbo, Italy

CHAPTER 1

Heliotropium, an Ethnomedicinal Plant: Past and Present Uses

César Donoso-Fierro[1], **José Becerra**[1,2], **Claudio Rojas-Leyton**[1] and **Claudia Pérez**[1,2,*]

[1] *Laboratorio de Química de Productos Naturales, Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepción, Chile*

[2] *Universidad de Concepción, Unidad de Desarrollo Tecnológico, UDT, Biobío 4191996, Chile*

Abstract: The genus *Heliotropium* is formed of herbaceous plants belonging to the family Boraginaceae. In Chile and around the world, many *Heliotropium* species are commonly used in traditional and complementary medicine to treat various diseases. Members of this genus are also recognized for unique biosynthesized phytochemicals, mainly terpenoids, phenolics and alkaloids. Due to important phyto-constituents, as well as their therapeutic potential, many *Heliotropium* species have been subjected to chemical, biological and pharmacological investigations. This review details the many ethnomedicinal uses for *Heliotropium*, with an emphasis on Chilean species, and analyzes their scientific validation based on the chemical constituents and pharmacological properties of *Heliotropium* reported in academic publications. In addition, we discuss the critical conclusions, as well as some suggestions for future phytochemical and biological studies with *Heliotropium* species.

Keywords: Boraginaceae, Ethnomedicine, *Heliotropium*, Pyrrolizidine alkaloids.

INTRODUCTION

Heliotropes are herbaceous plants that belong to the Boraginaceae family, and the subfamily Heliotropiaceae. Its name is derived from the Greek “helios-”, sun and “-tropium”, turning; thus, the name heliotrope evokes the ancient belief that inflorescence is oriented toward the sun [1].

The genus *Heliotropium* is made up of about 300 spp [2, 3]. Some heliotropes are popular garden plants and others are considered weeds [4]. They can be found in tropical, subtropical and temperate zones across all the continents [2, 5]. Neotropical representatives cover only 50 to 60 species. Members of both

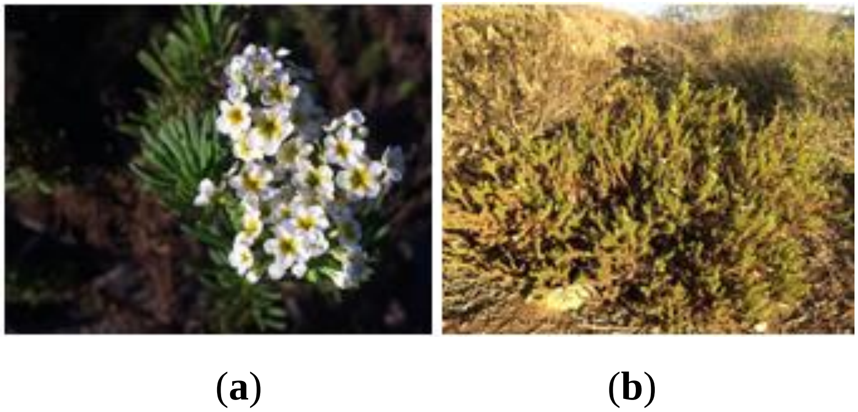
---

[*] **Corresponding author Claudia Pérez:** Laboratorio de Química de Productos Naturales, Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepción, Chile; E-mail: claudiaperez@udec.cl

Antonio Tiezzi, Elisa Ovidi and Tomasz M. Karpiński (Eds.)

sections, *i.e. Heliotropium* sect. *Heliothamnus* I.M. Johnst. (Andean, approximately 11 spp.) and H. sect. Cochranea (Miers) Kuntze (Atacama Desert, 17 spp.) are long-lived shrubs between 0.4-4.0 meters tall. The roughly 25 remaining species are quite divergent in their vegetative morphology. These species include small annual sub-bushes with succulent leaves (*H. amplexicaule* Vahl) (*e.g.*, *H. paronychioides* A. DC.), halophytic, clump-forming plants with massive roots (*H. curassa- vicum* L.), perennial herbs with root-tuber (*H. microstachyum*, Ruiz & Pav.), and decumbent perennial grasses with extensive shoots, abundant rooting and creeping (*H. veronicifolium*, Griseb.) [2].

The genus *Heliotropium* L. Sect. *Cochranea* (Miers) Kuntze is composed of four main lineages, three of which have their center of diversity in South America. According to its geographic distribution, it has been suggested that its origin could be related to the Andean uprising and the formation of arid environments in South America [6]. In Chile, *H. stenophyllum* (Hook & Arn) is distributed from the Atacama Region to the Metropolitan Region of Santiago, inhabiting arid to semi-arid zones, from the coastal zone to the interior valleys. It is a very bushy shrub, with rigid dark green branches and linear leaves covered with hair on their underside, alternates, sessile, and amphistomatic. Flowers are small (5 mm long), and disposed in terminal scorpiod monchasia (boragoids [7]). Calyx lobes are linear lanceolate, partially fused. The corolla is infundibuliform, exceeding the calyx, white with a yellow throat. A protuberant nectar disk at the base surrounds the ovary. The style is elongated. The gynoecium is glabrous. The stigma is elongated into a conical structure with a basal receptive area, which is typical of *Heliotropiaceae*. It can reach up to two meters in height (Fig. **1**).



(a) **(b)**

Fig. (1). *Helitropium stenophyllum* (Palito negro) (**a**) Flowers, (**b**) shrub.

There are varied ethnomedicinal uses for the different *Heliotropium* species. In general, they are used to treat skin diseases, poisonous bites, venereal diseases [8], renal, hepatic, and osteoarticular disorders [9], as antimalarial agents [10, 11], and for treating cancer [12]. Certain species are characterized by living in arid zones and being exposed to extreme environmental factors. For these reasons, they produce resinous exudates that cover the leaves and stem, secreted by special glands called trichomes. The resin is principally composed of terpenoids, lipids, waxes and flavonoids, which act as a protective barrier against high temperatures, UV radiation, water scarcity, herbivores and pathogens [4, 13]. Previous studies have attributed this resistance to the antioxidant, antimicrobial, antiviral and antifungal properties of the components of this resin [13], which are rich in secondary metabolites, with potential therapeutic uses due to their relevant biological activity. In this context, anticarcinogenic activity has been registered in compounds extracted from diverse plants around the world [12, 14 - 16]. For example, *H. indicum* is rich in pyrrolizidine alkaloids (PAs) [4], which are of great pharmacological, biological and chemotaxonomic interest, and have been isolated from a wide variety of plants, especially from the Boraginaceae family [17]. Within this group of anticarcinogenic alkaloids, the most important is *N*-oxide [18], as well as acetylindicine, indicinine, heleurine, heliotrine, supinine, supinidine and lindelofidine. This review examines the different ethnomedicinal uses of the genus, with an emphasis on Chilean species, and analyzes the scientific validation of said uses based on its chemical constituents and pharmacological properties as reported in academic studies.

Ethnomedicinal use of *Heliotropium* Species

Throughout human history, plants have played an important role in the health traditions of essentially every culture around the world, featuring in a wide variety of treatments, many of which may be incomprehensible by modern Western societies. However, these healthcare alternatives can be as valid today as they were 5,000 years ago [19]. In this regard, the World Health Organization (WHO) has estimated that more than 80% of the global population depends on medicinal plants to meet their primary healthcare needs, and that much of Traditional, Complementary and Alternative Medicine (T/CAM) involves the use of plant extracts or their active ingredients. As such, medicinal plants are a valuable natural resource contributing to disease treatment and prevention through T/CAM, especially in developing countries (up to 80% in African countries, 71% in Chile, and 40% in Colombia and China) [20 - 22].

