

Mahendra Rai
Ivan Kosalec *Editors*

Promising Antimicrobials from Natural Products

 Springer

Promising Antimicrobials from Natural Products

Mahendra Rai • Ivan Kosalec
Editors

Promising Antimicrobials from Natural Products

 Springer

Editors

Mahendra Rai
Sant Gadge Baba Amravati University
Department of Biotechnology
Amravati, Maharashtra, India

Ivan Kosalec
University of Zagreb
Faculty of Pharmacy and Biochemistry
Institute for Microbiology
Zagreb, Croatia

ISBN 978-3-030-83503-3 ISBN 978-3-030-83504-0 (eBook)
<https://doi.org/10.1007/978-3-030-83504-0>

© The Editor(s) (if applicable) and The Author(s), under exclusive license to Springer Nature Switzerland AG 2022

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

The publisher, the authors, and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, expressed or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

The enormous chemical diversity in the plant kingdom and the biochemical metabolic pathways yet to be discovered remain a challenging area of scientific research. They are a myriad of molecules yet to be discovered. With such immense natural diversity, it is a core business for phytochemists to define the structure-biology-activity relationship. The number of published papers in the peer-reviewed scientific literature on the subject of the biological activity of nature-based products is constantly growing, decade by decade, and will never decrease. The reason for this is some disadvantages in the synthetic *de novo* synthesis of molecules, such as the price, and on the other hand, the pool of countless unknown molecules on the planet Earth, as a source of completely new chemical identities.

Natural products as part of drug discovery are very well known to any student of biomedicine and biotechnology. From a historical perspective, there is a very large list of drugs on the market with known therapeutic efficacy and post-marketing pharmacovigilance data collected. There are almost all areas of natural sources covered by “scientific digging” for bioactive molecules. New technologies involving better analytical profiling, faster isolation and the use of various biological screening protocols have led to a revival of natural product research. With the reality of antimicrobial resistance and its impact on global health already becoming a global crisis, and therefore, there is a need for a major turnaround to bridge the gap in drug discovery.

One of the promising strategies to discover antimicrobial molecules could be summarised as follows: (a) use ethnobotanical data as a starting point for lay scientific evidence of indications; (b) test previously published results in the past with a more precise methodological approach and on different microbial models and against virulence factors; (c) use broader bio-screening targets; and (d) collect plausible bioactivities *in vitro* and retest them with different microbial models (such as multidrug-resistant microbes).

The aim of this book is to present some of the current areas of research on natural products from plant sources with antibacterial, antifungal and antiviral activities. There are reports from different geographical areas such as Nepal, Amazonia, Greece, Latin America and Argentina. These ethnopharmacological collected data

from the empirical use of some plants are still a good starting point for translational studies. On another side, some authors have focused on volatile compounds and others on specific compounds (berberine, cinnamon, hydroxytyrosol, oleuropein). Fungi as a source of bioactive compounds and the hive-based approach are another good example of the search for antimicrobial compounds.

The development of a broader range of modern antimicrobial-based methods to detect potential targets in whole-cell models or in biosynthetic pathways is crucial for a better understanding of the mechanisms of action. With an advance in analytical techniques and in combination with antimicrobial assays, our knowledge of structure-activity relationships could strengthen. Then, the doors from preclinical research to clinical trials are more likely to open.

As mentioned earlier, the chapters provide valuable information for researchers working on antimicrobial activities of natural products. We see this book as a foundation for furthering the search for antimicrobial phytochemicals.

As more than 70 authors contributed to this book, we would like to thank our colleagues around the world for sharing valuable information with the scientific audience.

We also thank everyone in the Springer team for their constant help and constructive suggestions, particularly Carolyn Spence, senior publishing editor at Springer, for her patience and cooperation. Finally, MR wishes to thank the Polish National Agency for Academic Exchange (NAWA) for financial support to visit the Department of Microbiology, Nicolaus Copernicus University, Toruń, Poland.

Amravati, Maharashtra, India
Zagreb, Croatia

Mahendra Rai
Ivan Kosalec

Contents

Part I Natural Antimicrobials from Plants

1	Natural Antimicrobials: An Introduction	3
	Ivan Kosalec and Mahendra Rai	
2	Nepalese Pteridophytes Used as Antimicrobials: Challenges and Opportunities	15
	Shandesh Bhattarai and Ripu M. Kunwar	
3	Antimicrobial Activity of Extracts and Essential Oils of Medicinal Plants Occurring in Amazonia: Nanotechnology as a Boon to Enhance Bioactivity	31
	Luiza Helena da Silva Martins, Andrea Komesu, Johnatt Allan Rocha de Oliveira, Carissa Michelle Goltara Bichara, Paulo Wender Portal Gomes, and Mahendra Rai	
4	Assessment of Cinnamon as an Antimicrobial Agent	53
	Merve Eylul Kiymaci and Banu Kaskatepe	
5	The Antimicrobial Activities of Oleuropein and Hydroxytyrosol	75
	Nataša Zorić and Ivan Kosalec	
6	Antimicrobial Properties of Selected Native Greek Aromatic Plants: An Ethnopharmacological Overview	91
	Christos G. Ganos, Olga Gortzi, Efrossini B. Chinou, Gioacchino Calapai, and Ioanna B. Chinou	
7	The Spectrum of Berberine Antibacterial and Antifungal Activities	119
	Ivan Kosalec, Maja Jazvinščak Jembrek, and Josipa Vlainić	

Part II Antimicrobial Agents/Secondary Metabolites

- 8 Plants to Drugs: A Case Study of Human Papilloma Virus and Traditional Chinese Medicine** 135
 Mohammed Rahmatullah, Taufiq Rahman, Anamul Hasan, Rownak Jahan, Md Shahadat Hossan, Khoshnur Jannat, and Tohmina Afroze Bondhon
- 9 Natural Products from Plants with Antimicrobial Action** 183
 Patrícia e S. Alves, Juliana S. de Figuerêdo, Felipe P. S. Santos, Pedro V. O. S. Furtado, Teresinha J. A. S. Andrade, Joaquim S. C. Júnior, Nerilson M. Lima, and Chistiane M. Feitosa
- 10 Volatile Organic Compounds and Their Capacity for Controlling Fungal Infection in Humans** 199
 Fernanda Achimón, Carolina Merlo, Romina P. Pizzolitto, José S. Dambolena, Alejandra Omarini, and Julio A. Zygado
- 11 Promising Antimicrobial Agents from Some Latin American Medicinal Plants Against Disease-Causing Pathogens in Humans and Animals** 241
 Amner Muñoz-Acevedo, María C. González, Osneider J. Castillo, Cindy P. Guzmán, Sandra Rodríguez-Acosta, Martha Cervantes-Díaz, Ricardo D. D. G. De Albuquerque, Bettina M. Ruppelt, Ninoska Flores, Alberto Giménez-Turba, Feliza Ramón-Farías, Leticia M. Cano-Asseleih, Elsa Rengifo, Gabriel Vargas-Arana, and Mahendra Rai
- 12 Natural Antifungal Agents Isolated from Argentine Plants. A Summary of Studies Developed in the Period 2000–2020** 281
 Gisela Seimandi, Estefanía Butassi, Melina Di Liberto, Estefanía Cordisco, Alan Blanc, Maximiliano Sortino, Laura Svetaz, and Marcos Derita

Part III Mushrooms/Beehive as Antimicrobials and Delivery Systems

- 13 Natural Antimicrobials from Basidiomycota Mushrooms** 323
 Vladimir Elisashvili, Mikheil D. Asatiani, Tamar Khardziani, and Mahendra Rai
- 14 Anti-quorum Sensing Properties of Mushrooms** 355
 Zdenka Bedlovičová and Imrich Strapáč
- 15 Beehives as a Natural Source of Novel Antimicrobials** 373
 Jelena Suran
- 16 Delivery Systems of Plant-Derived Antimicrobials** 397
 Enas Elmowafy, Eman M. El-Marakby, Haidy A. Gad, and Heba A. Gad

- Index** 443

Contributors

Fernanda Achimón Instituto Multidisciplinario de Biología Vegetal, Consejo Nacional de Investigaciones Científicas y Técnicas. IMBIV-CONICET, Córdoba, Argentina

Patrícia e S. Alves Post-Graduation Department in Chemistry, Federal University of Piauí, Terezina, Piauí, Brazil

Teresinha J. A. S. Andrade Nucleus of Applied Research to Sciences (NIAC), Federal Institute of Maranhão, Maranhão, Brazil

Mikheil D. Asatiani Institute of Microbial Biotechnology, Agricultural University of Georgia, Tbilisi, Georgia

Zdenka Bedlovičová Department of Chemistry, Biochemistry and Biophysics, University of Veterinary Medicine and Pharmacy, Košice, Slovakia

Shandesh Bhattarai Nepal Academy of Science and Technology, Khumaltar, Lalitpur, Nepal

Carissa Michelle Goltara Bichara Instituto de Saúde e Produção Animal (ISPA), Universidade Federal Rural da Amazônia, Belém, PA, Brazil

Tohmina Afroze Bondhon Department of Biotechnology & Genetic Engineering, University of Development Alternative, Lalmatia, Dhaka, Bangladesh

Estefanía Butassi Farmacognosia, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha, Rosario, Argentina

Gioacchino Calapai Dept of Biomedical and Dental Sciences and Morphological and Functional Imaging, University of Messina, Messina, Italy

Leticia M. Cano-Asseleih Centro de Investigaciones Tropicales, Universidad Veracruzana, Xalapa-Enríquez, México

Osneider J. Castillo Departamento de Química y Biología, Universidad del Norte, Barranquilla, Colombia

Martha Cervantes-Díaz Grupo Investigaciones Ambientales para el Desarrollo Sostenible, Universidad Santo Tomás, Bucaramanga, Colombia

Efrossini B. Chinou Lab of Clinical Microbiology, “St Savvas” Anticancer Hospital of Athens, Athens, Greece

Ioanna B. Chinou Lab of Pharmacognosy and Chemistry of Natural Products, Department of Pharmacy, National and Kapodistrian University of Athens, Univ. Campus of Zografou, Athens, Greece

Estefanía Cordisco Farmacognosia, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha, Rosario, Argentina

José S. Dambolena Instituto Multidisciplinario de Biología Vegetal, Consejo Nacional de Investigaciones Científicas y Técnicas. IMBIV-CONICET, Córdoba, Argentina

Facultad de Ciencias Exactas, Físicas y Naturales, Instituto de Ciencia y Tecnología de los Alimentos, Universidad Nacional de Córdoba, Córdoba, Argentina

Luiza Helena da Silva Martins Instituto de Saúde e Produção Animal (ISPA), Universidade Federal Rural da Amazônia (UFRA), Belém, Brazil

Ricardo D. D. G. de Albuquerque Laboratório de Tecnologia em Produtos Naturais, Universidade Federal Fluminense, Niterói, Brasil

Juliana S. de Figuerêdo Post-Graduation Department in Chemistry, Federal University of Piauí, Terezina, Piauí, Brazil

Johnatt Allan Rocha de Oliveira Institute of Health Sciences, Faculty of Nutrition (FANUT), Federal University of Pará (UFPA), Belém, PA, Brazil

Marcos Derita ICiAgro Litoral, Universidad Nacional del Litoral-CONICET, Kreder Esperanza, Argentina

Melina Di Liberto Farmacognosia, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Argentina

Vladimir Elisashvili Institute of Microbial Biotechnology, Agricultural University of Georgia, Tbilisi, Georgia

Eman M. El-Marakby Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt

Enas Elmowafy Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt

Chistiane M. Feitosa Post-Graduation Department in Chemistry, Federal University of Piauí, Terezina, Piauí, Brazil

Ninoska Flores Instituto de Investigaciones Fármaco Bioquímicas, Universidad Mayor de San Andrés, La Paz, Bolivia

Pedro V. O. S. Furtado Post-Graduation Department in Chemistry, Federal University of Piauí, Terezina, Piauí, Brazil

Haidy A. Gad Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt

Department of Pharmacognosy, Faculty of Pharmacy, King Salman International University, South Sinai, Egypt

Heba A. Gad Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt

Christos G. Ganos Lab of Pharmacognosy and Chemistry of Natural Products, Department of Pharmacy, National and Kapodistrian University of Athens, Univ. Campus of Zografou, Athens, Greece

Alberto Giménez-Turba Instituto de Investigaciones Fármaco Bioquímicas, Universidad Mayor de San Andrés, La Paz, Bolivia

Paulo Wender Portal Gomes Institute of Exaction and Natural Sciences (ICEN), Federal University of Pará (UFPA), Belém, PA, Brazil

María C. González Departamento de Química y Biología, Universidad del Norte, Barranquilla, Colombia

Olga Gortzi Food Chemistry, School of Agricultural Sciences, Department of Agriculture Crop Production and Rural Environment, University of Thessaly, Volos, Greece

Cindy P. Guzmán Departamento de Química y Biología, Universidad del Norte, Barranquilla, Colombia

Anamul Hasan Department of Biotechnology & Genetic Engineering, University of Development Alternative, Lalmatia, Dhaka, Bangladesh

Md Shahadat Hossan School of Pharmacy, University of Nottingham, University Park, Nottingham, United Kingdom

Rownak Jahan Department of Biotechnology & Genetic Engineering, University of Development Alternative, Lalmatia, Dhaka, Bangladesh

Khoshnur Jannat Department of Biotechnology & Genetic Engineering, University of Development Alternative, Lalmatia, Dhaka, Bangladesh

Maja Jazvinščak Jembrek Ruđer Bošković Institute, Zagreb, Croatia

Joaquim S. C. Júnior Federal Institute of Piauí (IFPI), Teresina, PI, Brazil

Banu Kaskatepe Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Ankara, Turkey

Tamar Khardziani Institute of Microbial Biotechnology, Agricultural University of Georgia, Tbilisi, Georgia

Merve Eylul Kiymaci University of Health Sciences Turkey, Gulhane Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Ankara, Turkey

Andrea Komesu Department of Marine Sciences (DCMar), Federal University of São Paulo (UNIFESP), Santos, SP, Brazil

Ivan Kosalec University of Zagreb, Faculty of Pharmacy and Biochemistry, Institute for Microbiology, Zagreb, Croatia

Ripu M. Kunwar Ethnobotanical Society of Nepal, Kathmandu, Nepal

Nerilson M. Lima Federal university of Juiz de Fora, Chemistry Department, Juiz de Fora, MG, Brazil

Carolina Merlo Instituto Multidisciplinario de Biología Vegetal, Consejo Nacional de Investigaciones Científicas y Técnicas. IMBIV-CONICET, Córdoba, Argentina
Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Córdoba, Argentina

Amner Muñoz-Acevedo Departamento de Química y Biología, Universidad del Norte, Barranquilla, Colombia

Alejandra Omarini Instituto de Ciencias de la Tierra y Ambientales de La Pampa, INCITAP-UNLPam- CONICET, La Pampa, Argentina

Romina P. Pizzolitto Instituto Multidisciplinario de Biología Vegetal, Consejo Nacional de Investigaciones Científicas y Técnicas. IMBIV-CONICET, Córdoba, Argentina; and Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Córdoba, Argentina

Taufiq Rahman Department of Pharmacology, University of Cambridge, Cambridge, UK

Mohammed Rahmatullah Department of Biotechnology & Genetic Engineering, University of Development Alternative, Lalmatia, Dhaka, Bangladesh

Mahendra Rai Department of Biotechnology, Sant Gadge Baba Amravati University, Amravati, Maharashtra, India

Department of Microbiology, Nicolaus Copernicus University, Torun, Poland

Feliza Ramón-Farias Facultad de Ciencias Biológicas y Agropecuarias, Universidad Veracruzana, Veracruz, México

Elsa Rengifo Instituto de Investigaciones de la Amazonía Peruana, Iquitos, Perú

Sandra Rodríguez-Acosta Grupo de Análisis Económico, Instituto de Estudios Económicos del Caribe, Departamento de Economía, Universidad del Norte, Barranquilla, Colombia

Bettina M. Ruppelt Departamento de Tecnologia Farmacêutica, Universidade Federal Fluminense, Niterói, Brasil

Felipe P. S. Santos Post-Graduation Department in Chemistry, Federal University of Piauí, Terezina, Piauí, Brazil

Gisela Seimandi ICiAgro Litoral, Universidad Nacional del Litoral-CONICET, Kreder, Esperanza, Argentina

Maximiliano Sortino Farmacognosia, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Argentina

Imrich Strapáč Department of Chemistry, Biochemistry and Biophysics, University of Veterinary Medicine and Pharmacy, Komenského, Košice, Slovakia

Jelena Suran Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

Laura Svetaz Farmacognosia, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Argentina

Gabriel Vargas-Arana Instituto de Investigaciones de la Amazonía Peruana, Iquitos, Perú

Josipa Vlainić Ruđer Bošković Institute, Zagreb, Croatia

Nataša Zorić Agency for Medicinal Products and Medical Devices of Croatia (HALMED), Zagreb, Croatia

Julio A. Zygodlo Instituto Multidisciplinario de Biología Vegetal, Consejo Nacional de Investigaciones Científicas y Técnicas. IMBIV-CONICET, Córdoba, Argentina

Facultad de Ciencias Exactas, Físicas y Naturales, Instituto de Ciencia y Tecnología de los Alimentos, Universidad Nacional de Córdoba, Córdoba, Argentina

Part I
Natural Antimicrobials from Plants

Chapter 1

Natural Antimicrobials: An Introduction



Ivan Kosalec and Mahendra Rai

Abstract From historical point of view, nature has always been a rich source of materials, and medicines also came from this vast and immeasurable resource. Ethnologically-based information still has great potential for future explanations of bioactivities of such medicines. Although epidemiological measures (vaccinations) and antimicrobial treatments curb infectious diseases, there is still an urgent need for well-defined molecules from nature. With the decline of *de novo* synthesis of new chemical entities, the main focus of nature-derived molecule research among the group of antimicrobials is clear definition of antimicrobial spectrum of activities, mechanism of action, stability, mutagenicity and genotoxicity. The possible road is also *in silico* studies of antimicrobial activities of natural molecules from natural products databases. Clearly displayed preclinical studies may lead to the *in vivo* studies which can prove the indication of such natural molecules. The main obstacles such as low bioavailability, short half-life and low PK/PD values will be a great challenge for future research. Considering the emerging new diseases, such as SARS-CoV-2 nowadays, and rising scientific awareness about testing known natural molecules, the area of natural antimicrobials is fast, prominent and still encouraging.