It is estimated that there are around 298,000 species of plants on the planet, of which 215,644 have been cataloged and 20% probably have medicinal potential that is important for human health [23]. Additionally, it is estimated that close to

CHAPTER 2

Essential Oils and their Chemical Constituents: The Potential Role in the Leukemic Diseases

Valentina Laghezza Masci[1], **Elisa Ovidi**[1,*] and **Stefania Garzoli**[2]

[1] *Department for the Innovation in Biological, Agrofood and Forestal Systems, University of Tuscia, Viterbo, Italy*

[2] *Department of Drug Chemistry and Technology, Sapienza University, Rome, Italy*

Abstract: Essential oils (EOs), or the volatile fraction produced by aromatic plants, possess a large number of biological activities. In the last few years, their possible use in anticancer therapy has been under investigation. The anticancer potential of EOs against leukemic diseases and their chemical composition have been reported in this chapter, highlighting the *in vitro* studies against different cell models with their cytotoxicity and the corresponding mechanism of action.

Keywords: Apoptosis induction, Chemical composition, Cytotoxicity, Essential oils, Leukemia cells, Leukemia diseases, Leukemia treatments.

INTRODUCTION

Chemical Composition of the EOs

The Essential Oils (EOs) are volatile and aromatic liquids obtained from plants. They can contain thousands of components whose concentrations can widely vary within the same species originating from different locations or regions [1]. The EOs consist mainly of monoterpenes and sesquiterpenes (hydrocarbon and oxygenated) and other functionalized derivatives of alcohols, ketones, aldehydes, esters, phenols and other substances including coumarins, anthraquinones and alkaloids [2]. Their chemical composition is influenced by multiple factors, such as the origin of the plant, its development stage, the organ of plant and the method of extraction.

In this regard, different techniques can be applied to obtain the EOs, such as steam distillation, solvent extraction, supercritical fluid (CO_2) extraction, micro-

---

[*] **Corresponding author Elisa Ovidi:** Department for the Innovation in Biological, Agrofood and Forestal Systems (DIBAF), University of Tuscia, 01100 Viterbo, Italy; E-mail: eovidi@unitus.it

Antonio Tiezzi, Elisa Ovidi and Tomasz M. Karpiński (Eds.)

wave assisted and ultrasound extraction, or mechanical methods, such as cold pressing.

EOs are widely used in the pharmaceutical and cosmetic fields as well as in food preservation and aromatherapy [3]. EOs production and trade are not regulated and their quality can be affected by the use of pesticides, and additives and by packaging, storage and handling. Therefore, an evaluation or control of the chemical composition is and essential before their use. Furthermore, preliminary research investigating the potential therapeutic effects of specific EOs is ongoing [4 - 6], and Table **1** shows some different chemical compositions of a selection of EOs which are active against leukemia diseases.

Table 1. Chemical composition of EOs.

Plant name from which EOs were derived	Family of plants	Origin	Main components identified	Ref
Croton regelianus	Euphorbiaceae	Brazil	p-cymene (27.97–18.97%), ascaridole (27.72 –14.51%), camphor (12.74–4.83%), α-terpinene (10.14–4.90%)	[7]
		Ceará State (Brazil)	ascaridole (33.9–17.0%), p-cymene (22.3–21.6%), camphor (13.0–3.1%) in Viçosa and Acarape sites respectively	[8]
Achillea wilhelmsii	Asteraceae	Iran	ρ-cymene (23.35%), 1,8-cineole (20.83%), dihydrocarvone (19.13%), camphor (6.67%), verbanol acetate (3.53%).	[9]
		Turkey	1,8-cineole (15.43%), o-cymene (19.56%), chrysanthenone (0.88%), camphor (6.36%), borneol (1.09%), carvenone oxide (1.84%), α-terpinene (19.30%) piperitone (26.93%).	[10]
		Iran	chrysanthenone (38.8%), trans-carveol (27.5%), linalool (26.1%), neoiso-dihydrocarveol acetate (25.2%), camphor (19.9%), filifolone (19.7%), 1,8-cineole (16.7%), borneol (13.6%), α-pinene (11.8), trans-piperitol (11.7%), (E)-caryophyllene (11.2%), (E)-nerolidol (10.8%), and lavandulyl acetate (10.0%).	[11]

(Table 1) cont.....

Plant name from which EOs were derived	Family of plants	Origin	Main components identified	Ref
Piper cernuum	Piperacea	Brazil	β-elemene (30.0%), bicyclogermacrene (19.9%), (E)-caryophyllene (16.3%), germacrene D (12.7%)	[12]
		Santa Catarina, Brazil	trans-dihydroagarofuran (30 to 36.7%), 4-epi-c-s-dihydroagarofuran (11.2 to 13.4%), γ-eudesmol (7.64 to 11.65%), β-caryophyllene (5.94 to 8.69%), elemol (5.89 to 9.15%), α-pinene (2.63 to 5.43%) and camphene (2.16 to 455%) in Winter, Spring, Summer and Autumn Season	[13]
		São Paulo city, Brazil	bicyclogermacrene (21.88%), β-caryophyllene (20.69%), α-pinene (7.16%) β-pinene (6.15%)	[14]
Porcelia macrocarpa	Annonaceae	Brazil	Leaves: germacrene D (47.0%), bicyclogermacrene (37.0%), α-copaene (2.2%)	[15]
		São Paulo, Brazil	germacrene D (29%–50%) bicyclogermacrene (24%–37%) (from January to December 2011)	[16]
		Jardim Botânico of São Paulo State	germacrene D (37.8%), bicyclogermacrene (27.5%), sptahulenol (3.0%)	[17]
Guatteria blepharophylla	Annonaceae	Brazilian Amazon	caryophyllene (55.7%), spathulenol (8.9%), palustrol (6.5%), elemicin (4.6%).	[18]
		Brazilian Amazon	caryophyllene oxide (70.0%), 14-hydroxy-9-epi-(E)-caryophyllene (2.5%), Ishwarone (2.0%)	[19]
Guatteria hispida	Annonaceae	Brazilian Amazon	α-pinene (31.0%), β-pinene (36.0%), (E)-Caryophyllene (21.0%), Caryophyllene oxide (3.0%),	[19]

CHAPTER 3

Insights into the Recent Application of Rosmarinic Acid in Therapy

Ilham Touiss[1,*], **Mahmoud A. Elfaky**[2], **Mohamed L. Ashour**[3] and **Hicham Harnafi**[1]

[1] *Laboratory of Bioresources, Biotechnologies, Ethnopharmacology and Health, Faculty of Sciences, University Mohamed I, 60 000, Oujda, Morocco*

[2] *Department of Natural Products and Alternative Medicine, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, KSA*

[3] *Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo11566, Egypt*

Abstract: Herbs are key players in many traditional health care systems that have been used in medical practices since ancient times. The beneficial therapeutic effects of these medicinal plants resulted from the combinations of their secondary metabolite. Nowadays, the use of natural compounds is increasing around the world due to their relatively mild potentials and low side effects, Polyphenols are the most important compounds that exhibit diverse activities. Among these polyphenols, rosmarinic acid (RA) attracted much attention from the researchers since it was isolated as the main compound in many plants, like those of the Boraginaceae and Lamiaceae families such as *Rosmarinus officinalis* (rosemary) and *Ocimum baslicum* (sweet basil). It is an ester of caffeic acid and 3,4-dihydroxy phenyl lactic acid, which has a wide spectrum of biological, pharmacological and medicinal properties that can be useful in many pathological conditions. Moreover, it presents anti-inflammatory effects, which are attributed to the inhibition of lipoxygenase and cyclooxygenases and interference with the complement cascade. Furthermore, RA has been shown to prevent cell damage caused by free radicals, thereby reducing the risk of cancer. On the other hand, it also exerts powerful hypolipidemic, antioxidant, anti-atherosclerotic, anticancer and even hepato-protective activities. The current chapter aims to highlight the therapeutic potential of RA against a wide range of diseases. Given the current evidence, rosmarinic acid can be used as part of the daily intake in the treatment of several diseases, with predefined doses preventing cytotoxicity.

Keywords: Antioxidant, Anti-inflammatory, Anti-cancer, Antimicrobial, Cardioprotective, Hepatoprotective, Neuroprotective, Oxidative stress, Rosemary, Rosmarinic acid.

---

[*] **Corresponding author Ilham Touiss:** Laboratory of Bioresources, Biotechnologies, Ethnopharmacology and Health, Faculty of Sciences, University Mohamed I, 60 000, Oujda, Morocco; E-mail touissilham@gmail.com

Antonio Tiezzi, Elisa Ovidi and Tomasz M. Karpiński (Eds.)

INTRODUCTION

Herbal medicine is considered one of the subgroups of complementary and alternative medicinal therapies and their natural products have a distinct role in the treatment of several diseases. They are not only limited to dietary uses, such as food and nutrition [1]. Actually, since antiquity, natural compounds have been used as traditional medicines. In fact, they have made an interesting contribution to pharmacotherapy. Nevertheless, these secondary metabolites present challenges for drug discovery. The dominant source of knowledge on the use of natural products from medicinal plants is the result of experiments carried out by man by trial and error for hundreds of centuries, through palatability tests or premature death, in search of food available for the treatment of diseases [2]. Moreover, the long usage of these natural products is explained by the knowledge passed from generation to generation.

Most of the early medicines were formed through traditional medicinal practices followed by subsequent clinical, pharmacological and chemical studies. The anti-inflammatory agent Aspirin (acetylsalicyclic acid) derived from the natural product, salicin isolated from the bark of the willow tree *Salix alba* L. was the most famous and well known example to date [3].

Shikimic acid is the key intermediate in the biosynthesis of the aromatic amino acids, L-phenylalanine, L-tyrosine and L-tryptophan, in plants [4]. These three aromatic amino acids are individually important precursors for various secondary metabolites such as rosmarinic acid (Fig. **1**).

Rosmarinic acid (RA) is a water-soluble phenolic compound, which is also known as labiatenic acid composed of an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid. For the first time, rosmarinic acid was isolated and characterized by Scarpati and Oriente [5], two Italian chemists, as a veritable agent and named as in conformity with the herb it was derived from, *Rosmarinus officinalis*. Some of the species from which this compound has been reported include sage (*Salvia officinalis*), peppermint (*Mentha piperita*), thyme (*Thymus vulgaris*), marjoram (*Origanum vulgare*), Sweet basil (*Ocimum basilicum*) and Prunella (*Prunella vulgaris*). Recently, it has been found in gaenari (*Forsythia koreana*), kadoké (*Hyptis pectinata),* Holy basil or Tulsi *(Ocimum tenuiflorum*), garden thyme (*Thymus mastichina*) [6, 7].

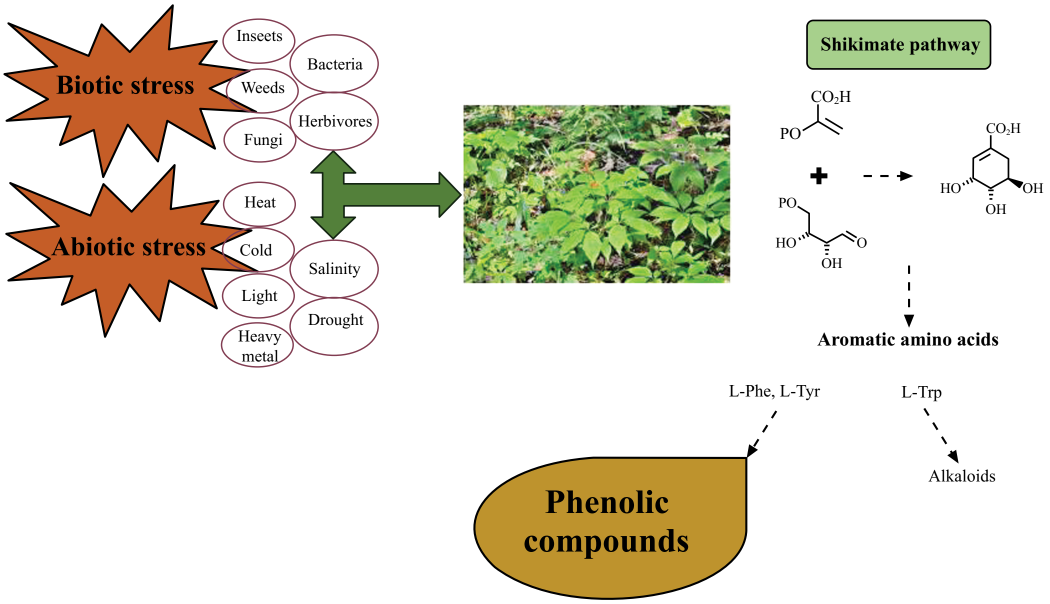


Fig. (1). Phenolic compounds promoted by biotic and abiotic stresses.

Chemical Structure

Rosmarinic acid is formally known as (2R)-3-(3,4-dihydroxyphenyl)-2-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxypropanoic acid and its molecular formula is $C_{18}H_{16}O_8$ (Fig. **2**). Structurally RA possesses two phenolic rings, with two vicinal OH groups in each aromatic ring. The main active groups of RA are the two phenolic hydroxyls in the rings A and B, in contrast with other flavonoids in which the main active position is in the ring B. Like other phenolic compounds, rosmarinic acid easily donates a hydrogen atom from an aromatic OH group to a free radical, because it is able to stabilize an unpaired electron through its delocalization. RA may act as a strong chelating agent. As chelating ability is an important property because it brings about the reduction of the concentration of transition metal that catalyzes lipid peroxidation. It is slightly soluble in water, but well-soluble in most organic solvents [8, 9].