Keywords Ethnobiology · Natural products · Bioactivity · Antimicrobials · Emerging diseases

I. Kosalec (✉)

University of Zagreb, Faculty of Pharmacy and Biochemistry, Institute for Microbiology,
Zagreb, Croatia

e-mail: ikosalec@pharma.hr

M. Rai

Department of Biotechnology, Sant Gadge Baba Amravati University,
Amravati, Maharashtra, India

Department of Microbiology, Nicolaus Copernicus University, Torun, Poland

e-mail: mahendrarai@sgbau.ac.in; mahendra.rai@v.umk.pl

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2022

M. Rai, I. Kosalec (eds.), *Promising Antimicrobials from Natural Products*,

https://doi.org/10.1007/978-3-030-83504-0_1

Abbreviation

CLSI	The Clinical & Laboratory Standards Institute
EUCAST	The European Committee on Antimicrobial Susceptibility Testing
MBcC	Minimal Bactericidal Concentration
MERS	Middle East Respiratory Syndrome
MFcC	Minimal Fungicidal Concentration
MIC	Minimal Inhibitory Concentration
MNDP	Marine Natural Products Database
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
QS	Quorum Sensing
SAR	Structure–Activity Relation
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
TTC	2,3,5-triphenyltetrazolium chloride
XTT	(2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide

1 Introduction

Nowadays, there is a steady decline of newly registered antimicrobials in the market. On the other side, many currently available antimicrobial drugs have failed to act on the target site in bacterial or fungal cells, thus exposing the patients to failure of the therapeutic outcome.

Moreover, the new and emerging diseases caused by viruses including the recent coronavirus 2 (SARS-CoV-2) have threatened the existence of human being (Dhama et al. 2020).

It is widely recognised that we are slowly losing the pace with microbes. It seems that we are one step backward, and that evolutionary pressure keeps microbes fittest with genetic and phenotypic changes and mutations that enable their propagation in environment with antimicrobials. Such a failure of therapeutic goal led to rethink on future of scientific approaches in search of new antimicrobial-active molecules. We are also faced with a slow decline of *de novo* synthesis of molecular entities which could be a promising molecule for new targets inside bacterial or fungal cells. The reason behind the slow decline of search for the new molecule is cost and uncertain future of such a molecule. Other researchers use known antimicrobial molecules with the goal of enhancing their activity by incorporating new substituents into the core molecule. This led to *in silico* approach where software-based computational structure–activity relationships (SAR) approach is possible and could predict new active molecules. After the positive outcome of *in silico* approach, scientists could demonstrate biological activity in preclinical steps. De Sousa Luis et al. (2020) emphasise the positive side of searching the databases of natural products, mainly because such a survey could reduce the time for the development of new medicinal

products. According to Sorokina and Steinbeck (2020), main topic to search for new drug candidates belongs to anticancer, antibacterial, antiviral, and anti-inflammatory area, with the most cited databases: ChemSpider, PubChem, ChEMBL, The Dictionary of Natural Products, Reaxys, Marine Natural Products database (MNDP), Universal natural Products Databases, Super Natural II, Traditional Chinese Medicine database @ Taiwan, AfroDb, AfroCancer, SANDCB, UEFS, NuBBE, and others. One good example includes the search for SARS-CoV protease inhibitors among natural products database which led to molecule SARS-M4367, a high-affinity natural product as a viral protease inhibitor (Liu and Zhou 2005). Another example is efflux-pumps MexAB and AcrAB inhibitory activity against *Pseudomonas aeruginosa* and *E. coli* by natural products molecules lanatoside C and daidzein (Liu and Zhou 2005; Aparna et al. 2014). Other example includes hamameletannin as quorum-sensing (QS) inhibitor RNAIII-inhibiting peptide of *Staphylococcus aureus*, as promising natural products molecule against device-associated infections (Kiran et al. 2008). If steps in QS signalling pathways are known, the promising *in silico* strategy is structure-based virtual screening for quorum-sensing inhibitors (Lu et al. 2019) among available natural products in public databases.

Traditional targets of antibiotics include cell membrane function distribution, cell wall synthesis, proteins and nucleic acid biosynthesis, and bacterial energetics (Hards and Cook 2017). Omics approaches have been used to find and identify new drug targets. This approach has revolutionised biology with new insights into the relationship between drug targets in microbial cells (Chernov et al. 2019).

The positive outcomes of combination, for example, beta-lactamase inhibitors with beta-lactam antibiotics open new possibilities to concomitant use of available molecules together with enzymatic inhibitors, thus act as a helper-molecule. This could decrease mortality rates caused by carbapenemase producers among Enterobacteriales (Lasko and Nicolau 2020). Advantages in the *in silico*-based approaches using bioinformatics, metagenomics, system-biology approach together with structural biological technologies (molecular dockings, the crystal structure of target sites, etc.) are also very promising and growing area as new antimicrobial discovery strategy (Hoffman 2020). There is a pressing need of new and effective antibacterial and antifungal drugs (Perfect 2016). A recent survey of bibliometric data (till 2020) showed that 30 antibacterial agents are in pipeline, but scientific literature reveals that microbial targets are wider and include (a) antivirulence strategy (virulence blockers), (b) targeting new proteins in microbial cells, (c) using nano-delivery strategy, (d) enzybiotics and peptidomimetics, and (e) antisense oligonucleotides (Vila et al. 2020).

New antimicrobials could come from natural sources as well. Historically, the fungi and bacteria were screened for metabolites with biological activity, and there are very successful examples of antibiotics and antimycotics in the current clinical practice. Still, there is a vast body of articles that deal with the structure–activity relationship of naturally isolated compounds. Alternative-to-antibiotics approach identified also some small molecules produced by bacteria such as lantibiotics, enterocins, bacteriocins, etc. with specific activity against pathogens (Seal et al. 2018).

From the centuries, plants (above the earth and in the seas) are not only the source of food, clothing and shelter but also as a medicine in the community (Tempesta and King 1994). Ethnobotanical data gathered from the rural and tribal community to biology-guided screening is still the main source for the development of newer antimicrobials (Tempesta and King 1994). Furthermore, indigenous knowledge concerning the use of medicinal plants among tribes could enhance research using retrospective studies about the effectiveness of herbal products and side effects. Such knowledge of use and practices should be regulated under the act of conservation (Sheng-Ji 2011; Shakya 2016).

The fact is that from 1% to 10% of known plant species has been scientifically investigated in depth as a potential source of antimicrobials (Borris 1996), and about 10,000 varieties of plants on Earth have been documented for medicinal use (Pandey and Agnihotry 2015). This fact is very encouraging and promising for future research and motivates young researchers to develop new strategies.

Many research works have focused on crude extract against recently recognised MDR bacterial and fungal pathogens, without defining the role of active compound(s) (Anand et al. 2020). Based on results of screenings of antimicrobial activity research, not only on MDR strains, researchers found its publishing space. The positive side of antimicrobial activity of crude extract is that emerging microbial resistance is not common when a mixture of compounds was applied for a long time. Other research teams used isolated molecules from the botanical origin and extensively tried to find the full spectrum of activities. Third research area is the modulation of the molecular pathways with phytochemicals which could lead to disruption of steps in microbial metabolism.

As stated in the review of current research on the topic of antimicrobial activity of medicinal plants by Rios and Recio (2005), the scientific production has been doubled between 1995 and 2004, in comparison with the period between 1966 and 1994, and still growing especially because of open access policy of some publishers.

2 Activity of Natural Antimicrobials: A Conservative Approach

The most common basic studies in natural antimicrobial research are the screening of the *in vitro* antibacterial and antifungal activities using liquid extracts in diffusion and dilution assay, with some modifications. The review of methods used for antimicrobial susceptibility testing for natural products was published as early as 1988 (Rios et al. 1988). There are generally accepted two prominent *in vitro* methods, both widely used but with the positive and negative sides. Diffusion assay was the most common one, easy to perform and with low costs and easy to read the outcome (zones of inhibition growth). There exist different types such as disk-diffusion assay (disks are placed onto the inoculated agar), diffusion from holes dug into inoculated agar and from cylinders as reservoir above the inoculated agar. Despite being simple to perform, this method has limitations when essential oils or other evaporated

substances are tested. Another limitation is that diffusion method cannot distinguish microbiostatic from microbicidal activity extracts/compounds (Balouiri et al. 2016). Lipophilic substances will hardly diffuse into inoculated agar plates, so the outcome could be false negative. Besides diffusion methods where active substance(s) diffuse into inoculated agar and show inhibition zones of inhibition after the incubation period, dilution methods in liquid media and agar are also in common use as screening methods. The advantages of dilution methods include the possibility to test lipophilic substances, after surfactants were added (such as polysorbates). Even as a typical screening method, it is possible to determine minimal inhibitory concentrations (MICs) including minimum bactericidal or fungicidal concentration (MBcC, MFcC) of crude extracts or isolated pure compounds. The inhibitory concentration of the test substances can be evaluated by two-fold serial dilution in liquid medium or in solid medium, such as agar plates. The most critical point in dilution methods is determination of endpoint (MIC and MBcC/MFcC). Since the most extracts absorb the light, the determination of endpoints (MIC, MBcC or MFcC) is sometimes challenging. To facilitate visualisation of the endpoints, there are several approaches. One of the simplest method is to read optical density at 540–620 nm and compare the turbidity with negative control (media with microbial inoculum). Other approaches include use of tetrazolium salts, such as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT), 2,3,5-triphenyltetrazolium chloride (TTC) and (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) which under enzymatic conversion turn to coloured formazan, whose absorbance is easy to determine using a spectrophotometer at 540 nm (Balouiri et al. 2016). The agar dilution method is also used in which extract or isolated compound is diluted within agar media, and more than one microbial strain is streaked on the surface of agar plates with diluted extract or isolated compound. The protocols for dilution methods were published as CLSI (Clinical & Laboratory Standards Institute) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations (Balouiri et al. 2016).

Using an FIC (fractional inhibitory concentration index), it is possible to predict the synergistic, additive or antagonistic activity of two compounds *in vitro*. The checkerboard techniques are commonly used to determine interaction with two compounds or extracts (EUCAST 2000; Odds 2003; Mun et al. 2014; Fratini et al. 2017).

2.1 Activity of Natural Antimicrobials: Target Sites in Microbial Cells and Their Communities

Conservative approach of antimicrobial activity-based screening of natural product must evolve to a second step which includes research based on target sites in microbial cells and biofilm disruption studies in microbial communities. After the spectrum of activity is known, the effect on target microbial species with special emphasis on the mechanism of action should be performed (Pandey and Agnihotry

Table 1.1 State-of-the-art of antimicrobial activity studies based on microbial target sites (Shahid et al. 2009; Savoia 2012; Pandey and Agnihotry 2015; Lu et al. 2019)

Approach	Examples for target sites	
Adhesion	Inhibition of adhesion (to epithelia or abiotic surfaces)	Inhibition of adhesins
Capsule formation	Inhibition of capsule formation	Inhibition or complex with cell wall envelope compounds
Cell wall	Inhibition of cell wall synthesis Disturbing physical structure of cell wall; disturbing the functional properties of cell wall	Complex with cell wall components; ROS production; inhibition of cell wall proteins
Cell membranes	Inhibition of membrane synthesis Disturbing physical structure of membranes	Cytoplasmic membrane function inhibitors; ROS production; inhibition of membrane-bound enzymes; disruption of permeability
Nucleic acid and protein synthesis	Inhibition of nucleic acid and protein synthesis	DNA intercalator molecules; DNA gyrase inhibition; RNA polymerase, gyrase and topoisomerase IV inhibition; disruption of mitochondrial activity
Metabolism	Inhibition of steps in metabolic processes	Free radicals irreversibly inhibit microbial proteins; binding to iron; anti-peroxidation activity; inhibition of sporulation or germination; modulation of microbial cytoskeleton
Efflux of xenobiotics from microbial cells by efflux pumps	Inhibition of efflux pumps	Highly selective inhibition of proteins (efflux pumps)
Virulence	Inhibition/disabling virulence factors	Inhibition of toxin, pigment or hemolysin production; inhibition of extracellular enzymes
Biofilm: Attachments to surfaces Maturing of biofilm Quorum sensing (QS)	Inhibition of adherence, attachment and coordination Disruption of mature biofilm formation Inhibition of QS signalling and QS molecules Inhibition of ECM generation and polymer matrix	Inhibition of <i>de novo</i> biofilm formation (by chemoprevention); reduction of the expression of QS-controlled genes; inhibition of planktonic-to-sessile switching process, etc.

2015). Some of the promising target site-based studies are presented in Table 1.1. As it can be realised, the discovery of new biologically active molecules among natural products has targeted the most sites in microbial cells and biofilm as conventional synthetic molecules. These target sites include the *in vitro* research into adhesion of microbial cells as the first step to infection in both planktonic and sessile

cells, cell wall and membrane interactions, disruption of nucleic acid and protein synthesis, virulence factors inhibition and modulation of efflux-pumps and expression of genes involved in regulation of efflux proteins. It is interesting to highlight the advancements and progress in clinical studies of natural products as medical devices or medicinal products for diseases connected with biofilm formation such as dental plaque, periodontal diseases, and dental stomatitis; indwelling urinary tract catheters and *Pseudomonas aeruginosa*-related lung cystic fibrosis (Lu et al. 2019).

It is well known that some natural products do not exhibit antimicrobial activity *per se*, but express some valuable and potent effects on microbial cells like inhibition of efflux-pumps, virulence factors of biofilm formation (Pandey and Agnihotry 2015). These could act as an advantage to combinational therapy with known antimicrobials, both from natural origin and synthetic drugs. Some of these molecules could potentiate microbicidal activity and disarm microbial cells and even stop or slow down the antimicrobial resistance.

3 Responses to the Emerging Microbial Infections

Apart from slow but progressive rise of multiple-drug resistant bacterial strains and clones, mainly among clinical settings, there are also threatening new emerging infectious diseases. Similar to the synthetic compounds, different biological activities could be found in the compounds of natural origin. This fact could act as starting point to build a public library bases with proved activities (from the *in vitro* screenings, not from dockings or *in silico* computational approach), and any future activity to be discovered. The good examples include anti-inflammatory activity together with antimicrobial, or antioxidant and inhibitory to acetylcholinesterase activity. Moreover, not only natural products isolated from marine organisms, bryostatin-1 from macrolide family showed anti-cancer activity but also anti-CoVs inhibitory activity as well (Yi et al. 2003).

From the other side, the scientific base of activity molecule data can be used to check activity against new and emerging diseases such as SARS, MERS and SARS-CoV-2 viruses which cause a global treat. The biology of later mentioned virus is well known from the outcomes caused by similar viruses from the same group, SARS or MERS. Research could focus more on the main targets of SARS-CoV-2 or on the interaction between host immune defences and the viral virulence/adherence/replication machinery. Google Scholar reveals more than 4000 articles with keywords SARS-CoV-2 and herbal published in 2020 and 2021, including the patent reviews as well. As pandemic emerges world-wide, there are also explosion of researches related to inhibition of viral adherence (as first step in infection) through inhibition of adherence to the ACE2 metalloprotease receptor on host cells, inhibition of 3C-like or papain-like proteases, block the spike-proteins on CoV-2 surface, viral replication/transcription Nsp-12 as RNA-dependent polymerase or CoV-2 helicase (Huang et al. 2020). Some natural

compounds, such as flavonoid baicalin, interact with the weakened host immune system as a corrective to lymphopenia in patients infected with SARS-CoV-2 (Zhang and Liu 2020). Some of natural compounds act as cytoprotective agents in *in vitro* culture infected with CoV-2, like furocoumarin-related compound ICEP4 isolated from *Angelica archangelica* L. (Apiaceae) roots and seeds (Galindo-Cardiel et al. 2020). In an extensive review, Vougiannopoulou et al. (2021) presented current state-of-the art of research or library screening approach to inhibitors of viral adherence/replication/transcription or cytopathic effects of different natural compounds.

4 Future Perspectives

Outbreaks of diseases that are not curable or where existing therapeutic options are not effective, or infections where pathogen resistance is expected, are the new areas for more extensive research with natural products. Some of the preclinical studies must be conducted in goal-based level. Such a goal could include interaction of microbial cells in guts of multicellular model organisms, after the extensive *in vitro* studies on spectrum on antimicrobial activity and mode of action successfully was provided. The *in vitro* studies must include all spectrum of possible activities: against planktonic and sessile cells (biofilm), mimicking as possible as it is possible the *in vivo* situation. Special care must be provided to methodology of susceptibility testing and definition of microbial models or synergism with known antimicrobials. Only pure, chemically defined natural products should be regarded as possible candidate molecules for next level of research. The biotransformation, secretion and bioavailability of natural products is crucial for activity on infections site(s). Stability in pharmaceutical preparations, interaction with microbiota after application and pharmacodynamic and pharmacokinetic studies are crucial for future application according to indication. Besides the traditional use of herbal medicines, future studies must be focused on rigorously defined studies both in preclinical or clinical phases. We have enough good examples from the past, just to mention on artemisinin isolated from *Artemisia annua* also as first or second treatment for uncomplicated *Plasmodium falciparum* malaria and for *Plasmodium vivax* resistant to chloroquine (WHO 2020). Artemisinin can also be used in micromolar concentrations to treat diseases other than malaria such as diseases caused by *Schistosoma*, *Fasciola*, *Clonorchis*, *Echinostoma*, *Leishmania* *Toxoplasma gondii*, *Neospora caninum*, *Eimeria tenella* and amoebae (Krishna et al. 2008). The mode of action of natural products their safety profile, and the mechanism of resistance to parasites at the cellular and molecular level should be clearly defined. Furthermore, to avoid problems of short half-life, bioavailability, etc. it is possible to design the drug delivery systems, including liposomes, niosomes, albumin, micelle and other nanoparticles (Efferth et al. 2016).

5 Conclusion

With an exceptional chemical diversity and a wide range of biological activities, the natural products derived from plants, microbes and other sources are the promising pool of new molecules that can lead to clinical studies. Among many activities proved during medicinal products discovery and development, antimicrobial research area of natural products is a highly propulsive field, especially among academic research teams and small and middle biotechnology companies. There are few basic steps in drug discovery and development that include basic screenings (mainly for unstudied plant species from exotic places), investigating the mechanisms of antimicrobial action through identified target sites on cellular and/or molecular level. Synergistic activity in combination with antimicrobial drugs is plausible effect and worth exploring in depth, especially *in vitro*. The future of plant-derived natural products will also include freely accessible data through open access databases which can lead to faster cooperation between academic and private sector. Furthermore, the synergism of plant-derived natural products with known antimicrobials warrants further research. The next step in scientific approach to antimicrobial effective natural products is to define their effectiveness *in vitro*, using all current knowledge to avoid the void of bioavailability, half-life PK/PD values, etc. Finally, the natural products are ascribed as multitarget or multifunctional molecules with more than one proved biological activities which is an added advantage.