CHAPTER 4

Plant-Derived Antimicrobial Compounds

Dilfuza Egamberdieva[1,2,*] and **Dilfuza Jabborova**[3]

[1] *Leibniz Centre for Agricultural Landscape Research, Müncheberg, Germany*

[2] *Faculty of Biology, National University of Uzbekistan, Tashkent, Uzbekistan*

[3] *Institute of Genetics and Plant Experimental Biology, Academy of Sciences of Uzbekistan, Uzbekistan*

Abstract: The medicinal plants are widely used to treat diverse ailments and diseases, including gastro-intestinal symptoms, cardiovascular diseases, skin disorders, respiratory and infectious diseases, accelerating in recent years. Plants are rich in a wide variety of secondary metabolites, such as alkaloids, tannins, flavonoids, and phenolic compounds, found *in vitro* to have antimicrobial properties and could assist in the discovery of novel drugs. The search for novel antimicrobial compounds, especially against multidrug-resistant pathogens from aromatic and herbal plants, is an essential line of scientific research. This review attempts to summarize the *in vivo* studies of the antimicrobial activity of medicinal plants and their biologically active compounds. The chemical composition of biologically active compounds with antimicrobial is also addressed.

Keywords: Antimicrobials, Herbal plants, Secondary metabolites.

INTRODUCTION

The cultivation of medicinal and aromatic plants to meet the great demand for biologically active compounds used by food, pharmaceutical industries, and health care is increasing worldwide. Plant-based medicines have been practiced in many countries and continents for treating various human diseases by traditional knowledge worldwide [1 - 3]. The remedies within natural resources, including forests, mountains, grassland, and desert plants widely used to treat diverse ailments and diseases, including gastro-intestinal symptoms, cardiovascular diseases, skin disorders, respiratory and urinary problems as an age-old tradition [4 - 6]. Medicinal plants contain a wide range of biologically active compounds, such as antimicrobials, essential oils, alkaloids, phenolic compounds, that are traditionally used as remedies for the treatment of various diseases [7 - 9].

---

[*] **Corresponding author Dilfuza Egamberdieva:** Leibniz Centre for Agricultural Landscape Research (ZALF), Müncheberg, Germany; E-mail: egamberdieva@yahoo.com

Antonio Tiezzi, Elisa Ovidi and Tomasz M. Karpiński (Eds.)

The problem with multidrug resistant bacterial pathogens (*e.g. Acinetobacter baumanni*, *Enterococcus faecium*, *Klebsiella pneumonia, Pseudomonas aeruginosa,* and *Staphylococcus aureus*), in many countries, is that they are considered as one of the crucial health threat, and new antimicrobials are needed to overcome it [10 - 12].

Thus, discovering novel biologically active compounds with antimicrobial activity from aromatic and herbal plants, which are promising sources of natural products, is an important strategy to maintain public health [13 - 16]. Many medicinal plants contain antimicrobial compounds against human pathogenic bacteria that are used for treating infectious diseases in traditional medicine [17 - 19]. Several studies reported antimicrobial activity of plant extracts against human pathogenic bacteria *Staphylococcus aureus e.g. Zingiber officinales* and *Thymus kotschyana* [20], *Hypericum perforatum* [3], *Allium sativum* [21], *Boerhaavia diffusa*, and *Soymida febrifuga* [22]. The biologically active compounds such as essential oils, flavonoids, alkaloids, tannins, phenolic compounds, saponins, and other compounds exhibited antimicrobial activity again a wide range of pathogenic bacteria [23].

Medicinal Plants with Antimicrobial Activity

Plants were used in traditional medicinal practices to treat infectious diseases [5, 24]. Several reports confirmed the antibacterial activity of ethanolic and methanolic extracts of medicinal plants against a wide range of pathogenic bacteria. The fruit extract inhibited *Staphylococcus aureus* and *Salmonella typhi* [25], *Enterococcus faecalis*, *P. aeruginosa*, *Salmonella typhi*, and *Staphylococcus epidermidis* [26]. According to Azizov *et al.* [27] *Arctium lappa* showed antimicrobial activity against many human pathogenic bacterial species. The plant extract of *H. perforatum* exhibited antibacterial activity against *P. aeruginosa, E. coli, E. faecalis, K. oxytoca, K. pneumoniae,* and *S. aureus* [3]. Plant extract of *Anethum graveolens* showed antimicrobial activity against *Candida albicans* and *Staphylococcus aureus* [28]. In contrast, *Ziziphora* species is rich in essential oils and exhibited antimicrobial activity [29]. Ishaq *et al.* [30] demonstrated antimicrobial activity of *Adiantum capillus-veneris* against *Escherichia coli, Staphylococcus aureus*, and *Klebsiella pneumonia*.

Nigussie *et al.* [31] recently observed the inhibitory activity of plant extract of *L. inermis* and *A. indica* leaves against *Streptococcus pyogenes*, *E. coli* isolate, and *S. aureus*. Agoramoorthy *et al.* [32] reported antimicrobial activity of fatty acids extracted from *Excoecaria agallocha* against various human pathogenic bacteria and fungi. Houngbèmè *et al.* [33] investigated the antimicrobial activity of *Ocimum gratissimum* L., *Acanthospermum hispidum* DC, *Caesalpinia bonduc* (L)

Roxb and *Calotropis procera* W. T. Aiton. The alcoholic extracts of plants showed antimicrobial properties against *Escherichia coli, Staphylococcus aureus*, *Salmonella typhi, Klebsiella pneumonia, Candida albicans*, and *Mycobacterium bovis*. The medicinal plants were also reported for their antiviral activity, *e.g.* alstotides from *Alstonia scholaris* plant are antiviral and cell-permeable to inhibit the early phase of infectious bronchitis virus and Dengue infection [34]. The oilseed crop plant *Ricinus communis* is used to treat rhinitis, bronchitis, dental caries, skin diseases, and infections in the digestive apparatus. The multiplatform-based metabolite profiling approach confirmed alkaloids, fatty acids, terpenes, phenolic compounds, steroids, and carotenoid derivatives in *R. communis* extracts which are some of the compounds with antimicrobial activity [35]. The medicinal plant from Africa, which is widely used to treat skin disease demonstrated antimicrobial activity, *e.g. Elephantorrhiza elephantine* against *Propioni bacterium acnes* (MIC - 0.05 mg/ml), *Diospyros mespiliformis* against *Trichophyton mentagrophytes* (MIC - 0.10 mg/ml), and *Microsporum canis* (MIC 0.50 mg/ml) [36].

Antimicrobial Compounds from Medicinal Plants

Plants with antimicrobial activity contain a range of lead compounds, enabling the development of novel antimicrobial agents as antimicrobial drugs [37]. Antibacterial properties of biologically active compounds isolated from *Hypericum perforatum* were reported by Dall'Agnol *et al.* [38]. *Prangos pabularia* contain coumarins, terpenoids, and glycosides, that exhibited antimicrobial activity [39]. In another study, diterpene and methyl carnosate isolated from the *Salvia officinalis* leaves showed antimicrobial properties against *Bacillus cereus* [40]. The methanol extract of *L. inermis* leaves had activity against *S. aureus, E. coli*, and *Streptococcus pyogenes* [41, 42]. The leaves, stems, and flowers of *Congea tomentosa* Roxb. showed antimicrobial activity against widely known human pathogenic bacteria and the identified biological active compound stigmasterol was found responsible for inhibiting bacterial growth [43]. The biologically active compound plumbagin isolated from *Aristea ecklonii* resulted in antimicrobial activity against human pathogenic bacteria *Trichophyton mentagrophytes* (MIC - 0.10 mg/ml) and *Microsporum canis* (MIC - 2.00 µg/ml and 16.00 µg/ml) [36]. Moreover, flavanone, stigmasterol, and quercetin compounds were isolated from *Acanthospermum hispidum.* The extract of *Caesalpinia bonduc* demonstrated antimicrobial activity against *Candida albicans* [33].

Essential Oils

In many studies, essential oils of medicinal plants demonstrated antimicrobial

CHAPTER 5

LC-HR-MS Based Approach to Identify Triterpenes in *Astragalus* Species

Nilufar Z. Mamadalieva[1,2,*] and **Hidayat Hussain**[2]

[1] *Institute of the Chemistry of Plant Substances, Academy Sciences of Uzbekistan, Tashkent, Uzbekistan*

[2] *Department of Bioorganic Chemistry, Leibniz Institute of Plant Biochemistry, Halle (Saale), Germany*

Abstract: *Astragalus* genus has been recorded as yielding a wide range of flavonoids, triterpenes, triterpene saponins, and polysaccharides. Cycloartane- and oleanane-type triterpenes are widely distributed in this genus with intriguing biological effects. Combining liquid chromatography (LC) with high-resolution mass spectrometry (HR-MS) has provided a valuable tool in the analysis of these triterpenes in plants. In this chapter, we summarized the main triterpenes studied by liquid chromatography high-resolution mass spectrometry (LC-HR-MS) associated with mass analysers, molecular weight information and mass fragmentation, presenting an interesting overview of increasing interest for cycloartane- and oleanane-type triterpenes.

Keywords: *Astragalus*, Biological activity, Chromatography, Fragmentation, Triterpenes, Orbitrap, LC-HR-MS, QTOF-MS.

INTRODUCTION

Astragalus is with about 2500–3000 species in 250 sections the largest genus of flowering plants. It belongs to the family of legumes (Leguminosae or Fabaceae), the third largest family of flowering plants, consisting of about 730 genera and approximately 19300 species after Orchidaceae and Asteraceae [1]. As annual or perennial herbs, subshrubs, or shrubs, the plants of *Astragalus* L. are widely distributed throughout the temperate and arid regions of the world, located principally in Europe, Asia, and North America [2]. Moreover, this genus is well known for its worldwide taxonomic problems and is under constant revision, particularly identification at the section level. Sections with primitive characters mostly contain mesophytic species widespread in mountains and relatively humid

---

[*] **Corresponding author Nilufar Z. Mamadalieva:** Institute of the Chemistry of Plant Substances, Tashkent 100170, M. Ulugbek Str 77, Uzbekistan; Tel: +998900237580; E-mail: nmamadalieva@yahoo.com

Antonio Tiezzi, Elisa Ovidi and Tomasz M. Karpiński (Eds.)

regions; in the more advanced sections, the species are adapted to dry or arid conditions [3]. *Astragalus* species are widely used as medicine, food, fodder, fuel, and ornamental plants in different ethnobotanical practices throughout the world. The most used part of *Astragalus* taxa is the gum tragacanth and Iran is the primary source of it (by supplying 70% of the commercially used gum tragacanth) in the world. Nowadays, several species of *Astragalus*, are reported to be commercially exploited for gum tragacanth [4].

Some species of this genus, such as *A. brachycalyx* (syn. *A. adscendens*), *A. fasciculifolius, A. glycyphyllos, A. gossypinus, A. gummifer, A. hamosus, A. microcephalus, A. mongholicus* and *A. tribuloides* are the most popular medicinal plants. Especially, in Traditional Chinese Medicine, Astragali Radix, the root of *A. membranaceus* and/or *A. mongholicus*, considers use in kidney and urinary problems, digestion, liver problems, female reproductive system problems, muscular, skin problems, cardiovascular and blood, immune and lymphatic system, nervous system, respiratory system, and for some specific disease. It helps protect the body against various types of stress such as physical and emotional stress. *Astragalus* root illustrated anti-aging properties and also helps to prevent bone loss [5].

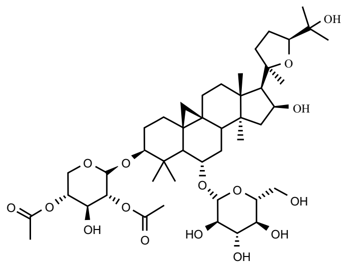
Astragalus genus has been recorded as yielding a wide range of flavonoids, triterpenes and polysaccharides. More than 300 compounds have been isolated from this genus [6]. Especially, cycloartane- and oleanane-type saponins are widely distributed in genus *Astragalus*. The interesting compounds, such as terpenoids and flavonoids are always in free or glycosidic forms. In addition, anthraquinones, alkaloids, amino acids, sterols, and metallic elements were also found in this genus [7].

Mass spectrometry has been used during the last decade to an increasing extent for the structure elucidation of cycloartane and oleanane- type glycosides. Structural characterization of saponin extracts is a challenging task because of the presence of numerous saponin molecules, with only subtle structural differences, in a given saponin extract making their characterization difficult. Today, saponin characterization takes more and more advantage of the capabilities of mass spectrometry-based methods and LC-MS experiments are very efficient for saponin mixture characterization, often using accurate mass HR-MS (high-resolution mass spectrometry) measurements and tandem mass spectrometry experiments (collision-induced dissociation - CID) [8].

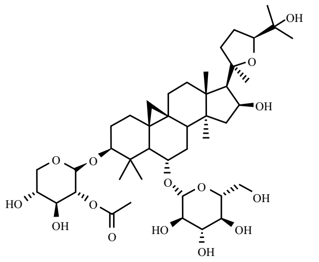
In this chapter, we summarized the main triterpenes studied by liquid chromatography high-resolution mass spectrometry (LC-HR-MS) associated with mass analysers, molecular weight information and mass fragmentation, presenting an overview of increasing interest in cycloartane- and oleanane-type triterpenes study by LC-HR-MS.

Astragalus Genus as a Source of Cycloartane- and Oleanane-Type Saponins

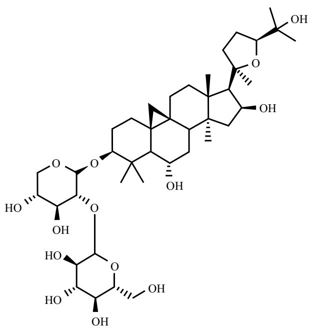
Many studies about *Astragalus* genus and its chemical constituents have been performed worldwide because of their diverse activities. The phytochemical and biological studies on *Astragalus* have resulted in isolation and characterization of >400 secondary metabolites, including triterpenoid saponins and sapogenins, flavonoids, phenylpropanoids, steroids, alkaloids, and some other compounds. Among them, isoflavonoids, triterpene saponins and polysaccharides are the three main types of beneficial compounds responsible for the pharmacological activities and therapeutic efficacy of *Astragalus* (*e.g. A. membranaceus*). Triterpenoid saponins are a unique class of high molecular weight glycosides with multi-sugar attached to a 30-carbon aglycone, which are widely distributed in the plant kingdom. Triterpenoid saponins are the most widely studied secondary metabolites in *Astragalus* and >200 triterpenoid saponins and sapogenins have been identified from this genus [9]. Chemical structures of some major oleanane-type saponins isolated from *Astragalus* are shown in Fig. **1**.