Acknowledgements Mahendra Rai is thankful to the Polish National Agency for Academic Exchange (NAWA) for financial support (Project No. PPN/ULM/2019/1/00117/A/DRAFT/00001) to visit the Department of Microbiology, Nicolaus Copernicus University, Toruń, Poland.

References

- Anand U, Nandy S, Mundhra A, Das N, Devendra Kumar P, Dey A (2020) Investigation on antimicrobial botanicals, phytochemicals and natural resistance modifying agents from the plant family of Apocynaceae; possible therapeutic approaches against multidrug resistance in pathogenic microorganisms. *Drug Resist Updat* 51:100695
- Aparna V, Dineshkumar K, Mohanalakshmi N, Velmurugan D, Hopper W (2014) Identification of natural compound inhibitors for multidrug efflux pumps of *Escherichia coli* and *Pseudomonas aeruginosa* using in silico high-throughput virtual screening and in vitro validation. *PLoS One* 9(7):e101840
- Balouiri M, Sadiki M, Ibensounda SK (2016) Methods for in vitro evaluating antimicrobial activity: a review. *J Pharm Anal* 6:71–79
- Borris RP (1996) Natural products research: perspectives from a major pharmaceutical company. *J Ethnopharmacol* 51:29–38
- Chernov VM, Chernova OA, Mouzykantov AA, Lopukhov LL, Aminov RI (2019) Omics of antimicrobials and antimicrobial resistance. *Expert Opin Drug Discovery* 14:455–468
- de Sousa Luis JA, Barros RPC, de Sousa NF, Muratov E, Scotti L, Scotti MT (2020) Virtual screening of natural products database. *Mini Rev Med Chem.* <https://doi.org/10.2174/1389557520666200730161549>

- Dhama K, Khan S, Tiwari R, Sircar S, Bhat S, Malik YS, Singh KP, Chaicumpa W, Bonilla-Aldana DK, Rodriguez-Morales AJ (2020) Coronavirus disease 2019-COVID-19. *Clin Microbiol Rev* 33:e00028-20. <https://doi.org/10.1128/CMR.00028-20>
- Efferth T, Romero MR, Bilia AR, Osman AG, El Sohly M, Wink M, Bauer R, Khan I, Bergonzi MC, Marin JGG (2016) Expanding the therapeutic spectrum of artemisinin: activity against infectious diseases beyond malaria and novel pharmaceutical developments. *World J Tradit Chin Med* 2:1–23
- EUCAST (2000) Terminology relating to methods for the determination of susceptibility of bacteria to antimicrobial agents. *Clin Microbiol Infect* 6:503–508
- Furneri PM, Piperno A, Sajia A, Bisignano G (2004) Antimycoplasmal activity of hydroxytyrosol. *Antimicrob Agents Chemother* 48(12):4892–4894
- Fratini F, Mancini S, Turchi B, Friscia E, Pistelli L, Giusti G, Cerri D (2017) A novel interpretation of the fractional inhibitory concentration index: the case *Origanum vulgare* L. and *Leptospermum scoparium* J. R. et G. Forst essential oils against *Staphylococcus aureus* strains. *Microbiol Res* 195:11–17
- Galindo-Cardiel JJ, Toledo Núñez A, Celaya Fernández M, Ramírez Labrada A, Uranga-Murillo A, Arias Cabrero M, Pardo J, Panzeri E (2020) First-described recently discovered non-toxic vegetal-derived furocoumarin preclinical efficacy against SARS-CoV-2: a promising antiviral herbal drug. *bioRxiv*. <https://doi.org/10.1101/2020.12.04.410340>
- Hards K, Cook GM (2017) Targeting bacterial energetics to produce new antimicrobials. *Drug Res Updat* 36:1–2
- Hoffman PS (2020) Impediments to discovery of new antimicrobials with new modes of action. In: Ahmad I, Ahad S, Rumbaugh R (eds) *Antibacterial drug discovery to combat MDR*. Springer, Singapore
- Huang J, Hume AJ, Abo KM, Werder RB, Villacorta-Martin C et al (2020) SARS-CoV-2 infection of pluripotent stem cell-derived human lung alveolar type 2 cells elicits a rapid epithelial-intrinsic inflammatory response. *Cell Stem Cell* 27:962–973
- Kiran MD, Adikesavan NV, Cirioni O, Giacometti A, Silvestri C, Scalise G, Ghiselli R, Saba V, Orlando F, Shoham M (2008) Discovery of a quorum sensing inhibitor of drug resistant staphylococcal infections by structure-based virtual screening. *Mol Pharmacol* 73:1578–1586
- Krishna S, Bustamante L, Haynes RK, Steines HM (2008) Artemisinin: their growing importance in medicine. *Trends Pharmacol Sci* 29:520–527
- Lasko MJ, Nicolau DP (2020) Carbapenem-resistant Enterobacterales: considerations for treatment in the era of new antimicrobials and evolving enzymology. *Curr Infect Dis Rep* 22:6
- Liu B, Zhou J (2005) SARS-CoV protease inhibitors design using virtual screening method from natural products libraries. *J Comput Chem* 26:484–490
- Lu L, Hu W, Tian Z, Yuan D, Yi G, Zhou Y, Cheng Q, Zhu J, Li M (2019) Developing natural products as potential anti-biofilm agents. *Chin Med* 14:11
- Mun SH, Kang OH, Joung DK, Kim SB, Choi JG, Shin DW, Kwon DY (2014) In vitro anti-MRSA activity of carvone with gentamicin. *Exp Ther Med* 7:891–896
- Odds FC (2003) Synergy, antagonism, and what the checkerboard puts between them. *J Antimicrob Chemother* 52:1. <https://doi.org/10.1093/jac/dkg301>
- Pandey A, Agnihotry V (2015) Antimicrobials from medicinal plants: research initiatives, challenges, and the future prospects. In: Gupta VK, Tuohy MG (eds) *Biotechnology and bioactive compounds. Sources and applications*. Wiley, New York, pp 123–150
- Perfect JR (2016) Is there an emerging need for new antifungals? *Expert Opin Emerg Drugs* 21:129–131
- Pereira JA, Pereira APG, Ferreira ICFR, Valentão P, Andrade PB, Seabra R, Estevinho L, Bento A (2006) Table olives from Portugal: phenolic compounds, antioxidant potential, and antimicrobial activity. *J Agric Food Chem* 54(22):8425–8431
- Rios JL, Recio MC (2005) Medicinal plants and antimicrobial activity. *J Ethnopharmacol* 100:80–84

- Rios JL, Recio MC, Villar A (1988) Screening methods for natural products with antimicrobial activity: a review of the literature. *J Ethnopharmacol* 23:127–149
- Savoia D (2012) Plant-derived antimicrobial compounds: alternatives to antibiotics. *Future Microbiol* 7:979–990
- Seal BS, Drider D, Oakley BB, Brussow H, Bikard D, Rich JO, Miller S, Devillard E, Kwan J, Beetin G, Reeves S, Swift SM, Raicek M, Gay CG (2018) Microbial-derived products as potential new antimicrobials. *Vet Res* 49:66
- Shahid M, Shahzad A, Sobia F, Sahai A, Tripathi T, Singh A, Kham HM, Umesh (2009) Plant natural products as a potential source for antibacterial agents: recent trends. *Anti-Infective Agents Med Chem* 8:211–225
- Shakya AK (2016) Medicinal plants: future source of new drugs. *Int J Herb Med* 4:59–64
- Sheng-Ji P (2011) Ethnobotanical approaches of traditional medicine studies: some experiences from Asia. *Pharm Biol* 39(sup1):74–79
- Sorokina M, Steinbeck C (2020) Review on natural products databases: where to find data in 2020. *J Chemoinformat* 12:20
- Tempesta MS, King SR (1994) Ethnobotany as a source for new drugs. *Ann Rep Med Chem* 29:325–330
- Tuck KL and Hayball PJ (2002) Major phenolic compounds in olive oil: metabolism and health effects. *J Nutr Biochem* 13:636–644
- Tassou CC and Nychas GJE (1995) Inhibition of *Salmonella enteritidis* by oleuropein in broth and in a model Food system. *Lett Appl Microbiol* 20:120–124
- Vila J, Moreno-Morales J, Balleste-Dalpiere C (2020) Current landscape in the discovery of novel antibacterial agents. *Clin Microbiol Infect* 26:596–603
- Vougianniopoulou K, Corona A, Tramontano E, Alexis MN, Skaltsounis A-L (2021) Natural and nature-derived products targeting human coronaviruses. *Molecules* 26:448
- WHO (2020) Malaria. <https://www.who.int/news-room/fact-sheets/detail/malaria>. Accessed 6 Feb 2021
- Yi Y, Lin H, Yao X, inventors; Univ Pla 2nd Military Medical, assignee. (2003 Nov 12) Use of total bryostatin for preparing medicine for anti SARS coronavirus. China patent CN 1454593
- Zhang L, Liu Y (2020) Potential interventions for novel coronavirus in China: a systematic review. *J Med Virol* 92:479–490

Chapter 2

Nepalese Pteridophytes Used as Antimicrobials: Challenges and Opportunities



Shandesh Bhattarai and Ripu M. Kunwar

Abstract Pteridophytes constitute the primitive vascular plant group, which are found scattered all over the world. There are 580 taxa of Nepalese pteridophytes, which are most diverse and adapted in different climatic zones forming an attractive component of the vegetation showing different ecological habits such as epiphytic, lithophytic, terrestrial, tree ferns, hanging club mosses, climbers, and hydrophytic. Human beings have been using pteridophytes because of their several useful properties, including food and medicine. Some important bioactive compounds have been identified from the Nepalese pteridophytes and the chemical compounds isolated have shown antimicrobial properties, which has revealed that pteridophytes play a potential role in Nepalese pharmacopoeia and drug discovery. Finally, the challenges and opportunities of pteridophyte research are discussed.

Keywords Bioactivity · Central Nepal · Ethnomedicine · Fern · Natural antimicrobial

Abbreviations

ATCC	American Type Culture Collection;
CITES	International Trade in Endangered Species of Wild Fauna and Flora
IUCN	The International Union for Conservation of Nature
MBC	Minimum Bactericidal Concentration
mg	Milligram
MIC	Minimum Inhibitory Concentration
mL	Milliliter

S. Bhattarai (✉)

Faculty of Science, Nepal Academy of Science and Technology, Lalitpur, Nepal

R. M. Kunwar

Ethnobotanical Society of Nepal, Kathmandu, Nepal

mm	Millimeter
MRSA	Methicillin-Resistant <i>Staphylococcus aureus</i>
MTCC	Microbial Type Culture Collection
µg	Microgram

1 Introduction

Pteridophytes have a long geological history on our planet (Pandey et al. 1996). They are known from as far back as 380 million years. Fossils of pteridophytes have been obtained from rock strata belonging to Silurian and Devonian periods of the Paleozoic era (Pandey et al. 1996).

According to one school of thought, the pteridophytes like bryophytes have evolved from algae and both the groups are parallel to each other in their origin and they are not linked phylogenetically (Pandey et al. 1996). But, some researchers do not trace their origin from any particular group of algae (Eames 1936). Some believe that pteridophytes originated from a particular group of marine algae, that is, Phaeophyceae (Church 1919; Arnold 1947). Pteridophytes originated from erect and parenchymatous green algae with an isomorphic life cycle (Pandey et al. 1996). The pteridophytes resemble the bryophytes in many respects, and some researchers assumed that the pteridophytes have originated from bryophytes. The two groups (bryophytes and pteridophytes) are phylogenetically connected, but they are divergent evolutionary lines evolved from hypothetical terrestrial plants of primitive archegonia type (Zimmermann 1938; Pandey et al. 1996). They have arisen from an anthocerotean type of bryophytes.

Pteridophytes are nonflowering plants with well-developed vascular system. The most characteristic feature of pteridophytes is the presence of independent gametophytes and sporophytes at maturity. They have leaves (known as fronds), roots and sometimes true stems, and tree ferns have full trunks, constitute an essential and major group of plant kingdom possessing extinct and extant members (Pandey et al. 1996; www.plantlist.org). The root system is always adventitious. The stem is either underground or aerial. The leaves may be microphylls or megaphylls. Pteridophytes do not have seeds or flowers either; instead, they also reproduce via spores. They are divided into four classes: Psilopsida, Lycopsidea, Sphenopsida, and Pteropsida (Sinnot 1935; Pandey et al. 1996).

Human beings have been using pteridophytes because of their several useful properties, including food and medicine. Some important bioactive compounds have been identified from the Nepalese pteridophytes and the chemical compounds isolated have shown antimicrobial properties, which has revealed that pteridophytes play a potential role in Nepalese pharmacopoeia and drug discovery. The principal aim of this chapter is to collect the dispersed data about the Nepalese pteridophytes used as antimicrobials. Finally, the challenges and opportunities of pteridophyte research are discussed.

2 Diversity and Distribution

There are around 13,271 species of living pteridophytes in the world (Hassler 2018; www.plantlist.org), which accounts for nearly 3% of the world flora. The pteridophytes constitute an important component of the Nepalese flora. Fraser-Jenkins et al. (2015) listed 580 taxa of Nepalese pteridophytes (550 species and an additional 30 subspecies), based only on material seen and verified. Within Nepal, the region with the greatest number of species recorded is Central Nepal followed by Eastern Nepal. Western Nepal has the lowest number of species recorded (Fraser-Jenkins et al. 2015). Some fern species of Nepal are shown in Figs. 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, and 2.8.



Fig. 2.1 *Adiantum capillus-veneris*



Fig. 2.2 *Adiantum philippense*



Fig. 2.3 *Aleurites bicolor*



Fig. 2.4 *Angiopteris helferiana*

Ferns are abundantly found in humid and shady forests. They are most diverse in the tropics, and in Nepal, in different climatic zones, they form an attractive component of the vegetation of its hills and forests showing different ecological habit as epiphytic, lithophytes, terrestrial, tree ferns, hanging club mosses, climbers, and hydrophytes (Gurung 1984; Hogan 2004). Fern flora, which usually prefers to grow on tree bases, tree trunks, branches, and tree tops clothed with mosses and leafy liverworts, are termed as the epiphytic ferns (Rajbhandary 2016). Of the epiphytes, the most common and widely distributed species are *Asplenium nidus*, *Drynaria quercifolia*, *Microsorium punctatum*, *Lepisorus thunbergianus*, *Pyrrosia lanceolata*, *Nephrolepis cordifolia*, etc. Fern and fern allies usually occur in rock crevices



Fig. 2.5 *Asplenium dalhousae*



Fig. 2.6 *Diplazium esculentum*

or mossy cliffs or those that occupy bare or humus-rich rocks in shady areas are called lithophytic ferns. The most common and widely distributed lithophytic species are *Adiantum capillus-veneris*, *Cheilanthes farinose*, *Onychium siliculosum*, *Odontosoria chinensis*, *Pteris vittata*, *Selaginella* spp., *Asplenium* spp., *Dryopteris cochleata*, etc.

The terrestrial area included exposed areas, shady areas, stream banks, and hill slopes. In evergreen and semievergreen forests where the forest flora is rich in humus and organic nutrients, some ferns and fern allies are found growing and they are called as terrestrial ferns. Some of the terrestrial ferns are *Lycopodium japonicum*, *Dryopteris juxtaposite*, *Osmunda japonica*, and *Nephrolepis auriculata*



Fig. 2.7 *Lygodium flexuosum*



Fig. 2.8 *Oeosporangium teinufolia*

(Rajbhandary 2016). Some species of climbing ferns found in in Nepal are *Lygodium flexuosum*, *L. japonicum*, *Stenochlaena palustris*, etc. Some ferns grow on ponds, puddles, banks of streams, ravines, waterfalls, and swampy areas: these are termed as hydrophytes. Some examples are *Isoetes coromandelina*, *Equisetum ramosissimum*, *Ceratopteris thalictroides*, *Marsilea minuta*, and *Diplazium polypodioides* (Kandel and Fraser-Zenkins 2020).

3 Bioactive Compounds

Secondary metabolites are important compounds which are produced to confer a selective advantage to the organism. The secondary metabolites, flavonoids, have anti-inflammatory properties through the inhibition of the cyclo-oxygenase pathways (Liang et al. 1999). *Selaginella* species has a large number of bioactive compounds, which contains alkaloid, phenols, sterol, aliphatic acid, and terpenoid. Biflavonoids, such as amentoflavone, sumafavone, robustafavone, ginkgetin, hinokiflavone, and isocryptomerin, are the most important valuable natural products of *Selaginella* (Li et al. 2014a). Some bioactive flavones such as amentoflavone, robustafavone, biapigenin, hinokiflavone, podocarpusflavone A, and ginkgetin, in *Selaginella* sp., were reported to have antioxidant, antiviral, and anticancer activities (Shi et al. 2008; Liu et al. 2011; Li et al. 2014b). Flavonoids and phenolic compounds are the most common group of bioactive compounds present in plants, occurring in almost all plant parts, but mainly in photosynthesizing cells. These compounds are the main sources of coloration in blooming plants (Koes et al. 2005).

4 Antimicrobial Activity

Antimicrobial medicines can be grouped according to the microorganisms they act primarily against (Bhattarai et al. 2008, 2009). For example, antibiotics are used against bacteria, and antifungals are used against fungi. Recent studies comparing natural derivatives from plants with synthetic antimicrobials have shown that natural substances could be safer (Dorman and Deans 2000; Burt and Reinders 2003; Hygreeva et al. 2014).

There are many antibiotics currently available for the treatment of bacterial and fungal infections, but they are not always reliable against pathogenic organisms (Gearhart 1994). This situation has forced scientists to search for new bioactive compounds from plants (Kumar et al. 2006). In recent years, the search for phytochemicals with antioxidant, antimicrobial, or anti-inflammatory properties has been on the rise due to their potential use for the treatment of various chronic and infectious diseases (Halliwell 1996). Plant extracts have numerous secondary metabolites, having antimicrobial and antioxidant properties. Traditionally, the biomedical system, Ayurveda system, and Unani system of medicine all have suggested the medicinal use of ferns (Baskaran et al. 2018).