Astragaloside I



Astragaloside II



Astragaloside III

CHAPTER 6

The Use of the Nematode *Caenorhabditis Elegans* to Study Antioxidant and Longevity-Promoting Plant Secondary Metabolites

Mariana Roxo[1] and **Michael Wink**[1,*]

[1] *Institute of Pharmacy and Molecular Biotechnology, Heidelberg University, Heidelberg, Germany*

Abstract: Oxidative stress is implicated in the pathophysiology of aging and age-related health conditions, such as cancer, cardiovascular disorders and neurodegenerative diseases; therefore, the supplementation of diet with plant-derived antioxidants is recognized as a potential strategy to delay or even prevent the onset of age-related diseases. Among the model organisms commonly used in the early phases of anti-aging drug discovery, the nematode *Caenorhabditis elegans* has proven useful for investigating the bioactivity of complex extracts and isolated plant secondary metabolites. In this chapter, we have reviewed recent studies on the antioxidant, longevity-promoting and neuroprotective activities of polyphenol-rich plant extracts in *C. elegans*, with particular focus on the highly conserved insulin/IGF-1 signaling pathway.

Keywords: Age-related diseases, Alzheimer's disease, Amyloid beta, Anti-aging, Antioxidant, *Caenorhabditis elegans*, DAF-16, GFP, HSF-1, Huntington's disease, IIS pathway, Neuroprotection, oxidative stress, Pplant extracts, Polyglutamine, Polyphenols, Protein aggregation, ROS, Secondary metabolites, SKN-1.

INTRODUCTION

Reactive oxygen species (ROS) are involved in essential physiological processes, including cell signaling, immune response, inflammation and cell differentiation [1]. However, if their concentration is above physiological levels, these by-products of aerobic respiration can cause damage to cellular macromolecules, in particular to proteins, DNA and lipids, leading to a state of oxidative stress in which the intracellular redox homeostasis is disrupted.

---

[*] **Corresponding author Michael Wink:** Heidelberg University, IPMB, Heidelberg, Germany; E-mail: wink@uni-heidelberg.de

Antonio Tiezzi, Elisa Ovidi and Tomasz M. Karpiński (Eds.)

Throughout the aging process, the innate antioxidant defense mechanisms that allow us to cope with oxidative stress are gradually compromised, increasing the risk of developing ROS-related health conditions, such as cancer, cardiovascular disorders and neurodegenerative diseases, collectively named age-related diseases [2]. Accordingly, in the last years, the rapid increase in human life expectancy has led to a global rise in the prevalence of such age-related diseases, raising the need for effective and easily available anti-aging interventions [3].

Numerous epidemiological and clinical studies have provided evidence for the potential of an antioxidant-rich diet in the prevention and management of age-related disorders [4 - 9]. In this context, the supplementation of a diet with plant-derived antioxidants (polyphenols, carotenoids, mustard oils, allicin, vitamins C and E) is widely accepted as a potential strategy to delay or even prevent the onset of age-related diseases as well as to improve the overall health status.

Due to their unique structural diversity and multi-target mechanism of action, phytochemicals are able to act at multiple cellular and molecular levels that go far beyond the simple scavenging of free-radicals [10]. In fact, a robust and ever-growing body of evidence has demonstrated the capacity of plant secondary metabolites to modulate highly conserved signalling pathways that regulate aging, stress resistance and protein homeostasis across species, in particular the insulin/IGF (insulin-like growth factor)-like signaling (IIS) pathway [11 - 17].

Since much of our knowledge on the IIS pathway components and regulation has come from pioneering studies using the multicellular model organism *Caenorhabditis elegans* [18], it is not surprising that the worm has been increasingly regarded as a model of choice to screen the anti-aging potential of phytochemicals and plant extracts [19 - 21].

C. ELEGANS AS A MODEL ORGANISM

C. elegans Anatomy and Life Cycle

As all nematodes, *C. elegans* is an unsegmented slender worm with a body cavity of circular cross section, consisting of an outer tube or body wall and an inner tube separated by a pseudocoelomic cavity [22]. The body wall is composed of a collagenous transparent cuticle; excretory system; four quadrants of body wall muscles longitudinally distributed along the whole body, and neuronal cell bodies located in the head, ventral cord and tail. The inner tube encloses the alimentary system (pharynx and intestines) and the reproductive system (gonads) (Fig. **1**). Vascular and circulatory systems are absent.

C. elegans has two naturally occurring sexes, males (XO) and self-fertilizing hermaphrodites (XX), which are dimorphic in cell number, anatomy, size and behavior [23]. After the reduction of the original cell number by apoptosis, the adult male contains 1031 somatic cells and the hermaphrodite 959 somatic cells. Males can be visually differentiated from hermaphrodites due to the presence of a copulatory apparatus in the tail and smaller body size. Wild-type and laboratory populations are composed of 99.99% hermaphrodites [24]; therefore, the following descriptions will focus on hermaphrodites.

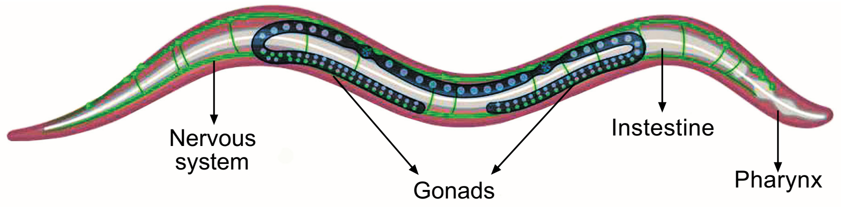


Fig. (1). Anatomy of a *C. elegans* adult hermaphrodite. Figure created with BioRender.com.

Under optimal conditions of high food availability, temperate environment and sparse population, the development of *C. elegans* from egg to adult takes approximately 3 days at 20ºC. After hatching, the worm enters 4 stages of larval development (L1 to L4) culminating in a reproductive adult of approximately 1 mm in length with a lifespan of around 2 to 3 weeks. The nematode lays around 300 eggs during the whole reproductive period of about 5 days. Upon harsh conditions, L1 worms may enter a non-growing stage called "dauer" that are capable of surviving for up to four months and, once the conditions are again favorable, posteriorly develop into adult worms.

C. elegans can be easily maintained in large numbers under laboratory conditions. Large populations can be grown under controlled incubation on Nematode Growth Media (NGM) plates inoculated with a bacteria lawn, usually of *Escherichia coli* OP50, a strain with growth limited by uracil availability (uracil auxotroph). Experiments with *C. elegans* are not considered animal experiments and do not require a special permit.

C. elegans in Aging and Antioxidant Research

One of the first evidence of the relevance of *C. elegans* to aging research was the similarity of its aging process to that of mammals. The worm also gets old and, due to its transparency, small size and short lifespan, the signs of aging can be easily observed under a dissecting microscope. Some of the aging-markers readily observed are the decreased rate and coordination of movement and the impaired organization of the internal organs.

CHAPTER 7

Olive Oil: A Natural Source of Benefits for Human Health

Fabio Mastrogiovanni[1,*], **Roberta Bernini**[2] and **Antonio Tiezzi**[1]

[1] *Department for the Innovation in Biological, Agrofood and Forest Systems, University of Tuscia, Viterbo, Italy*

[2] *Department of Agriculture and Forest Sciences, University of Tuscia, Viterbo, Italy*

Abstract: Since ancient times, olive oil has been part of human culture. It is the main source of fatty acids in the Mediterranean diet and has beneficial effects on human health. Due to its high content of monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and polyphenols, it is generally considered one of the best sources of fat. Among its health properties, olive oil can counteract LDL cholesterol, and protect against cardiovascular diseases and furthermore, its biomolecules (such as polyphenols) can positively interact with oxidative stress, carcinogenesis and arteriosclerosis. Oleuropein and hydroxytyrosol (HTyr) are characteristic phenols of olive oil. Oleuropein is hydrolyzed by endogenous β-glycosidases to produce HTyr. The beneficial effect of olive oil on oxidative stress is largely due to HTyr and its derivatives. Furthermore, HTyr exhibits antioxidant, antitumor, anti-inflammatory, cardioprotective and neuroprotective activities. The search for synthetic compounds derived from HTyr can be an interesting way to enhance their biological activities and / or to discover other properties. HTyr can be obtained from crusher wastewater, which is considered a good source of Htyr.

Keywords: Biological assets, Health benefits, Hydroxytyrosol, Hydroxytyrosol derivatives, MUFA, *Olea europaea*, Oleuropein, Olive oil, Polyphenols.

INTRODUCTION

Humans have developed the ability to adapt to many climates and habitats over the years, and the ability to obtain nourishment and meals has enabled them to build life circumstances conducive to the survival and proliferation of their offspring. In the world, many diseases affect man, and plants represent a useful tool against diseases and a fundamental resource for improving the quality of life [1]. The great variability of plant species located in different regions of the planet

---

[*] **Corresponding author Fabio Mastrogiovanni**: Department for Innovation in Biological, Agro-food and Forest Systems (DIBAF), University of Tuscia, 01100 Viterbo, Italy; E-mail: fabio.m@unitus.it

Antonio Tiezzi, Elisa Ovidi and Tomasz M. Karpiński (Eds.)

has been exploited in many different ways and has fully contributed to the traditions of the different cultures and ethnic groups in the world. Nowadays, large numbers of humans are populating the planet; however, heart disease, stroke, cancer, neurodegenerative disease and diabetes are widespread, and, consequently, more effective pharmacological interventions are required. It is now established that there is a relationship between quality of life and pathologies. The quality of one's life is determined by one's lifestyle and, in terms of diet, the quality of one's food and the significant consumption of plant foods with a minimum content of chemicals and pesticides are essential. Nutraceutical is a recent scientific field where the chemical composition of foods has acquired relevance within the human population and currently, an increasing number of people observe more specific and balanced diets. Particular attention is paid to the possibility of preventing diseases and pathologies through the use of specific foods that represent a large source of molecules potentially containing pharmaceutical properties. The concept “foods as natural pharmaceuticals” is spreading widely, especially in advanced countries, and a careful search is underway for plants and plant derivatives used as traditional foods.

Since ancient times, different cultures and religions have flourished in the Mediterranean area and, depending on the great availability in the various Mediterranean countries, there was a wide use of olive oil in the diet, in pharmaceutical uses and also in religious ceremonies. Consequently, olive oil has been thoroughly studied and its importance for a balanced diet has been consolidated. Here we review the biological properties of olive oil and the importance of its chemical components. Furthermore, attention is also focused on the new pharmaceutical perspectives offered by olive oil molecules and their derived molecules obtained with chemical procedures developed in laboratories. Interesting perspectives seem to be offered by this new research approach and more precise and defined investigations at both the research and clinical levels can take place in a short time to support the human need for new tools against the disease.

BOTANICAL DESCRIPTION OF *OLEA EUROPAEA*

The Oleaceae family includes about 30 genera and 600 species and is widespread in the temperate and tropical regions of the world [2, 3]. Fraxinus, Jasminum, Lingustrum and other genera are economically important, however the Olive (*Olea europaea*) is the main cultivated one, above all for its edible fruits and the oil derived from it [3].

Olea europaea (O. e.) is part of the genus Olea and includes six subspecies (Fig. **1**):

- *O. e.* subsp. *europaea*
- *O. e.* subsp. *cuspidata*
- *O. e.* subsp. *laperrinei*
- *O. e.* subsp. *maroccana*
- *O. e.* subsp. *cerasiformis*
- *O. e.* subsp. *Guanchica*

Kingdom: *Plantae*
Phylum: *Magnoliophyta*
Class: *Rosopsida*
Order: Lamiales

Family: *Oleaceae*
Sub-family: *Oleideae*

Genus: *Olea*
  Sub-genera: *Paniculatae*
    Tetrapilus
    Olea

    Sections: *Ligustroides*

    Olea
    Sub-species: *cuspidata*
      laperrinei
      maroccana
      cerasiformis
      guanchica
      europaea
        Varieties: *sylvestris* (wild olive)
          europaea (cultivated olive)

Fig. (1). Taxonomic scheme of *Olea europaea* subsp. *europaea* var *europaea* and *sylvestris* [2].

Furthermore, *Olea europaea* subsp. europaea can be further recognized as var. europaea for the cultivated olive tree (Fig. **2a**) and var. sylvestris for the wild olive tree, also called wild olive (Fig. **2b**) [4]. Both, europaea and sylvestris, are a diploid species (2n = 2x = 46) [2].

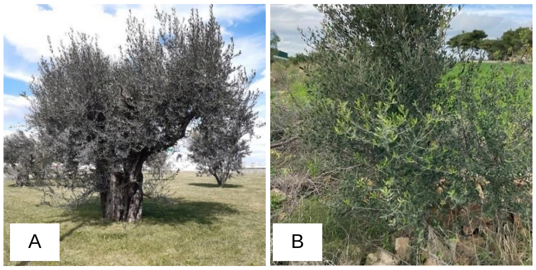


Fig. (2). (A) *Olea europaea* subsp. europaea var. europaea; **(B)** *Olea europaea* subsp. europaea var. sylvestris.

CHAPTER 8

Hydrolates: Characteristics, Properties, and Potential Uses in the Food Industry

Diana De Santis[1,*] and **Giovanni Turchetti**[1]

[1] *Department for the Innovation in Biological, Agrofood and Forestal Systems, University of Tuscia,01100 Viterbo, Italy*

Abstract: Hydrodistillation is a traditional method that has long been used to extract essential oils and hydrolates from plant matrices known to be rich in bioactive compounds. The use of water as a solvent in the process does not require the recovery of the solvent, with a saving in terms of time, energy, loss of volatile compounds, and a lower environmental impact. Very often, and for some matrices rich in essential oils, hydrolates are considered a by-product. They contain a variety of organic phytochemicals which can be extracted and used for other medicinal, cosmetic, dermatological, and commercial applications. Extracts derived from different plant parts, such as leaves, roots, stems, bulbs, fruits, flowers, and seeds, vary in characteristics and composition. Many other variables can influence the composition of hydrolates, such as geographic location, cultivation techniques, seasonality, and process variations. Plant hydrolates contain significant quantities of bio compounds with different activities: they can be used as antimicrobial, sanitizing, or antitumor agents for the control of weeds, nematodes, larvae, and various parasites affecting crops of agronomic interest, as well as anesthetizing in aquaculture. These extracts are also used in aromatherapy or as components in cosmetic formulations. The hydrolate represents a matrix of considerable interest due to the greater ease of diffusion on specific substrates and the lower toxicity compared to essential oil.