5 Activity of Ferns

Plant extracts have been widely used since ancient times for treating human illness. Numerous studies of the antimicrobial activity of different natural products have been reported (Bhattacharjee et al. 2006; Parekh and Chanda 2006, 2007). Although the medicinal value of pteridophytes has been known to traditional cultures for more than 2000 years, they are used only on a small scale in modern chemotherapy. Studies of the bioactivity of pteridophytes are still in their early years compared with angiosperms (Baskaran et al. 2018).

5.1 Activity Against Bacteria

The methanol extracts of *A. capillus-veneris* and *A. venustum* have been tested for their antimicrobial activity against five Gram-positive, six Gram-negative (including multiresistant *S. aureus*) bacterial and eight fungal strains using standard micro-dilution assay. Maximum activity was exhibited by *A. venustum* followed by *A. capillus-veneris*. The extract of *A. capillus-veneris* had a very low MIC value (0.48 µg/mL) against *Escherichia coli*, whereas *A. venustum* extract activity against *Aspergillus terreus* showed an MIC of 0.97 µg/mL (Singh et al. 2008).

The antagonistic potential of crude extract of *Adiantum philippense* was studied using agar cup/well diffusion method against food pathogens (*S. aureus*, *E. coli*, *P. aeruginosa*, and *S. flexneri*). Antibacterial activity results were portrayed in the form of zone of inhibition and revealed substantial antagonistic activity against all the tested bacterial strains. *S. aureus* was found to be more susceptible when compared to *E. coli*, *P. aeruginosa*, and *S. flexneri* (Adhan et al. 2020).

Adiantum caudatum was evaluated for its antibacterial potential and phytochemical contents in various solvent extracts of the plant in increasing polarity toward bacterial species involved in skin diseases. The test organisms include *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *Serratia marcescens*. Water extracts did not show any antibacterial activity toward tested organisms; the same condition was observed with petroleum ether extracts. Acetone extract of *A. caudatum* showed moderate level of inhibition toward *S. aureus*; the plant showed lower level of inhibition toward *E. coli* compared to the other bacterial strains. *P. aeruginosa* and *S. marcescens* are the most sensitive organisms toward the methanol extract of the plant. The plant extracts did not show any antibacterial activity against *E. coli* (Thomas 2014). Petroleum ether extract contained nonpolar compounds dissolved in it, and these compounds did not have antibacterial activity. Likewise, water extract contained highly polar compounds and these compounds also showed lowest level of antibacterial activity. Methanolic extract of *A. caudatum* showed maximum action against *P. aeruginosa*, Gram-negative bacteria. The present antibacterial analysis of the plant supports the ethnobotanical importance of *A. caudatum*. The plant showed antibacterial activity in methanol extract. The methanol extract of the

plant showed maximum level of activity toward *P. aeruginosa*. Petroleum ether and water extracts did not show any antibacterial activity toward any of the tested organisms. Methanolic extract of the plant exhibited minimum inhibitory concentration as 50 mg/ml and minimum bactericidal concentration as 25 mg/ml toward *P. aeruginosa* (Thomas 2014). Bioactivity studies showed strong antibacterial activity (MIC = 31.3–62.5 µg/mL) of *Blechnum orientale* against five Gram-positive bacteria (Lai et al. 2017).

The Malasar tribes in the Valparai hills, India, use *Dicranopteris linearis* due to their wound-healing activity (Santhosh et al. 2014). Also, wound-healing activity has been reported for *Nephrolepis cordifolia* (Upreti et al. 2009). The 1:10 dilution of the essential oil of *Equisetum arvense* was shown to possess a broad spectrum of a very strong antimicrobial activity against *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *Salmonella enteritidis* (Radulović et al. 2006). *Dryopteris cochleata* possesses antibacterial principles, soluble in acetone, which hinder the growth and multiplication of some multidrug-resistant bacterial strains (Thomas 2009).

Yenn et al. (2018) aimed to evaluate the antibacterial potential of *Helminthostachys zeylanica* on foodborne *Bacillus cereus*. The ethanolic extract showed significant inhibitory activity on *B. cereus* with a sizeable clear zone detected on disc diffusion assay. On broth microdilution assay, the MIC of the extract on *B. cereus* was 6.25 mg/ml and the MBC was 12.5 mg/ml. The inhibitory activity of the extract on *B. cereus* was bactericidal. In the growth dynamic study, the antibacterial efficacy of the extract was concentration dependent, where a lower colony-forming unit count was obtained with increased extract concentration. The GCMS analysis of the extract showed that the major constituents of the extract were phenol (36.26%) and quercetin (29.70%). This study is important as it shows the potential use of *H. zeylanica* as an effective agent to control *B. cereus*-related infections (Yenn et al. 2018).

On the other hand, *L. clavatum* was formerly reported to contain various phenolic acids such as dihydrocaffeic, vanillic, p-hydroxy-benzoic, syringic, p-coumaric, and ferulic acids (Towers and Maass 1965) and phenolic acids are known to display antimicrobial activity against a variety of microorganisms (Herald and Davidson 1983; Stead 1993). For instance, ferulic acid has been shown to have antimicrobial activity by several researchers against *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *C. albicans* as well as *Listeria monocytogenes* (Fernandez et al. 1996; Kwon et al. 1997; Panizzi et al. 2002; Wen et al. 2003). In one study, syringic, caffeic, isovanillic, ferulic, and p-hydroxycinnamic acids were found to exhibit antimicrobial activity (Fernandez et al. 1996). For that reason, the presence of these compounds in this plant may explain their antibacterial activity. In the past, plants have afforded a number of anti-infective agents including emetine, quinine, and berberin (Iwu et al. 1999). The *Lycopodium* genus is also known to be rich in alkaloids with high toxicity (Ayer 1991). This may also contribute to the antimicrobial activity of the LC extracts (Ainge et al. 2002).

Antibacterial and antifungal activities of the *L. clavatum* extracts were tested against standard and isolated strains of the following bacteria: *E. coli*, *P. aeruginosa*, *P. mirabilis*, *A. baumannii*, *K. pneumoniae*, *S. aureus*, and *B. subtilis*. All the

extracts possessed noteworthy activity against ATCC strains of *S. aureus* (Orhan et al. 2007a). Phytochemical analysis of water extracts of *Lygodium flexuosum* confirmed the presence of glycosides and carbohydrates, but alkaloids, terpenoids, steroids, saponins, tannins, and flavonoids were absent (Nayak et al. 2013).

Verma et al. (2015) used four bacterial strains viz. *E. coli*, *K. pneumoniae*, *E. faecalis*, *S. aureus* for antimicrobial assay of the extracts and isolated compounds. Hexane and acetone extracts of *Selaginella bryopteris* and compounds isolated from it were tested for their antimicrobial activity, excluding (+)-syringaresinol, amentoflavone, due to paucity of the sample. The zone of inhibition and MIC values for extract as well as isolated compounds were measured against some Gram-positive and Gram-negative bacteria. No antimicrobial activity was observed in the n-hexane extract, but the acetone extract of *S. bryopteris* showed activity against all the microbes tested in this study. The maximum effect of the acetone extract was observed against *K. pneumoniae* and *S. aureus*. Usually, the n-hexane extract contains mainly nonpolar compounds such as oils and fats, while the acetone extract contains polar compounds, including phenols, glycosides, alkaloids, steroids, terpenoids, antioxidants, etc.

Vanillic acid is also the most potent antimicrobial agent present in *S. bryopteris*. It has activity against both Gram-positive and Gram-negative bacteria as well as against different fungal strains. Only β -sitosterol β -D-glucoside showed some activity against bacteria. Thus, it seems that addition of the glucoside moiety to β -sitosterol increases its potency as an antimicrobial agent (Verma et al. 2015).

The antimicrobial activity of *Tectaria macrodonta* may be attributed to various phytochemicals, namely, saponins, tannins, anthocyanin, flavonoid, phenol, and alkaloid (Masal and Dongare 2010). The aqueous, ethanol, and methanol extracts of the rhizomes of *T. macrodonta* were evaluated for its potential antibacterial properties. Potential antibacterial activities were exhibited by the methanol and ethanol extracts of *T. macrodonta* (Poudyali and Singh 2013).

5.2 Activity Against Fungi

Antifungal activities of the extracts of *Lycopodium clavatum* were tested against standard and isolated strains of *Candida albicans* and *C. parapsilosis*. The *L. clavatum* extracts showed reasonable antifungal effects (Orhan et al. 2007a, b).

Verma et al. (2015) used three fungal strains, viz. *C. albicans*, *C. krusie*, and *C. tropicalis*, for antifungal assay of the extracts and isolated compounds. Hexane and acetone extracts of *Selaginella bryopteris* and compounds isolated from it were tested for their antifungal activity. The zone of inhibition and MIC values for extract as well as isolated compounds were measured against some *Candida* species. No antimicrobial activity was observed in the n-hexane extract, but the acetone extract of *S. bryopteris* showed activity against all the microbes tested. It was concluded that Vanillic acid is the most potent antifungal agent present in *S. bryopteris* and

β -sitosterol, β -sitosterol β -D-glucoside were effective against fungal strains (Verma et al. 2015).

5.3 Activity Against Viruses

Using vesicular stomatitis virus in monkey cell cultures as test organism, the extracts of *A. capillus-veneris* were found to exhibit antiviral activity (Husson et al. 1986). The antihyperglycemic and analgesic activity of the leaves of *A. philippense* has been studied by Tanzin et al. (2013). The whole plants of *A. capillus-veneris* were used by the tribes of the Valparai hills, Western Ghats, and Tamil Nadu, India, for their hypoglycemic and anticancer activity (Santhosh et al. 2014). Both leaves and rhizomes of *A. capillus-veneris* have been used in the preparation of herbal drugs for treating diabetes in India and Europe (Baskaran et al. 2018).

Some bioactive flavones, such as amentoflavone, robustaflavone, biapigenin, hinokiflavone, podocarpusflavone A, and ginkgetin, in *Selaginella* sp., were reported to have antiviral and anticancer activity (Ma et al. 2001; Shi et al. 2008; Liu et al. 2011; Li et al. 2014b). *Lygodium flexuosum* have shown significant effects against viral disease and jaundice. Butylated hydroxytoluene is primarily used as a food additive that exploits its antioxidant properties. It is also documented as an antioxidant additive in such diverse products as cosmetics and pharmaceuticals. It has been reported to have antiviral effects particularly in use against herpes family viruses and in combination with L-lysine and Vitamin C (Snipes et al. 1975; Brugh 1977; Kim et al. 1978; Richards et al. 1985; Pirtle et al. 1986; Chetverikova et al. 1989; Chetverikova and Inozemtseva 1996). Flavonoid derived from *Cheilanthes tenuifolia* possesses potent anticancerous and antioxidant activities that are responsible for their chemo-preventive potential (Jarial et al. 2018).

Lycopodium clavatum CHCl extract exerted good antiviral effect toward the DNA virus HSV (16–8 lg/ml) with the MNTC of 16 lg/ml, similar to that of acyclovir (16 to <0.25 lg/ml), except for its therapeutic range of LC-CHCl₃ was narrower. As to PI-3, LC-PE and LC-CHCl₃-Alk exhibited some inhibition (16–4 and 32–4 lg/ml, respectively). In particular, the alkaloid fraction of *L. clavatum* showed quite similar anti-PI-3 effect and MNTC value to that of oseltamivir (32 to <0.25 lg/ml) (Orhan et al. 2007a, b).

6 Challenges

Pteridophytes have remained the exclusive domain of academicians, rarely heard outside the academic world. This chapter discusses about the antimicrobial properties of pteridophytes. Moreover, the bioactive compounds and traditional usage of Nepalese ferns have also been discussed. Various anthropogenic activities like

deforestation for agricultural land expansion, logging, urbanization, and roads/trails building activities have resulted in tremendous pressure on the natural habitat of fern species.

Some of the ferns including tree fern are also not devoid of economic exploitation in some parts of the world, as it has high market demand due to its multiple socioeconomic uses viz. ornamental, horticulture, food, and medicinal uses (Chandra and Fraser-Jenkins 2008; Rout et al. 2009; Singh and Singh 2012), resulting in the rapid decline of the wild population resulted many species of tree ferns under threatened category in IUCN Red Data book and Appendix II of CITES. Tree ferns are being suffered from the unsustainable harvesting of fronds for food and fodder without being aware of the taking into consideration of its own natural viability challenges and its conservation status. Little knowledge on the importance of tree fern stands as one of the major factors for the declining its populations in that area. Currently, construction of rural roads in Nepal has become a major threat to the habitat as well as species itself as such construction practices are not well monitored. Keeping the aforesaid statements in view, the existing threats for tree fern expand across the social, economic, and environmental dimensions both globally and locally due to which they continue to be under great threat of extinction.

The family Cyatheaceae is listed in Appendix II of Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), 1975 in order to protect these epibiotic tree ferns from being sold randomly and overexploited. It is also listed in threatened category of IUCN Red Data Book in 1998. Despite the family being listed in CITES and IUCN Red Data Book, it has not been assessed for special protection in Nepal (Thapa et al. 2017).

Very limited researches have been conducted for the fern conservation in Nepal. Researches on reproductive biology of fern in Nepal are almost null due to which there is a huge gap in scope for effective conservation and management of fern species. Beside the natural conditions to be considered for fern conservation, the actual threats from the human use in Nepal are still not known clearly. This is a huge knowledge gap to be addressed to identify the effective conservation measures for ferns. The awareness in communities on the importance and conservation of ferns is very limited. Despite their dependency on fern dominantly for food and fodder purpose and few for medicinal values, people lack strong rationale for initiating some effective and concrete conservation initiatives to protect the remaining natural patches. There is a crucial need of generation of knowledge on fern and its dissemination to the communities and developing a conservation and management plan. This step will provide a foundation on which the conservation efforts for protecting the tree fern species can be extended in the areas of the country and the region.

7 Opportunities

The pteridophytes constitute an important component of the Nepalese flora: the greatest number of species is recorded in Central Nepal. In recent years, the importance of ferns and their allies in plant science research has been increasing continuously (Baskaran et al. 2018); however, only a small number of species have been analyzed for the pharmaceutical property. In the context of Nepal, pteridophytes research is in preliminary phase. We hope that this chapter will encourage the Nepalese scientists to search more ferns for discovery of novel antimicrobials. Further research on high valued pteridophytes for exploration of chemical constituents and their commercialization into national level is recommended.

The present contribution attempts to describe the scope of pteridophytes in antimicrobial drug discovery, pharmacology, and ethnopharmacopoeias. Such initiative is crucial for conserving traditional knowledge and paving the way forward for antimicrobial drug discovery.

8 Conclusions

Some identified important isolated compounds from the Nepalese pteridophytes have shown antimicrobial properties, which has revealed that pteridophytes play a potential role in Nepalese pharmacopoeia and drug discovery. In recent years, the importance of ferns and their allies in plant science research has been increasing continuously; however, only a small number of species have been analyzed for their pharmaceutical property. In the context of Nepal, pteridophytes research is in preliminary phase. We hope that this chapter will encourage the Nepalese scientists to explore more ferns that helps in the discovery of novel antimicrobial drugs for human benefits. A descriptive research on high valued pteridophytes for exploration of chemical constituents and their commercialization into national level is recommended.

Acknowledgments We are thankful to Mr. Dhanraj Kandal, Scientific Officer at National Herbarium and Plant Laboratories, Department of Plant Resources, Godawari, Lalitpur, for providing us the photographs of ferns. The first author thanks Nepal Academy of Science and Technology, Khumaltar, Lalitpur, for various support.

References

- Adhan M, Patel M, Deshpande S, Alreshidi M, Siddiqui AJ, Reddy MN, Emira N, Feo VD (2020) Effect of *Adiantum philippense* extract on biofilm formation, adhesion with its antibacterial activities against foodborne pathogens and characterization of bioactive metabolites: an *in-vitro-in silico* approach. *Front Microbiol* 11:1–19

- Ainge GD, Lorimer SD, Gerard PJ, Ruf LD (2002) Insecticidal activity of huperzine A from the New Zealand clubmoss, *Lycopodium varium*. *J Agric Food Chem* 50:491–494
- Arnold (1947) <https://www.askiitians.com/biology/plant-kingdom/pteridophytes.html>
- Ayer W (1991) The Lycopodium alkaloids. *Nat Prod Rep* 8:455–463
- Baskaran X-r, GeoVigila A-v, Zhang S-z, Feng S-x, Liao W-b (2018) A review of the use of pteridophytes for treating human ailments. *J Zhejiang Univ Sci B* 19(2):85–119
- Bhattacharjee I, Chatterjee SK, Chatterjee SN (2006) Antibacterial potentiality of *Argemone mexicana* solvent extracts against some pathogenic bacteria. *Mem Inst Oswaldo Cruz* 101(6):645–648
- Bhattarai S, Chaudhary RP, Taylor RSL (2008) Antibacterial activity of selected ethnomedicinal plants of Manang District, Central Nepal. *J Theor Exp Biol* 5(1&2):01–09
- Bhattarai S, Chaudhary RP, Taylor RSL, Ghimire SK (2009) Biological activities of some Nepalese medicinal plants used in treating bacterial infections in human beings. *Nepal J Sci Tech* 10:83–90
- Brugh MJR (1977) Butylated hydroxytoluene protects chickens exposed to Newcastle disease virus. *Science* 197(4310):1291–1292
- Burt SA, Reinders RD (2003) Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. *Lett Appl Microbiol* 36:162–167
- Chandra S, Fraser-Jenkins CR (2008) Threatened Pteridophytes of India. In: Verma SC, Khullar SP, Cheema HK (eds) *Perspectives in pteridophytes*. Bishen Singh Mahendra Pal Singh, Dehradun, pp 207–233
- Chetverikova LK, Inozemtseva LI (1996) Role of lipid peroxidation in the pathogenesis of influenza and search for antiviral protective agents. *Vestn Ross Akad Med Nauk* 3:37–40. (in Russian)
- Chetverikova LK, Kildivatov II, Inozemtseva LI, Kramskaia TA, Filippov VK (1989) Factors of antiviral resistance in the pathogenesis of influenza in mice. *Vestn Akad Med Nauk SSSR* 11:63–68. (in Russian)
- Church (1919) Assessed from. <https://plantlet.org/origin-of-pteridophytes/>
- Dorman HJD, Deans SG (2000) Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J Appl Microbiol* 88:308–316
- Eames AJ (1936) *Morphology of vascular plants: lower groups (Psilophytales to Filicales)*. McGraw-Hill, New York
- Fernandez MA, Garcia MD, Saenz MT (1996) Antibacterial activity of the phenolic acid fractions of *Scrophularia frutescens* and *S. sambucifolia*. *J Ethnopharmacol* 53:11–14
- Fraser-Jenkins CR, Kandel DR, Pariyar S (2015) *Ferns and fern-allies of Nepal-volume 1*, vol 508. Department of Plant Resources, National Herbarium and Plant Laboratories, Ministry of Forests and Soil Conservation, Kathmandu
- Gearhart MO (1994) Worsening of lung function with fluconazole and review of azole antifungal hepatotoxicity. *Ann Pharmacother* 28:1177–1181
- Gurung VL (1984) Ferns of Nepal. In: Majupuria TC (ed) *Nepal nature's paradise*. White Lotus Press, Kathmandu, pp 198–211
- Halliwell B (1996) Antioxidants in human health and disease. *Ann Rev Nutr* 16(1):33–50
- Hassler M. 2018. Checklist of ferns and lycophytes of world
- Herald PJ, Davidson PM (1983) Antibacterial activity of selected hydroxycinnamic acids. *J Food Sci* 48:1378–1379
- Hassler M (2018) *World Ferns: Checklist of Ferns and Lycophytes of the World* (version Apr 2018). In: *Species 2000 & ITIS Catalogue of Life, 2018 Annual Checklist* (Roskov Y., Abucay L., Orrell T., Nicolson D., Bailly N., Kirk P.M., Bourgoin T., DeWalt R.E., Decock W., De Wever A., Nieukerken E. van, Zarucchi J., Penev L., eds.). Digital resource at <https://www.catalogueoflife.org/annual-checklist/2018>. Species 2000: Naturalis, Leiden, the Netherlands. ISSN 2405-884X.
- Husson GP, Vilagines R, Delaveau P (1986) Research into the antiviral properties of some natural extracts. *Ann Pharm Fr* 44:41–48