Keywords: Acaricidal activity, Anesthetizer, Antibacterial activity, Antifungal activity, Antifeeding activity, Antioxidant activity, Antitumor activity, Aromatherapy, Dry distillation, Essential oil, Hydrodistillation, Hydrolate, Ixodicidal activity, Larvicidal activity, Nematicidal activity, Pest control, Sanitizer, Steam distillation.

INTRODUCTION

Consumers' increasing demand for healthy foods, grown and/or produced with respect for health and the environment and the consequent greater attention of res-

---

[*] **Corresponding author Diana De Santis:** Department for the Innovation in Biological, Agrofood and Forestal Systems, University of Tuscia,, 01100 Viterbo, Italy; Tel: +39 0761357371; E-mail: desdiana@unitus.it

Antonio Tiezzi, Elisa Ovidi and Tomasz M. Karpiński (Eds.)

earch towards safe products, as an alternative to the numerous chemical additives used in the food industry, has encouraged interest in plant extracts [1].

Extracts from specific plant matrices can offer a wide range of biologically active components, some of which can guarantee safety and efficacy in hindering many alterations in food [2]. Natural biodiversity ensures various plant constituents are very different from the structural and functional points of view. Among these compounds, we recognize primary metabolites such as carbohydrates, while others, including tannins, alkaloids, *etc.*, are by-products of secondary metabolism [3].

The production and accumulation of essential oils (EO) in the tissues of some plants are also possible in response to stressful situations of the plant, and many of these, can be used to alleviate ailments, for general health, and well-being, having nutraceutical and pharmacological properties. Among the extracts of greatest interest, we find EOs and all compounds recovered through distillation processes or similar. EOs can be extracted by different tissue districts of a plant, through various extraction methods. The process for the extraction of the EO and the preparation of the sample, will be appropriately selected according to the botanical material used, also considering the state and shape. The extraction method is one of the first factors determining the quality of EOs collected. An inappropriate extraction procedure can damage or alter the chemical composition of EO, resulting in the loss of bioactivity and natural characteristics.

The recovery of the EO from the plant material occurs mainly through conventional techniques such as hydro-distillation, steam distillation, solvent extraction, and new technologies with different degrees of complexity. The hydrodistillation techniques with the increased interest in the protection of the environment, represent a very interesting topic in the studies of plant matrices and the possibilities of recovery and use.

Hydrolates, also called hydrosols, are the pure and natural waters, produced during the steam distillation of plant materials such as leaves, flowers, peels, berries, wood, stems, and roots, recovered during the distillation process that also extracts the EOs.

The use of water to solubilize the plant material, and the strength of the steam generated in heating, effectively lead to isolating the volatile and particularly active portion of the substrate. During this process, if the matrix contains any, the EO and the hydrolate can be recovered simultaneously. If the matrix is essential-oil-less, only hydrolate can be recovered. These two products are often discussed together because EOs cannot be extracted without producing a hydrolate. Steam distillation is the most used method for extracting Eos and hydrolates from plant tissues [4]. Overall, through steam distillation, about 90% of the EO is extracted, to increase the extraction yield, other methods will have to be used [5]. The technique involves immersing the plant matrix in boiling or steam-heated water. The action of heat allows the destruction of the cellular structure of the plant tissue with the release of aromatic or essential compounds [6, 7].

Hydrolates generally represent a secondary production of the process of extracting EOs from aromatic plants. In detail, hydrolates mainly consist of all those volatile and hydrophilic components capable of forming weak bonds with water, which are carried away by the steam during distillation, and collected through the final condensation. Chemically, they consist of variable concentrations, generally very low, of the same chemical constituents of EOs and volatile and water-soluble secondary metabolites, even if the composition is affected by many variables [8, 9].

The process greatly influences the chemical composition of the extract and, consequently, its biological properties. Therefore, sometimes, different authors report dissimilar properties for the extracts of the same plant. An example of this is represented by consistent composition differences between solvent extracts and hydro distilled. A substantial loss of volatile compounds occurs in the extraction with solvent, especially during the removal of the solvent, while the hydrodistillation does not require this step. Furthermore, as reported by Cu and colleagues [10], the different solvents used in the extraction process, influence the chemical composition of the extract.

Among the emerging techniques for the recovery of EOs and plant extracts, Ultrasound-Assisted Extraction (UAE), Microwave Assisted Extraction (MAE), Supercritical/Subcritical Fluid Extraction (SFE), and Subcritical Aqueous Extract [11, 12] stand out. Although the EOs extraction process through supercritical fluid gives optimal results in terms of yield and minimization of component decomposition, requiring large investment, therefore it is especially suitable for commercial purposes.

Despite the fact that new techniques can offer undeniable advantages in terms of time, yield, and reduction of artifacts, the classic process, remains an advantageous method, according to the principles of green chemistry, fully compatible with food uses, economic, and environmentally friendly [13].

The use of water as a solvent in the distillation and hydrodistillation processes does not require solvent recovery processes, with savings in terms of time and energy. The process can be carried out mainly in two ways, by immersing the plant matrix in water and bringing it to a boiling point (hydrodistillation) or by investing it with a flow of steam produced in an adjacent kettle (steam

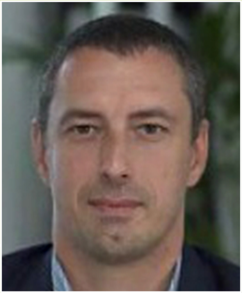
SUBJECT INDEX

Antonio Tiezzi

Antonio Tiezzi is Professor of Botany and Head of the Laboratory of Plant Cytology and Biotechnology of Natural Substances at Tuscia University, Dept. for the Innovation in Biological, Agrofood and Forestal systems, Viterbo, Italy. He graduated in Biology from the University of Siena, Italy, in 1979 and was Visiting Scholar at the Biozentrum, Basel, Switzerland, under an EMBO fellowship. In 1982, he gained experience in the vaccine industry at Sclavo (then Novartis and presently GSK) and in 1983 he undertook research at the Einstein College of Medicine, NY, USA. In 1985, he worked at Siena University and since 1992 he has been serving as Professor at Tuscia University. His main research areas include plant cytoskeleton – he discovered the motor protein kinesin in plants and became a board member of the European Cytoskeleton Forum – and bioactive plant substances.

Elisa Ovidi

Elisa Ovidi is an Assistant Professor at the Laboratory of Plant Cytology and Biotechnology of Natural Substances, Department of Innovation of Biological Systems, Food, and Forestry, Tuscia University, Italy where she got her Ph.D. degree in Genetics and Cellular Biology. She is involved in investigations concerning the biology and chemistry of natural substances to identify potential active biomolecules for the development of new drugs and active principles for pharmaceuticals. Furthermore, her research activities are also focused on the biology of reproduction in higher plants and on the study of plant cell structural and molecular components. She has also been involved in numerous research projects and collaborations with laboratories from different countries.



Tomasz. M. Karpinski

Tomasz M. Karpiński is a professor at Poznań University of Medical Sciences, Poland. He is the Head of the Department of Medical Microbiology. He is a specialist in medical microbiology. His research focus is on human microbiome, infectious diseases, and microbial drug resistance. Moreover, he is engaged in in silico and in vitro research on the antibacterial, antifungal and anticancer activity of plant extracts, natural compounds and synthetic substances. He is the author or co-author of over 100 scientific articles.