- Hygreeva D, Pandey MC, Radhakrishna K (2014) Potential applications of plant based derivatives as fat replacers, antioxidants and antimicrobials in fresh and processed meat products. *Meat Sci* 98:47–57
- Iwu MM, Duncan AR, Okunji CO (1999) New antimicrobials of plant origin. In: Janick J (ed) Perspectives on new crops and new uses. ASHS Press, Alexandria
- Jarial R, Shard A, Thakur S, Sakinah M, Zularisam AW, Rezaia S, Kanwar SS, Singh L (2018) Characterization of flavonoids from fern *Cheilanthes tenuifolia* and evaluation of antioxidant, antimicrobial and anticancer activities. *J King Saud Univ* 30:425–432
- Kandel DR, Fraser-Zenkins CR (2020) Ferns and fern allies of Nepal. Department of Plant Resources, Ministry of Forest and Environment, Nepal. <https://www.plantlist.org>. Accessed on August 2020
- Kim KS, Moon HM, Sapienza V, Carp RI, Pullarkat R (1978) Inactivation of Cytomegalovirus and Semliki forest virus by Butylated hydroxytoluene. *J Infect Dis* 138(1):91–94
- Koes R, Verweij W, Quattrocchio F (2005) Flavonoids: a colourful model for the regulation and evolution of biochemical pathways. *Trends Plant Sci* 10(5):236–242
- Kumar RS, Sivakumar T, Sundaram RS, Sivakumar P, Nethaji R, Gupta M, Mazumdar UK (2006) Antimicrobial and antioxidant activities of *Careya arborea* Roxb. Stem bark. *Iran J Pharmacol Ther* 5:35–41
- Kwon YS, Kobayashi A, Kajiya SI, Kawazu K, Kanzaki H, Kim CM (1997) Antimicrobial constituents of *Angelica dahurica* roots. *Phytochemistry* 44:887–889
- Lai HY, Lim YY, Kim KH (2017) Isolation and characterisation of a proanthocyanidin with antioxidative, antibacterial and anti-cancer properties from fern *Blechnum orientale*. *Pharmacogn Mag* 13:31–37
- Li J, Lei X, Ke-li C (2014a) Comparison of cytotoxic activities of extracts from *Selaginella* species. *Pharmacogn Mag* 10(40):529–535
- Li S, Zhao M, Li Y, Sui Y, Yao H, Huang L, Lin X (2014b) Preparative isolation of six antitumour bioflavonoids from *Selaginella doederleinii* Hieron by high-speed counter current chromatography. *Phytochem Anal* 25(2):127–133
- Liang YC, Huang YT, Tsau SH, Lin-Shiau SY, Chen CF, Lin JK (1999) Suppression of inducible cyclo oxygenase and inducible nitric oxide synthase by apigenin and related flavonoid in mouse macrophages. *Carcinogenesis* 20(10):1945–1952
- Liu H, Hu P, Ji Z, Zhao S, Zhang Y, Wu J, Fan J, Liao J (2011) Reactive oxygen species mediated mitochondrial dysfunction is involved in apoptosis in human nasopharyngeal carcinoma CNE cells induced by *Selaginella doederleinii* extract. *J Ethnopharmacol* 138(1):184–191
- Ma SC, But PP, Ooi VE, He YH, Lee SH, Lee SF, Lin RC (2001) Antiviral amentoflavone from *Selaginella sinensis*. *Biol Pharm Bull* 24(3):311–312
- Masal VP, Dongare MM (2010) Studies on the ethnomedicinal uses of pteridophytes of Ratnagiri, Maharashtra, India. *Indian Fern J* 27(12):88–93
- Nayak N, Rath S, Mishra M, Ghose G (2013) Antibacterial activity of the fern *Lygodium flexuosum* (L.) Sw. against multidrug resistant enteric and uro-pathogenic bacteria. *J Acute Disease* 2:270–276
- Orhan IE, Erdem SA, Kartal M, Karaoglu T (2007a) Antioxidant and antimicrobial actions of the clubmoss *Lycopodium clavatum* L. *Phytochem Rev* 6:189–196
- Orhan I, Ozcelik B, Aslan S, Kartal M, Karaoglu T, Sener B, Terzioğlu S, Choudhary IM (2007b) Antioxidant and antimicrobial actions of the clubmoss *Lycopodium clavatum* L. *Phytochem Rev* 6:189–196
- Pandey SN, Trivedi PS, Misra SP (1996) A textbook of botany Bryophyta, Pteridophyta, Gymnosperms, and Paleobotany volume II. Vikas Publishing House PVT LTD, New Delhi
- Panizzi L, Caponi C, Catalano S, Cioni PL, Morelli I (2002) Invitro antimicrobial activity of the extracts and isolated constituents of *Rubus ulmifolius*. *J Ethnopharmacol* 79:165–168
- Parekh J, Chanda S (2006) Screening of some Indian medicinal plants for antibacterial activity. *Indian J Pharm Sci* 68(6):835–838

- Parekh J, Chanda S (2007) Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *Afr J Biomed Res* 10:175–181
- Pirtle EC, Sacks JM, Nachman RJ (1986) Antiviral effectiveness of butylated hydroxytoluene against pseudorabies (Aujeszky's disease) virus in cell culture, mice and swine. *Am J Vet Res* 47(9):1892–1895
- Poudyal B, Singh B (2013) Potential antibacterial and antioxidant properties of aqueous, ethanol and methanol extracts of *Tectaria macrodonta* C.Chr. *Int J Pharm Sci Res* 10(8):3785–3794
- Radulović N, Stojanović G, Palić R (2006) Composition and antimicrobial activity of *Equisetum arvense* L. essential oil. *Phytother Res* 20(1):85–88
- Rajbhandary S (2016) Fern and fern allies of Nepal. In: Jha PK, Siwakoti M, Rajbhandary S (eds) *Frontiers of botany*. Central Department of Botany, Tribhuvan University, Kathmandu, pp 124–150
- Richards JT, Katz ME, Kern ER (1985) Topical butylated hydroxytoluene treatment of genital herpes simplex virus infections of guinea pigs. *Antivir Res* 5(5):281–290
- Rout SD, Panda T, Mishra N (2009) Ethnomedicinal studies on some pteridophytes of simlipal biosphere reserve, Orissa, India. *Int J Med Med Sci* 1(5):192–197
- Santhosh KS, Samyudurai P, Nagarajan N (2014) Indigenous knowledge on some medicinal pteridophytic plant species among the Malasar tribes in Valparai Hills, Western Ghats of Tamil Nadu. *Am J Ethnomed* 1(3):164–173
- Shi S, Zhou H, Zhang Y, Huang K (2008) Hyphenated HSCCC-DPPH for rapid preparative isolation and screening of antioxidants from *Selaginella moellendorffii*. *Chromatographia* 68(3–4):173–178
- Singh M, Singh N, Prem K, Rawat A (2008) Antimicrobial activity of some important *Adiantum* species used traditionally in indigenous systems of medicine. *J Ethnopharmacol* 17:115(2):327–329
- Singh S, Singh R (2012) Ethno medicinal use of Pteridophytes in reproductive health of tribal women of Pachmarhi Biosphere Reserve, Madhya Pradesh, India. *Int J Pharm Sci Res* 3(12):4780
- Sinnot (1935) <https://www.askiitians.com/biology/plant-kingdom/pteridophytes.html>
- Snipes W, Person S, Keith A, Cupp J (1975) Butylated hydroxytoluene inactivates lipids containing viruses. *Science* 188(4183):64–66
- Stead D (1993) The effect of hydroxycinnamic acids on the growth of wine-spoilage lactic acid bacteria. *J Appl Bacteriol* 75:135–141
- Tanzin R, Rahman S, Hossain MS et al (2013) Medicinal potential of pteridophytes-an antihyperglycemic and antinociceptive activity evaluation of methanolic extract of whole plants of *Christella dentate*. *Adv Nat Appl Sci* 7(1):67–73
- Thapa I, Kalu S, Budha PB, Lekhak K (2017) Community based tree fern conservation strategy: with special reference to panchase region. The Rufford Foundation, London, p 18
- Thomas T (2009) Preliminary antibacterial and phytochemical evaluation of *Dryopteris cochleata* (D.Don) C.Chr. *J Glob Pharm Technol* 3(6):4–8
- Thomas T (2014) Antibacterial activity of fronds of *Adiantum caudatum* L. towards bacterial involved in cutaneous diseases. *Indian J Pharm Sci Res* 4(2):80–82
- Towers GHN, Maass WSG (1965) Phenolic acids and lignins in the Lycopodiales. *Phytochemistry* 4:57–66
- Upreti K, Jalal JS, Tewari LM et al (2009) Ethnomedicinal uses of pteridophytes of Kumaun Himalaya, Uttarakhand, India. *J Am Sci* 5:167–170
- Verma M, Gangwar M, Sahai M, Nath G, Singh TD (2015) Antimicrobial activity of phytochemicals isolated from *Selaginella bryopteris*. *Chem Nat Comp* 51(2):341–345
- Wen A, Delaquis P, Stanich K, Toivonen P (2003) Antilisterial activity of selected phenolic acids. *Food Microbiol* 20:305–311
- Yenn TW, Ring LC, Zahan KA, Rahman MSA, Tan W-N, Alaudin BJS (2018) Chemical composition and antimicrobial efficacy of *Helminthostachys zeylanica* against foodborne *Bacillus cereus*. *Nat Prod Sci* 24(1):66–70
- Zimmermann W (1938) *Die Telomtheorie*. *Biologie* 7:385–391

Chapter 3

Antimicrobial Activity of Extracts and Essential Oils of Medicinal Plants Occurring in Amazonia: Nanotechnology as a Boon to Enhance Bioactivity



Luiza Helena da Silva Martins, Andrea Komesu, Johnatt Allan Rocha de Oliveira, Carissa Michelle Goltara Bichara, Paulo Wender Portal Gomes, and Mahendra Rai

Abstract The Brazilian Amazon is the largest biome in the country and encompasses about 40% of the forest remnants of the humid tropic. The use of homemade medicines from medicinal plants has been practiced since the dawn of human civilization. In prehistoric age, man sought to alleviate his pain or treat his illnesses through the action of bioactive compounds present in plants, although in an intuitive way based on random discoveries. Such secondary metabolites of medicinal plants

L. H. da Silva Martins (✉)

Instituto de Saúde e Produção Animal (ISPA), Universidade Federal Rural da Amazônia (UFRA), Belém, Brazil

e-mail: luiza.martins@ufra.edu.br

A. Komesu

Department of Marine Sciences (DCMar), Federal University of São Paulo (UNIFESP), Santos, Brazil

J. A. R. de Oliveira

Institute of Health Sciences, Faculty of Nutrition (FANUT), Federal University of Pará (UFPA), Belém, Brazil

C. M. G. Bichara

Instituto de Saúde e Produção Animal (ISPA), Universidade Federal Rural da Amazônia, Belém, Brazil

e-mail: carissa.bichara@ufra.edu.br

P. W. P. Gomes

Institute of Exaction and Natural Sciences (ICEN), Federal University of Pará (UFPA), Belém, Brazil

M. Rai

Nanobiotechnology Laboratory, Department of Biotechnology, Sant Gadge Baba Amravati University, Amravati, India

Department of Microbiology, Nicolaus Copernicus University, Torun, Poland

e-mail: mahendra.rai@v.umk.pl; mahendrarai@sgbau.ac.in

have proven to have antimicrobial activity due to the action of their bioactive compounds. The extraction of bioactive compounds is an especially important step, not only for the separation of compounds, but also during the analysis of solid materials. There are several conventional and unconventional techniques for the extraction of bioactive compounds from plant matrices, those that use a solvent. Nanotechnology emerges as a potential technology to enhance the action of bioactive compounds present in plant matrices, as it can maintain its characteristics and stability, making these compounds used in several areas. As many of the biologically active compounds have insolubility and hydrophobicity, nanoencapsulation facilitates the delivery of these poorly bioavailable compounds when applied to functional products and drugs, which increases their absorption into cellular structures through properties of favorable particles of shape, size, and surface. Nanotechnology has proved to be a great tool to potentiate the action of such bioactive compounds.

Keywords Nanoencapsulation · Bioactive compounds · Antimicrobial activity · Ethnopharmacology · Brazilian Amazon

1 Introduction

The Amazon Forest is the largest humid tropical forest on Earth, being considered the region with the greatest biodiversity of animal and plant species. Regarding species of tree plants, it is estimated that approximately 16,000 occur along the Amazon basin (Oliveira Piva et al. 2020). The Brazilian Amazon constitutes the largest biome in the national territory and comprises around 40% of the remaining forests of the humid tropic having its relevance recognized in terms of maintaining biodiversity, regional hydrology, and climatic functions (Rodrigues et al. 2020).

The study carried out by Breitbach et al. (2013) showed that many medicinal plants in the Amazon have not been studied in more detail while some have not been studied at all. The collections of bibliographic and botanical samples that were collected by von Martius in the Amazon represent the abundance of high value materials for the development and conservation of this region. The work carried out by the same authors in 2013 was one of the additional incentives, to value the traditional knowledge of the people of the Amazon region and is also useful in protecting collective intellectual property rights.

The use of homemade medicine from medicinal plants has been practiced since the dawn of human civilization. In prehistory, man sought to alleviate his pain or treat his illnesses through the action of the active principles existing in plants. This conduct can also be observed among primitive, isolated people: for example, some indigenous tribes in South America (Rodrigues et al. 2020). The therapeutic effects of medicinal plants are due to their bioactive secondary metabolites, which act in the maintenance of tissues and prevention of a series of diseases.

Bioactive compounds can be found mainly in plant matrices as secondary metabolites; however, recently, some primary metabolites are already being considered as bioactive compounds. Such compounds have been used to prevent degenerative diseases and to even treat a wide range of other diseases. In plants, bioactive compounds act as a protector against biotic and abiotic stress and are present in different amounts. It is important to develop their production in order to obtain the largest amount possible and find viable techniques for their extraction (Azmir et al. 2013; Cvjetko Bubalo et al. 2015; Banožić et al. 2020).

There are many conventional techniques for the extraction of bioactive compounds from plants: those that use solvent (ethanol, methanol, ethyl acetate, acetone, methylene chloride, hexane, mixtures, and others) and those that use thermal extraction, heat reflux, and bioenzymatic extraction (Banožić et al. 2020). Emerging extraction techniques have also been studied to replace conventional ones, those that tend to cause negative impacts on health and the environment. Such techniques include the use of green solvents, ultrasound, subcritical water extraction, and pressurized liquid extraction among other techniques; however, these still need further studies and are better consolidated (Carvalho et al. 2018).

According to Bazana et al. (2019), nanotechnology has emerged as a potential tool to enhance the action of bioactive compounds present in plants, as it is able to maintain its characteristics and stability, making these compounds usable in a range of areas. As many of the biologically active compounds have insolubility and hydrophobicity, nanoencapsulation facilitates the delivery of these poorly bioavailable compounds when applied to functional products and drugs, which increases their absorption into cellular structures through favorable particle properties of shape, size, and surface. Such properties cause the nanoparticles to increase the solubilization potential; thus, by altering their absorption pathways and modifying the rate and location of the release, they influence gastrointestinal dispersion and prevent premature metabolic degradation of bioactive compounds.

In this context, this chapter aims to address the therapeutic properties of medicinal plants from the Brazilian Amazon region, mentioning the main herbs used and possible technologies that could be applied for their extraction (from conventional to emerging). Nanotechnology as an emerging technology to enhance the activity of bioactive compounds will also be addressed.

2 Medicinal Plants from Amazon: A Major Hot Spot of Biodiversity

The Portuguese colonized Brazil in the year 1500. The Jesuit priests were the first to establish contact with the native population of the Amazon, who passed on information from various medicinal plants in the region (Souza et al. 1938). However, Brazil was under a strong colonial regime, due to which the knowledge of medicinal plants from the Amazon until the end of the nineteenth century was hidden

(Breitbach et al. 2013). Over the years, many scientists and naturalists traveled around Brazil in search of registering animals and natural products. Among them, we highlight Carl Friedrich Philipp von Martius. He collected 22,267 species of Brazilian medicinal plants, with rich ethnobotanical and ethnopharmacological descriptions (Wuschek 1989; Riederer 2007). Until now, this collection is extremely important for research that seeks new drugs for the treatment of diseases.

With about 80,000 plant species well distributed throughout the Amazon (Morales and Vinicius 2003), it is not surprising that the region has the largest collection of natural products capable of curing or preventing diseases that threaten life on Earth. About 25% of all modern drugs are derived directly or indirectly from medicinal plants, mainly through the application of modern technologies to traditional knowledge (Brasil 2012). However, only 1% of the species in the tropical forest were tested. This leads us to say that the more we learn about the biology and chemistry of the plants that surround the habitat of Amazon, the curative power that comes from plants increases exponentially, especially against different types of cancer.

According to the US National Cancer Institute, the plants identified with anticancer properties, 70% are found in the Amazon (data available at <http://www.ars-grin.gov/duke/>). Although we are just beginning to know the power behind the Amazon rainforest, indigenous people have used plants for many generations as an alternative form of cure (Lewis 1992). Unfortunately, we still do not understand scientifically the mechanism of action of these species, but the evidence continues to suggest an incredibly effective potential against several diseases. Among the thousands of species that make up the Amazonian flora, we present some of the most important medicinal plants that are used by the people of the forest to treat various prophylaxis (Table 3.1).

3 Antimicrobial Action of Medicinal Plants from the Amazon

Nowadays, an increasing number of infectious agents are becoming resistant to commercial antimicrobials (Hancock et al. 2012; Oliveira et al. 2013). The necessity to develop new drugs requires varied strategies; among them, the bioprospection of secondary metabolites produced by medicinal plants (Oliveira et al. 2013) is more important.

The use of plants for treating diseases is as old as the human species. Popular observations on the use and efficacy of medicinal plants significantly contribute to the disclosure of their therapeutic properties, so that they are frequently prescribed, even if their chemical constituents are not always completely known (Silva and Fernandes Júnior 2010).

Brazil has the world's largest biodiversity, accounting for over 20% of the total number of known species (Silva and Fernandes Júnior 2010). In the Amazon region,

Table 3.1 Medicinal species from the Brazilian Amazon

Botanical name	Vernacular name ^a	Occurrence state ^a	Traditional use ^a	Correlated studies
<i>Manihot esculenta</i> Crantz	Mandioca	Pará	Lymphatic system, fresh leaves are antidote	Antibacterial (Mustarichie et al. 2020); Antioxidant (Bahekar and Kale 2016)
<i>Carapa guianensis</i> Aubl.	Andiroba	Amazonas	Exanthema, especially that originating from bites of insects of the family Simuliidae. Decoctions against <i>Ascaris</i> (internally), dermatophytosis	Antileishmanial (Oliveira et al. 2018); Antiplasmodial (Miranda Júnior et al. 2012)
<i>Virola sebifera</i> Aubl.	Ucuúba	Guyana, Pará	Colic, dyspepsia; rheumatic pain, arthritic tumors	Antioxidant (Rezende et al. 2005); Antiproliferative (Denny et al. 2007)
<i>Paullinia pinnata</i> L.	Timbó	Pará	Anorexia, nervous headache, dry skin. Aphrodisiac but decreases the fertility of Sperm. Poisonous to the brain and kidneys. Against hydrophobia, melancholia, and other types of mental illness	Antifungal (Ngandeu et al. 2019); Analgesic and anti-inflammatory (Ior et al. 2011)
<i>Phyllanthus niruri</i> L.	Quebra-pedra	Pará	Fight kidney and gallbladder stones. Diabetes mellitus	Anti-inflammatory and antinociceptive (Porto et al. 2013); Antimicrobial (Ibrahim et al. 2013)

Note: ^aData adapted from Breitbach et al. (2013) with the permission of Journal of Ethnopharmacology

there is a large biodiversity of medicinal plants used empirically, but whose prescription has been consolidated through centuries of cultural interaction. Among many medicinal plants, some have been used specifically as anti-inflammatory and antimicrobial agents, including Andiroba (*Carapa guianense*), Alfavaca (*Ocimum micranthum*), Copaiba (*Copaifera multijuga*), Crajiru (*Arrabidaea chica*), Jambu (*Spilanthes acmella*), and Juca (*Libidibia ferrea*) (Carvalho et al. 1996; Conde et al. 2015). Conde et al. (2015) observed that extracts of Juca, Crajiru, Alfavaca, and Copaiba essential oils demonstrated antimicrobial activity against biofilm forming bacteria occurring in the mouth.

Machado et al. (2003) studied different plant species and plant fractions (*P. granatum*, *T. avellanedae*, *Cissampelos sympodialis*, *Croton salutaris*, *Piper nigrum*, *Zanthoxylum rhoifolia*, *Tibouchina granulosa*, *Alpinia zerumbet*, *Amburana cearensis*, *Lobelia thapsoides*, *Melissa officinalis*, *Spilanthes oleraceae*, *Schinus* sp., *Eugenia* sp.) against multiresistant hospital bacteria. *Staphylococcus aureus* strains

are susceptible to extracts of *Punica granatum* and *Tabebuia avellanedae*. A mixture of ellagitannins isolated from *P. granatum* and two naphthoquinones isolated from *T. avellanedae* demonstrated antibacterial activity against all *S. aureus* strains tested. The results indicate that these natural products can be effective candidates for the development of new strategies to treat methicillin-resistant infections.

In another study, Suffredini et al. (2004) screened 705 organic and aqueous extracts of plants obtained from different Amazon Rainforest and Atlantic Forest plants for antibacterial activity at 100 µg/ml, using a microdilution broth assay against *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Escherichia coli*. In another work, 1220 organic and aqueous extracts were screened against *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *E. coli*. Seventeen organic and aqueous extracts obtained from 16 plants showed activity against both Gram-positive bacteria. None of the extracts showed relevant activity against the Gram-negative *E. coli* and *Pseudomonas aeruginosa* (Suffredini et al. 2006).

Oliveira et al. (2013) evaluated the antimicrobial activity of medicinal plants of family Fabaceae collected from the Amapa state (members of Caesalpiniaceae sub-family), Rubiaceae, Ochnaceae, Apocynaceae, and Clusiaceae families. *Hymenelobium petraeum* showed inhibitory activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Salmonella enterica* serovar Typhi, and *Candida albicans*. Aqueous extracts of *Vatairea guianensis* and *Symphonia globulifera* presented inhibitory activity exclusively for *Staphylococcus aureus*. Also, the aqueous extract of *Ptychopetalum olacoides* and *Pentaclethra macroloba* inhibited the growth of *Klebsiella ozaenae* and *Acinetobacter baumannii* (Oliveira et al. 2013).

In another study, the antimycobacterial activity of various plants' extracts was tested against three strains of *Mycobacterium tuberculosis*. The extracts of *Duroia macrophylla*, *Ferdinandusa rudgeoides*, *Ferdinandusa* sp., and *Palicourea guianensis* (Rubiaceae) were active against *M. tuberculosis* RMPPr strain. The activity of these extracts may be associated with the possible presence of flavonoids, since this substance is present in these species, which is commonly found in Rubiaceae family species, whose antimicrobial activity against *Escherichia coli* has been demonstrated (Carrion et al. 2013).

The antimicrobial activity of essential oils from leaves of *G. blepharophylla*, *Guatteropsis friesiana*, and *Guatteropsis hispida*, three species of Amazon Brazilian plants, were evaluated against 11 species of microorganisms. The oil of *G. friesiana* exhibited significant antimicrobial activity against all microorganisms tested, whereas *G. hispida* and *G. blepharophylla* showed potent activity against *Rhodococcus equi*. The major constituents of each oil were also tested separately, which showed lower activity compared to the oils (Costa et al. 2008).

Santos et al. (2012) studied *in vitro* antimicrobial activity of Andiroba and Copaiba essential oils against *Paenibacillus* species, including *P. larvae*. The results showed for the first time that these oils presented a high activity against *Paenibacillus* species, showing that Copaiba oil may be a candidate for the treatment or prevention of American foulbrood disease.

Studies of antimicrobial activity and inhibition of the enzyme acetylcholinesterase of fixed oils and hexane extracts of the fruits, abiu (*Pouteria caimito*), acerola (*Malpighia emarginata*), araçá (*Psidium cattleianum*), bacupari (*Rheedia gardneriana*), biribá (*Rollinia mucosa*), camu-camu (*Myrciaria dubia*), fruta-do-conde (*Annona squamosa*), graviola (*Annona muricata*), and taperebá (*Spondias mombin* L.), were carried out against *Candida albicans* ATCC 18804, *Staphylococcus aureus* ATCC 29212, *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 25922, and *Salmonella typhimurium* ATCC 14028 (Fernández et al. 2020). Of these microorganisms, the best inhibition results were obtained for yeast *C. albicans* with inhibition of 94.46% by taperebá barks extracts, acerola barks (87.12%), araçá seeds (85.23%), and taperebá pulp (85.22%). The % inhibition of bacteria tested was low as compared to antifungal activity (Fernández et al. 2020).

In another work, the antioxidant, cytotoxic, antimicrobial, and schistosomicidal activities of the methanolic extract of *Cassia grandis* L. (Fabaceae), a native tree from Amazon Forest, was evaluated. The ethyl acetate fraction exhibited both antibacterial activity against multidrug-resistant *S. aureus*, and schistosomicidal activities, which could be attributed to the presence of flavonoids, such as catechin derivatives, quercetin, and luteolin (Magalhães et al. 2020).

Antimicrobial and phytotoxicity activities of aqueous crude extract from the Amazonian ethnomedicinal plant *Bellucia grossularioides* (L.) Triana (popularly known as Muúba or Angry-Jambo) were tested against four human pathogenic microorganisms. The results showed no antimicrobial potential against *Staphylococcus aureus*, *Candida albicans*, and *Candida krusei*, which cause furunculosis and leukorrhea, respectively. Additionally, growth inhibition of the toxigenic fungus *Aspergillus parasiticus* was assayed *in vitro* and the results showed no inhibitory activity for any of the tested concentrations. These findings contradict the traditional knowledge and may assist in the targeting of future therapeutics practices (Martins et al. 2016).

Rodrigues et al. (2014) reported the antifungal activity of twenty-eight species of plants extracts of Amazon forest belonging to twenty botanical families. The minimum inhibitory concentrations of the one hundred fourteen crude extracts of dichloromethane, methanol, and water were evaluated against three *Candida* species: *Candida albicans*, *Candida glabrata*, and *Candida parapsilosis*. Seventy-four extracts showed activity, with minimum inhibitory concentration between (0.06 and 1) mg/mL, against the three species evaluated. The results observed in this study, mainly about the families Arecaceae, Apocynaceae, Salicaceae, and Urticaceae, showed that these extracts are promising for the development of new drugs that can be used in the treatment against opportunistic fungal infections (Rodrigues et al. 2014).

Pires et al. (2016) analyzed and compiled information regarding the species *Bauhinia variegata*, *Cecropia obtusa*, *Cecropia palmata*, *Conarus perrottetii* var. *angustifolius*, *Chrysobalanus icaco*, and *Mansoa alliacea*. These common species of the Brazilian Amazon region, widely used in folk medicine, show antioxidant, antibacterial, cytotoxic, hypoglycemic, antifungal, antiangiogenic, antitumor, anti-inflammatory, and antiallergic activities (Pires et al. 2016).

Silva et al. (2018) compiled information of the antileishmanial activity of extracts, isolated compounds, and essential oils commonly used by the local population in the Brazilian Amazonian region to treat several illnesses. Scientific research widely involved natural products in the form of extracts, essential oils, and purified compounds. Moreover, it is important to emphasize that medicinal plants in the Brazilian Amazonian region are used in the form of teas and infusions based on limited access to highly purified compounds due to their high production cost (Silva et al. 2018).

The antimicrobial activity of plants was proven by various examples, in the form of both essential oils and extracts. Thus, this property can be a promising ally in the development of medicine necessary to combat the increasing number of bacterial strains that become resistant to conventional antibiotics (Silva and Fernandes Júnior 2010).

Brazil is the country with the greatest potential for plant research. Recent data show that the Brazilian Amazon region has at least 45,000 different species of plants and many of them are rich in active ingredients (Pires et al. 2016). However, the genetic resources of this vast biodiversity are still poorly explored (Pires et al. 2016). This should be changed using incentives to follow up promising findings: with more investment, especially in the Amazonian region (Silva et al. 2018).

4 Medicinal Plants to Combat Antibiotic-Resistant Human Pathogens

As previously mentioned, many studies with medicinal plants are being developed to combat human pathogens, either due to their natural resistance to control methods, or by developing resistance against commercially used antibiotics, a great concern in the health field.

In 2017, a list of pathogenic microorganisms highly resistant to antibiotics was published, such microorganisms were related to 12 families of bacteria that were among those that caused the greatest threats to the health of the population. These groups were divided into following categories: the first category (critical): *Acinetobacter baumannii*, resistant to carbapenem; *Pseudomonas aeruginosa*, resistant to carbapenem; *Enterobacteriaceae*, resistant to carbapenem, producing ESBL. Second category (high): *Enterococcus faecium*, resistant to vancomycin; *Staphylococcus aureus*, resistant to methicillin, intermediate to vancomycin, and *Helicobacter pylori* resistant, resistant to clarithromycin; *Campylobacter* spp., *Salmonella* resistant to fluoroquinolone, resistant to fluoroquinolone; *Neisseria gonorrhoeae*, resistant to cephalosporin, resistant to fluoroquinolone. Third category (medium): *Streptococcus pneumoniae*, not sensitive to penicillin; *Haemophilus influenzae*, resistant to ampicillin; *Shigella* spp., Resistant to fluoroquinolones. These data were obtained from the World Health Organization (WHO 2017).

In CDC's landmark report (CDC 2019), a similar list is published, but now with 18 microbial groups divided into the following: **Urgent Threats:** Carbapenem-resistant *Acinetobacter*, *Candida auris*, *Clostridioides difficile*, Carbapenem-resistant *Enterobacteriaceae*, Drug-resistant *Neisseria gonorrhoeae*. **Serious Threats:** Drug-resistant *Campylobacter*, Drug-resistant *Candida*, ESBL-producing *Enterobacteriaceae*, Vancomycin-resistant *Enterococci*, Multidrug-resistant *Pseudomonas aeruginosa*, Drug-resistant nontyphoidal *Salmonella*, Drug-resistant *Salmonella* serotype Typhi, Drug-resistant *Shigella*, Methicillin-resistant *Staphylococcus aureus*, Drug-resistant *Streptococcus pneumoniae*, Drug-resistant *Mycobacterium tuberculosis*. **Concerning Threats:** Erythromycin-resistant group A *Streptococcus*, Clindamycin-resistant group B *Streptococcus*.

According to the report, more than 2.8 million antibiotic-resistant infections occur in the United States each year, and more than 35,000 people die as a result. In addition, nearly 223,900 people in the United States required hospital care for *C. difficile* and at least 12,800 people died in 2017 (CDC 2019).

Staph bacteria, including methicillin-resistant (MRSA), are also one of the most common causes of healthcare-associated infections. In 2017, CDC estimated 323,700 cases of hospitalized patients and 10,600 estimated deaths.

Therefore, many studies aim at alternatives to combat infections originating from these microbial groups, and natural plant molecules are very promising. Recently, Reis et al. (2020) evaluated antibiofilm, antibacterial, and antioxidant activities of *Brosimum acutifolium* flavonoids, with highly promising results in control preformed *S. aureus* biofilms of importance in the medical field.

Recent studies using plants from other Brazilian region have also shown satisfactory results in the fight against bacterial resistance. Among them, Matias et al. (2016) evaluated the antibacterial activity of *Cordia verbenacea* extracts and found a moderate antibacterial activity against *S. aureus*, *Escherichia coli*, and *P. aeruginosa*. However, they observed a synergistic effect between the fractions of plants with antibiotics, and this could be an alternative to these treatments.

Freitas et al. (2020) reported the antibacterial and antibiotic-modulating activity of *Baccharis coridifolia* essential oil against *P. aeruginosa* and *S. aureus*, where they also found a synergism between the combinations of subinhibitory doses of the oil with conventional antibiotics, indicating potentiation of the antibacterial effect.

Gomes et al. (2020) investigated extracts and residues from the Brazilian pepper tree against multidrug-resistant strains of hospital origin (*S. aureus*, *Enterococcus faecium*, *E. faecalis*, *P. aeruginosa*, and *Acinetobacter baumannii*) and found promising results, where the methanolic fraction and the hydroethanolic extract were the most active mainly against *S. aureus*, *E. faecium*, and *E. faecalis*. In another study, Cruz et al. (2020) isolated a flavonoid from *Croton piauhiensis* leaves to evaluate the antimicrobial action against *E. coli*, *P. aeruginosa*, and *S. aureus*. The results revealed that the compound had no antibacterial activity at concentrations <1024 µg/mL, but the combination of 128 mg/mL of flavonoid with gentamicin presented synergistic effects against *S. aureus* and *E. coli*, as Amikacin also showed synergistic effects against both organisms.

5 Techniques for Extraction

5.1 Conventional Techniques

Medicinal plants have a multitude of compounds of interest, which can have the most diverse technological applications with emphasis on the medical and cosmetic areas. Countless antioxidant compounds of interest can be found in fruits and vegetables, including phenolics, carotenoids, anthocyanins, and tocopherols (Jakubowski and Bartosz 1997). These have various applications, ranging from a simple treatment of skin blemishes to cancer treatment. As a result, there are countless studies aimed at the components found in plants with possibilities of technological application.

However, to obtain these chemical components, extraction is necessary. Extraction can be defined as the separation of medically active portions of the plant using selective solvents through standard procedures (Handa et al. 2008).

There are many methodologies for preparing plant extracts and for isolating their chemical constituents. However, hydroalcoholic extraction (ethanol/water 50/50, v/v), is usually initially applied, for chemical-pharmacological analysis. In the case of possible biological effects of interest, a systematic study method should be used. In this case, the most suitable solvent for obtaining the crude extract is methanol, as it allows the extraction of a greater number of compounds (Cechinel Filho and Yunes 1998).

For many years, conventional methods, such as maceration, boiling, immersion, hydrodistillation, and Soxhlet, were used with great success. There are more and more chemical and pharmacological studies involving medicinal plants that aim to obtain new compounds with different therapeutic properties, which have driven a great scientific advance in this area.

There are multitudes of methods that can be used to extract these compounds, but from an industrial point of view, there is still a strong focus on conventional extraction methods. The extraction of very useful products is an issue of great interest and importance from the point of view of their exploitation. However, the content of these compounds can differ between cultivars and extraction methods. In this way, several extraction methods have been studied to maximize both the quality and quantity of the components obtained.

Among the conventional extraction methods, we can highlight those that use water, with or without heating, and a mixture of solvents such as ethanol, methanol, and organic acids. The different combinations and conditions applied with these solvents include most of the conventional extraction methods for chemical components of plants with an emphasis on phenolic compounds.

The extraction of phenolics can be carried out using organic, or inorganic, solvents. According to Wong and Kitts (2006), highly polar solvents, such as methanol, are highly effective in obtaining antioxidant compounds. Many parameters can influence the performance of phenolic, among which the extraction time, temperature, solvent/sample ratio, number of repeated extractions of the sample, and type of

solvent are important. Another important factor is the recovery of the phenolic compounds obtained, which is different from one sample to the next and depends on the type of plant and its active compounds of interest. The yields of the extracted phenolic compounds are influenced by the solvents used such as water, acetone, ethyl acetate, alcohols (methanol, ethanol, and propanol), and their mixtures (Garcia-Salas et al. 2010).

It is important to note that the various solvents used will behave differently according to the material from which the compound of interest is being extracted: for example, an investigation into the effect of different solvents on the extraction of phenolics from aerial parts of *Potentilla atrosanguinea* showed that 50% aqueous ethanol was more efficient than pure aqueous forms or 50% methanol and acetone (Kalpana et al. 2008). That is, although there is evidence that methanol would be more efficient for most of the extraction processes, this behavior may vary depending on the material used. It was observed that the highest levels of phenolics extracted from *Vitis vinifera* and sunflower with the use of pure methanol and 80% aqueous acetone (Casazza et al. 2010; Taha et al. 2011).

It is verified that for the extraction, for example, of flavonoids, methanol was better than heptane, which was observed as a poor solvent, while ethanol and acetone are moderate solvents for the extraction (Sathishkumar et al. 2012). Sathishkumar et al. (2012) further reported that maximum amount of flavonoids can be extracted with 75% ethanol, and further increase in the concentration of ethanol was not helpful when it came to increased extraction of flavonoids. The addition of a liquid modifier can enhance the extraction efficiency by reducing the extraction time and improving the recovery of different types of natural products from plant materials (Liu et al. 2008).

One of the benefits of choosing ethanol in extraction processes is because it has a benign character from an environmental point of view, it is relatively safe for human health and it interacts with the flavonoid probably through noncovalent interactions, leading to a rapid diffusion in the solution (Shi et al. 2003). The choice of the solvent used in the extraction of compounds of interest is based on the polarity of the solute of interest. A solvent of polarity similar to the solute dissolves the solute more efficiently. Many solvents can be used sequentially to limit the number of analogous compounds in the desired yield. It is possible to use several solvents sequentially or simultaneously; in the first situation, this can be done to limit the number of analogous compounds in the desired yield. According to Altemimi et al. (2017), the polarity of some common solvents from less polar to more polar is as follows: Hexane < Chloroform < Ethylacetate < Acetone < Methanol < Water.

The extraction of medicinal compounds from plants through the Soxhlet apparatus is the most commonly used, mainly to extract phenolic compounds, due to its lower processing cost, simplicity of operation, good recovery of extracts, and less consumption of time and solvent compared to other conventional methods, such as maceration or percolation (Seidel 2012).

The Soxhlet method generally uses solvents such as ethanol and methanol, which usually present good yields, with methanol being more efficient according to Scherer and Godoy (2014). This high yield of bioactive compounds by the Soxhlet

method can be explained using the high temperature that increases the intensity of solution. The content of phenolic compounds obtained from medicinal plants is generally affected by the solvent used, with methanol being the best when compared to ethanol and ethyl acetate (Scherer and Godoy 2014). It is a well-established technique that has an excellent performance when compared to other conventional extraction methods (Luque de Castro and García-Ayuso 1998).

The ethyl acetate solvent and the chloroform/dichloromethane solvent showed lower yields when compared to methanol (Moure et al. 2000). A decreasing yield is observed in the literature for phenolic compounds extracted with the solvent methanol, ethanol, and ethyl acetate, with maceration with methanol, in general, being the one that provides the highest yield of phenolic compounds.

In Soxhlet extraction, the extraction time is essential in reducing energy and cost of the extraction process, being one of the most important factors, which alter the recovery of phenolic compounds from the plant matrix, since if the sample is in an overexposure situation, phenolic compounds tend to degrade, so it is necessary to determine a time considered appropriate for a greater recovery of the compounds (Mojzer et al. 2016).

According to Alara et al. (2018) in the extraction with Soxhlet and ethanol, it is possible to find maximum yields after 2 hours of extraction, with additional times promoting a reduction in the recovery yields of the compounds (total phenolic content and total flavonoid content). This can be explained by the degradation of phenolic compounds, due to the excessive heating of the plant samples (Dahmoune et al. 2014). For this type of observation, it is possible to apply Fick's second law of diffusion, in which the final balance between the extraction solvent and the plant sample will be achieved at a certain time of the extraction.

Most of the modifications carried out to date in the conventional Soxhlet extraction method developed in recent years aimed to make its performance more similar to that of recent techniques for the treatment of solid samples: with reducing the time of the leaching step, energy and automation (Luque de Castro and García-Ayuso 1998).

There are many divergences between the different methods used; however, it is of great importance that each material used is evaluated for different extraction methods, which will include different solvents. For example, according to Anokwuru et al. (2011) acetone and N, N dimethylformamide (DMF) are highly effective in extracting antioxidants. Koffi et al. (2010) found that methanol was more effective in a large amount of phenolic content of nut fruits when compared to ethanol.

Ethanol extracts from plants in Côte d'Ivoire were reported to extract higher concentrations of phenolics compared to acetone, water, and methanol (Koffi et al. 2010) Multiple solvents were commonly used to extract phytochemicals, and scientists often use dry powder from plants to extract bioactive compounds and eliminate water interference at the same time.

Another widely used method for extracting medicinal compounds from plants is maceration. According to Vongsak et al. (2013), one of the more useful methods would be, convenient, and relatively cheaper for small- and medium-sized companies when compared to other modern extraction methods; however, there is a

wastage of chemicals during the application of the technique. The isolation and purification of extracts are quite complex and time consuming. This method, which is commonly used in wine production, was adopted for application in medicinal plants. According to Handa et al. (2008), this method is characterized by immersing the material, in whole or powder form, in containers, together with the solvent, for a minimum period of 3 days, after which the content is pressed or filtered. This method is generally used at room temperature. They further reported that the method of infusion and decoction uses the same principle of maceration, because in both, the material is immersed in cold or boiled water. Rathi et al. (2006) stated that the decoction is more suitable for the extraction of heat-stable compounds (hard parts of the plant: root and bark) and generally results in compounds with greater oil solubility. It is also important to remember the percolation process, which is usually applied to coffee, in this process the samples in the form of dry powder, encounter boiling water, using a strainer, and the compound of interest is extracted at a moderate rate.

5.2 Nonconventional Technologies for Obtaining Extracts

In the literature, the comparison between conventional and nonconventional methods of extraction is quite common; for example, de Oliveira et al. (2020) obtained yields of superior phenolic compounds by enzymatic extraction compared to a conventional extraction performed with the association of methanol: water: acetic acid (5:4:1), which demonstrates that it is necessary to evaluate different extraction methods for the same plant matrix when maximum extraction efficiency is being sought.

There is a tendency to reduce the use of conventional organic solvents, since such solvents are harmful to health and the environment. However, researchers have been looking for new extraction alternatives such as green extraction techniques, which work using lower temperatures, shorter residence times, more accessible separation and purification, higher yield, and better efficiency during extraction. In general, these techniques have better environmental, health, and safety properties. However, it is still impossible to use a totally solvent-free technique, because they have an irreplaceable impact on mass transfer during extraction. Thus, new extraction techniques have great potential to replace conventional techniques, but their application and optimization still needs further studies (Banožić et al. 2020).

A nonconventional method of extracting bioactive compounds is that of ultrasound waves. In this process, cavitation bubbles will be generated within the medium; following a cellular collapse, millions of these microscopic bubbles are capable of releasing energy and can create localized areas of high pressure and temperature. Such a mechanism is known as the cavitation effect (Panja 2018) (Fig. 3.1).

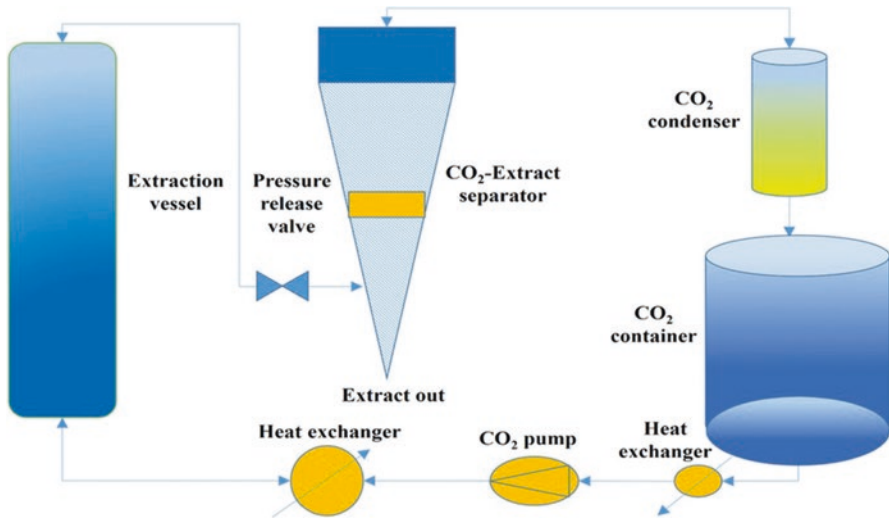


Fig. 3.1 Sketch of the mechanism of release of bioactive compounds from plant cells using ultrasound waves taken from Panja (2018) with permission from *Current Opinion in Food Science*

6 Nanotechnology as an Emerging Tool to Enhance Bioactivity

6.1 Nanoencapsulation of Bioactive Compounds

Nanotechnology is well known for production and application of structures, devices, and systems that have a scale size of 10^{-9} m. This technology has many applications in various areas and has been developing at an accelerated rate in recent years, with its wide applications, sectors such as medicine, pharmacy, and food especially in the stages of processing, packaging, storage, transportation, functionality, and others. Thus, industry and academic researchers have carried out many studies for the application of nanotechnology to encapsulate, protect, and release biologically active compounds from plants (fruits, leaves, roots, and seeds, among others) (Rai et al. 2017; Bazana et al. 2019).

The emergence of different diseases such as diabetes, cancer, obesity, and Alzheimer's has increased severely in recent years and this has led people to think better about having healthier habits (such as a diet rich in healthy plant foods). The plants contain bioactive compounds that act as antioxidants and help prevent these diseases. Nanotechnology came as a technology that increased health promotion through the manufacturing of functional products (such as drugs, supplements, etc.). In the process of using nanotechnology, some of the main physicochemical properties of the particles are changed, reducing their size to nanodimension. European Food Safety Authority (EFSA) claims that particles below 100 nm are

nanoparticles. However, in general, particles smaller than 1000 nm can be considered as nanoparticles (Esfanjani and Jafari 2017).

Much work has been carried out concerning the use of nanotechnology in the line of research on nanoencapsulation of bioactive compounds. Although these compounds promote disease prevention, as well as improvements in human health, they are poorly absorbed. Interestingly, these nanostructures can improve many characteristics of these elements. In addition, they can also promote protection against degradation, solubility, stability, and bioavailability, among others. However, the development of nanostructure has many challenges, ranging from choosing the best method for obtaining it to identifying the ideal type of nanomaterial for a bioactive compound of interest. In addition, the characterization of toxicological effects is sought through specific regulations for safety in human consumption and the environment, such as the use of green synthesis (Bazana et al. 2019).

Bioactive compounds are particularly important for the maintenance of human health; they are mostly hydrophobic and/or poorly soluble in water. The most known compounds are phenolics, carotenoids, essential oils, essential fatty acids, and insoluble vitamins. One of the biggest bottlenecks in the industry is the application of these compounds in the pharmaceutical and food industries due to their low bioavailability and stability. By incorporating functional elements in food, its bioavailability can be increased. Thus, nanotechnology has become a useful tool for this purpose, with nanoemulsions and nanocomposites in development (Hamad et al. 2018; Rezaei et al. 2019).

Nanoencapsulation is an emerging and interesting alternative for application in the preservation and protection of bioactive compounds (against inappropriate environmental circumstances) and with this increase in their bioavailability and stability, the bioactive compounds could be applied in food and pharmaceutical products (Rezaei et al. 2019). The encapsulation can decrease volatility and increase chemical and thermal stability; it can also protect these compounds from factors such as oxygen, light, pH, moisture, and gastric digestion; another interesting fact is that nanoencapsulation can also disguise the unpleasant taste and aroma of the compounds. It can also promote controlled release and improve the solubility of lipophilic compounds in aqueous media, allowing prolonged absorption of nutrients. Such advantages are possible by reducing the size of the nanoparticles, thus increasing the area per unit volume (Rezaei et al. 2019; Bazana et al. 2019).

Nanoencapsulation is the action of involving a substance using nanocarriers by absorption, incorporation, chemical interaction, or dispersion. There are several nanoencapsulation techniques to produce bioactive and nutraceutical compounds. Bazana et al. (2019) developed different nanocarriers, such as nanoemulsions; they can be nanostructured lipid transporters, nanosuspensions, solid lipid nanoparticles, nanometric liposomes and phytosomes, solid biopolymer lipid nanoparticles, and micelles made of proteins, polysaccharides, or their complete conjugates (Bazana et al. 2019).

The literature also discusses other nanotransporters, such as inclusion complexes by means of cyclodextrins, amylose and yeast cells, nanogels, nanofibers, nanosponges, or nanoparticles made of lipids and biopolymers. Some nanoencapsulation

techniques require specialized equipment, such as electrospinning, electrospray, nanospray dryers, and microfluidic devices. Various natural and synthetic polymers are used for nanoencapsulation. Natural encapsulating materials include polymers such as chitosan, alginates, cyclodextrins, and phospholipids, among others. Synthetic polymers include biodegradable esters, such as lactic-co-glycolic polyacid, poly-and-caprolactone polymers, and methacrylate. Few studies have compared various methods or materials for a specific bioactive compound or ingredient, making this difficult (Bazana et al. 2019).

6.2 Nanoencapsulation Process of Phenolic Compounds and Antioxidants

The study on the nanoencapsulation of phenolic compounds and antioxidants is very promising, because this technique can increase surface area, causing an orientable release, protection against different stresses during the processes, and storage of biomolecules when compared to microencapsulation. In addition, it causes an improvement in the bioavailability of such molecules, in relation to combating free radicals, diseases, and enhancing antimicrobial activity. The bioavailability of phenolic and antioxidant compounds can be increased with greater solubility, absorption, and permeation of such substances in the body and in food and medication formulations through the nanoencapsulation process (Fig. 3.2).

When humans consume nanoencapsulated products, many physical and chemical changes occur during their passage through the digestive system from the mouth-stomach to the intestine, which can affect their digestion and/or absorption capacity. Phenolic and nanoencapsulated antioxidants are more stable in stomach conditions, as the pH and enzymes are low, compared to their unencapsulated form. Nanoencapsulation can allow phenolics and antioxidants to be passively absorbed from the lumen of the intestine into the blood and lymphatic circulatory system; therefore, its bioavailability can increase considerably (Esfanjani and Jafari 2017).

According to Esfanjani and Jafari (2017), nanoencapsulation of phenolic and antioxidant compounds can provide the following main benefits:

1. Provide a larger surface area for interaction with biological substrates.
2. Delivers greater encapsulation efficiency compared to the microencapsulation process.
3. Increase the solubility of antioxidants and phenolics that are poorly soluble in water: this can occur in nanoparticles based on biopolymers. In fact, poorly soluble compounds are trapped within the nanoparticles and are coated by polar groups on the surface of the particles.
4. Increase the absorption of phenolics and antioxidants by interrupting hermetic junctions and / or direct uptake by epithelial cells through endocytosis.
5. Protect phenolics and antioxidants against oxidation / degradation in the gastrointestinal tract.

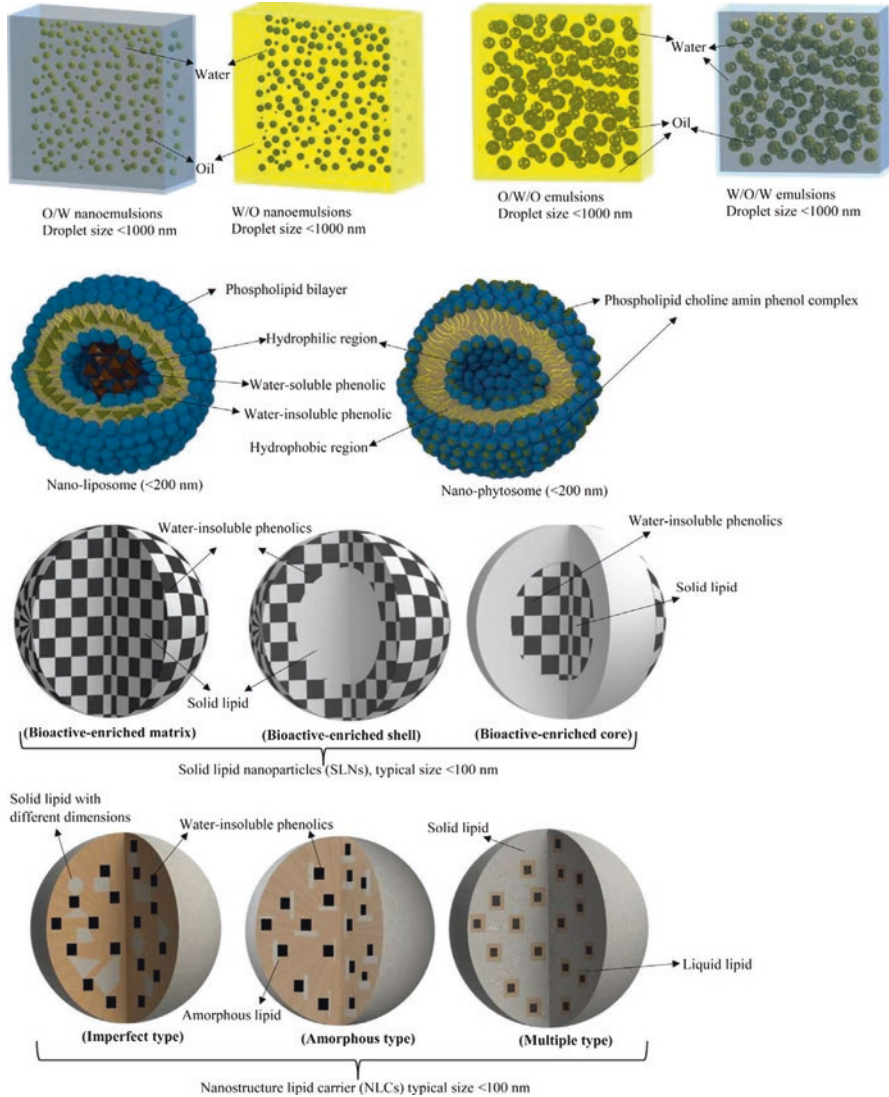


Fig. 3.2 Presents a schematic of a nanoencapsulation of phenolics by lipid-based formulation method. (Reproduced from Esfanjani and Jafari (2017) with permission of *Nanoencapsulation of food bioactive ingredients*)

- Temperature, pH, oxygen, light, mixing, enzymes, proteins, and metal ions are among environmental and process stresses that phenolics and antioxidants must withstand. This can help it last longer in storage and processing.
- Multilayer nanoencapsulation technologies, for example, can release and target phenolic chemicals in food formulations for a longer period of time.

8. It can mask the bitter taste of phenolics and antioxidants, limiting its use in food compositions such beverages and chewing gums.
9. Commercialization of transparent drinks enriched with phenolics and antioxidants; these goods can be made using nanoemulsions (100 nm) in which other taste constituents are disrupted in the emulsion's oil or water phase.
10. Nanoencapsulated powders produced using drying nanoprojection, electro-spray, and electrospinning devices provide easy handling and packaging of phenolic and nanoencapsulated antioxidants.

7 Conclusion

The importance of ethnobotanical information on medicinal plants in the Amazon is extremely important for the identification of bioactive compounds and the production of possible drugs from these raw materials. The available literature shows that these bioactive compounds have already been tested on a wide range of microorganisms. Amazon has a great biodiversity and within that diversity, it represents several potential herbs that are utilized in the maintenance of health. Several techniques for the extraction of bioactive compounds from plants have already been reported; however, greater attention is now being paid to unconventional techniques. Nanotechnology has emerged as a great tool to help potentiate the action of bioactive compounds; however, the toxicity and safety of nanomaterials should also be evaluated before its use.

Acknowledgments All authors thankfully acknowledge their institutions: Federal Rural University of Amazonia, Federal University of Pará, and SGB Amravati University for supporting research and for the access to didactic materials and scientific papers. Mahendra Rai is thankful to the Polish National Agency for Academic Exchange (NAWA) for financial support (Project No. PPN/ULM/2019/1/00117/A/DRAFT/00001) to visit the Department of Microbiology, Nicolaus Copernicus University, Toruń, Poland.

References

- Alara OR, Abdurahman NH, Olalere OA (2018) Ethanolic extraction of bioactive compounds from *Vernonia amygdalina* leaf using response surface methodology as an optimization tool. *J Food Meas Charact* 12(2):1107–1122
- Altemimi A, Lakhssassi N, Baharlouei A, Watson D, Lightfoot D (2017) Phytochemicals: extraction, isolation, and identification of bioactive compounds. *Plan Theory* 11:12–17
- Anokwuru CP, Anyasor GN, Ajibaye O, Fakoya O, Okebugwu P (2011) Effect of extraction solvents on phenolic, flavonoid and antioxidant activities of three Nigerian medicinal plants. *Nat Sci* 9:53–61
- Azmir J, Zaidul ISM, Rahman MM, Sharif KM, Mohamed A, Sahena F, Jahurul MHA, Ghafoor K, Norulaini NAN, Omar AKM (2013) Techniques for extraction of bioactive compounds from plant materials: a review. *J Food Eng* 117(4):426–436

- Bahekar SE, Kale RS (2016) Evaluation of antioxidant activity of *Manihot esculenta* Crantz in wistar rats. *J Pharm Bioallied Sci* 8(2):119
- Banožić M, Babić J, Jokić S (2020) Recent advances in extraction of bioactive compounds from tobacco industrial waste—a review. *Ind Crop Prod* 144:112009
- Bazana MT, Codevilla CF, de Menezes CR (2019) Nanoencapsulation of bioactive compounds: challenges and perspectives. *Curr Opin Food Sci* 26:47–56
- Brasil (2012) Departamento de Atenção Básica. Secretaria de Atenção à Saúde. Ministério da Saúde. Práticas integrativas e complementares: plantas medicinais e fitoterapia na atenção básica. Normas e Manuais Técnicos—Caderno de Atenção Básica. Available at: http://bvsms.saude.gov.br/bvs/publicacoes/praticas_integrativas_complementares_plantas_medicinais_cab31.pdf. Accessed on 13 Apr 2021
- Breitbach UB, Niehues M, Lopes NP, Faria JE, Brandão MG (2013) Amazonian Brazilian medicinal plants described by CFP von Martius in the 19th century. *J Ethnopharmacol* 147(1):180–189
- Carrion LL, Ramos DF, Martins D, Osório MIC, Cursino LMC, Mesquita DWO, Nunez CV, Silva PEA (2013) Antimycobacterial activity of Brazilian Amazon plants extracts. *Int J Phytomed* 5(4):479–485
- Carvalho JC, Teixeira JR, Souza PJ, Bastos JK, Santos Filho D, Sarti SJ (1996) Preliminary studies of analgesic and anti-inflammatory properties of *Caesalpinia ferrea* crude extract. *J Ethnopharmacol* 53:175–178
- Carvalho MT, Bergamasco R, Gomes RG (2018) Métodos de extração de compostos bioativos: aproveitamento de subprodutos na agroindústria. *Revista UNINGÁ Rev* 33(1):66–84
- Casazza AA, Aliakbarian B, Mantegna S, Cravotto G, Perego P (2010) Extraction of phenolics from *Vitis vinifera* wastes using non-conventional techniques. *J Food Eng* 100:50–55
- CDC. Centers for Disease Control and Prevention (2019) Antibiotic resistance threats in the United States, 2019. U.S. Department of Health and Human Services, CDC, Atlanta. Available online at www.cdc.gov/DrugResistance/Biggest-Threats.html
- Cechinel Filho V, Yunes RA (1998) Strategies for obtaining pharmacologically active compounds from medicinal plants. Concepts on structural modification to optimize activity. *Química Nova* 21(1):598–603
- Conde NCO, Pereira MSV, Bandeira MFCL, Venâncio GN, Oliveira GP, Sampaio FC (2015) In vitro antimicrobial activity of plants of the Amazon on oral biofilm micro-organisms. *Rev Odonto Cienc* 30(4):179–183
- Costa EV, Teixeira SD, Marques FA, Duarte MCT, Delarmelina C, Pinheiro MLB, Trigo JR, Maia BHLNS (2008) Chemical composition and antimicrobial activity of the essential oils of the Amazon Guatteropsis species. *Phytochemistry* 69:1895–1899
- Cruz BG, Santos HS, Bandeira PN, Rodrigues THS, Matos MGC, Nascimento MF, Carvalho GGC, Braz-Filho R, Teixeira AMR, Tintino SR, Coutinho HDM (2020) Evaluation of antibacterial and enhancement of antibiotic action by the flavonoid kaempferol 7-O-β-D-(6'-O-cumaroyl)-glucopyranoside isolated from *Croton piauhiensis* Müll. *Microb Pathog* 143:104–144
- Cvjetko Bubalo M, Vidović S, Radojčić Redovniković I, Jokić S (2015) Green solvents for green technologies. *J Chem Technol Biotechnol* 90(9):1631–1639
- Dahmoune F, Spigno G, Moussi K, Remini H, Cherbal A, Madani K (2014) *Pistacia lentiscus* leaves as a source of phenolic compounds: microwave-assisted extraction optimized and compared with ultrasound-assisted and conventional solvent extraction. *Ind Crop Prod* 61:31–40
- de Oliveira JAR, Komesu A, Martins LH d S, Rogez H, Pena R d S (2020) Enzyme-assisted extraction of phenolic compounds from murucizeiro leaves (*Byrsonima crassifolia*). *Scientia Plena* 16:1–9
- Denny C, Zacharias ME, Kohn LK, Foglio MA, De Carvalho JE (2007) Antiproliferative activity of extracts and fractions from *Virola Sebifera* Aubl. Leaves (myristicaceae) [atividade Antiproliferativa Dos Extratos E Da Fração Orgânica Obtidos Das Folhas De *Virola Sebifera* Aubl.(myristicaceae)]. *Rev Bras Farmacogn* 17(4):598–603
- Esfanjani A, Jafari S (2017) Nanoencapsulation of phenolic compounds and antioxidants. *Nanoencapsul Food Bioact Ingrid* 85(5):63–101

- Fernández IM, Chagas EA, Maldonado SAS, Takahashi JA, Alemán RS, Melo Filho AA, Santos RC, Ribeiro PRE, Fuentes JAM, Chagas PC, Melo ACGR (2020) Antimicrobial activity and acetylcholinesterase inhibition of oils and Amazon fruit extracts. *J Med Plants Res* 14(3):88–97
- Freitas PR, Araújo ACJ, Barbosa CRS, Muniz DF, Rocha JE, Neto JBA, Silva MMC, Pereira RLS, Silva LE, Amaral W, Deschamps C, Tintino SR, Ribeiro-Filho J, Coutinho HDM (2020) Characterization and antibacterial activity of the essential oil obtained from the leaves of *Baccharis coridifolia* DC against multiresistant strains. *Microb Pathog* 145:104223
- García-Salas P, Morales-Soto A, Segura-Carretero A, Fernández-Gutiérrez A (2010) Phenolic-compound-extraction systems for fruit and vegetable samples. *Molecules* 15:8813–8826
- Gomes RBA, Souza ES, Barraqui NSG, Tosta CL, Nunes APF, Schuenck RP, Ruas FG, Ventura JA, Filgueiras PR, Kuster RM (2020) Residues from the Brazilian pepper tree (*Schinus terebinthifolia* Raddi) processing industry: chemical profile and antimicrobial activity of extracts against hospital bacteria. *Ind Crops Prod* 143:111–430
- Hamad AF, Han JH, Kim BC, Rather IA (2018) The intertwine of nanotechnology with the food industry. *Saudi J Biol Sci* 25(1):27–30
- Hancock RE, Nijnik A, Philpott DJ (2012) Modulating immunity as a therapy for bacterial infections. *Nat Rev Microbiol* 10:243–254
- Handa SS, Khanuja SPS, Longo G, Rakesh DD (2008) Extraction technologies for medicinal and aromatic plants, vol 66, 1st edn. United Nations Industrial Development Organization and the International Centre for Science and High Technology, Trieste
- Ibrahim D, Hong LS, Kuppan N (2013) Antimicrobial activity of crude methanolic extract from *Phyllanthus niruri*. *Nat Prod Commun*:8–4. <https://doi.org/10.1177/1934578X1300800422>
- Ior LD, Uguru MO, Olotu PN, Ohemu TL, Ukpe A (2011) Evaluation of analgesic and anti-inflammatory activities and phytochemical screening of the leaves extract of *Paullinia Pinnata* (Sapindaceae). *J Chem Pharm Res* 3(4):351–356
- Jakubowski W, Bartosz G (1997) Estimation of oxidative stress in *saccharomyces cerevisiae* with fluorescent probes. *Int J Biochem Cell Biol* 29:1297–1301
- Kalpna K, Kapil S, Harsh PS, Bikram S (2008) Effects of extraction methods on phenolic contents and antioxidant activity in aerial parts of *Potentilla atrosanguinea* Lodd. and quantification of its phenolic constituents by RP-HPLC. *J Agric Food Chem* 56:10129–10134
- Koffi E, Sea T, Dodehe Y, Soro S (2010) Effect of solvent type on extraction of polyphenols from twenty three Ivorian plants. *J Anim Plant Sci* 5(3):550–558
- Lewis WH (1992) Plants used medicinally by indigenous peoples. In: *Phytochemical resources for medicine and agriculture*. Springer, Boston, pp 33–74
- Liu B, Shen B, Guo F, Chang Y (2008) Optimization of supercritical fluid extraction of dl-tetrahydropalmatine from rhizome of *Corydalis yanhusuo* W.T. Wang with orthogonal array design. *Sep Purif Technol* 64(2):242–246
- Luque de Castro M, García-Ayuso L (1998) Soxhlet extraction of solid materials: an outdated technique with a promising innovative future. *Anal Chim Acta* 369:1–10
- Machado TB, Pinto AV, Pinto MCFR, Leal ICR, Silva MG, Amaral ACF, Kuster RM, NettodosSantos KR (2003) In vitro activity of Brazilian medicinal plants, naturally occurring naphthoquinones and their analogues, against methicillin-resistant *Staphylococcus aureus*. *Int J Antimicrob Agents* 21:279–284
- Magalhães LPM, Ramos BA, Silva MV, Correia MTS, Sena KXFR, Silva TD, Aires AL, Albuquerque MCPA, Militão GCG, Silva TG, Araújo RM, Albuquerque JFC, Ximenes RM (2020) Antibacterial, cytotoxic, and schistosomicidal activities of the methanolic extract from *Cassia grandis* L.f. (Fabaceae) stem bark and its fractions. *J Med Plants Res* 14(6):265–282
- Martins RTMC, Borges AKP, Armiato AM, Pimenta RS (2016) Antimicrobial and phytotoxicity activities of aqueous crude extract from the Amazonian ethnomedicinal plant *Bellucia grossularioides* (L.) Triana. *J Med Plants Res* 10(10):130–138
- Matias EFF, Alves EF, Silva MKN, Carvalho VRA, Medeiros CR, Santos FAV, Bitu VCN, Souza CES, Figueredo FG, Boligon AA, Athayde ML, Costa JGM, Coutinho HDM (2016)

- Potiation of antibiotic activity of aminoglycosides by natural products from *Cordia verbenacea* DC. *Microb Pathog* 95:111–116
- Miranda Júnior RNC, Dolabela MF, Silva MND, Póvoa MM, Maia JGS (2012) Antiplasmodial activity of the andiroba (*Carapa guianensis* Aubl., Meliaceae) oil and its limonoid-rich fraction. *J Ethnopharmacol* 142(3):679–683
- Mojzer BE, Knez Hrnčič M, Škerget M, Knez Ž, Bren U (2016) Polyphenols: Extraction methods, antioxidative action, bioavailability and anticarcinogenic effects. *Molecules* 21(7):901
- Morales K, Vinicius T (2003) Amazon rainforest: biodiversity and biopiracy. *Student BMJ* 13:386–387
- Moure A, Franco D, Sineiro J, Domínguez N, Lema MJ (2000) Evaluation of extracts from *Gevuina avellana* hulls as antioxidants. *J Agric Food Chem* 48:3890–3897
- Mustarichie R, Sulistyaningsih S, Runadi D (2020) Antibacterial activity test of extracts and fractions of cassava leaves (*Manihot esculenta* Crantz) against clinical isolates of *Staphylococcus epidermidis* and *Propionibacterium acnes* causing acne. *Int J Microbiol*. Article ID 1975904
- Ngandeu A, Gonsu H, Afagnigni A, Voundi S, Chafa A, Abange W, Nyegue M (2019) In vitro assessment the ant/ifungal activity of *Dissotis multiflora* (Melastomataceae) and *Paullinia pinnata* (Sapindaceae) leaves extracts on *Candida* species-experimental study. *Microbiol Res J Int* 29(3):1–8, Article no. MRJI.51816
- Oliveira Piva LR, Sanquetta CR, Wojciechowski J, Dalla Corte AP (2020) Fitossociologia em comunidades florestais do projeto RADAMBRASIL no bioma Amazônia. *BIOFIX Sci J* 5(2):264–271
- Oliveira AA, Segovia JFO, Sousa VYK, Mata ECG, Gonçalves MCA, Bezerra RM, Junior POM, Kanzaki LIB (2013) Antimicrobial activity of amazonian medicinal plants. *Springer Plus* 2:371
- Oliveira IDSDS, Moragas Tellis CJ, Chagas MDSDS, Behrens MD, Calabrese KDS, Abreu-Silva AL, Almeida-Souza F (2018) *Carapa guianensis* Aublet (andiroba) seed oil: chemical composition and antileishmanial activity of limonoid-rich fractions. *Biomed Res Int*. Article ID 5032816, 10 pages
- Panja P (2018) Green extraction methods of food polyphenols from vegetable materials. *Curr Opin Food Sci* 23:173–182
- Pires FB, Dolwitsch CB, Prá VD, Monego DL, Schneider VM, Loose RF, Schmidt MEP, Bressan LP, Mazutti MA, Rosa MB (2016) An overview about the chemical composition and biological activity of medicinal species found in the Brazilian Amazon. *J Appl Pharm Sci* 6(12):233–238
- Porto CR, Soares LA, Souza TP, Petrovick PR, Lyra IL, Júnior RFA, Guerra GC (2013) Anti-inflammatory and antinociceptive activities of *Phyllanthus niruri* spray-dried standardized extract. *Rev Bras* 23(1):138–144
- Rai M, Paralikar P, Jogee P, Agarkar G, Ingle AP, Derita M, Zacchino S (2017) Synergistic antimicrobial potential of essential oils in combination with nanoparticles: emerging trends and future perspectives. *Int J Pharm* 519(1-2):67–78
- Rathi BS, Bodhankar SL, Baheti AM (2006) Evaluation of aqueous leaves extract of *Moringa oleifera* Linn for wound healing in albino rats. *Indian J Exp Biol* 44:898–901
- Reis SV, Couto NMG, Brust FB, Trentin DS, Silva JKR, Arruda MSP, Gnoatto SCB, Macedo AJ (2020) Remarkable capacity of brosimine b to disrupt methicillin-resistant *Staphylococcus aureus* (MRSA) preformed biofilms. *Microb Pathog* 140:103–967
- Rezaei A, Fathi M, Jafari SM (2019) Nanoencapsulation of hydrophobic and low-soluble food bioactive compounds within different nanocarriers. *Food Hydrocoll* 88:146–162
- Rezende KR, Davino SC, Barros SB, Kato MJ (2005) Antioxidant activity of aryltetralone lignans and derivatives from *Virola sebifera* (Aubl.). *Nat Prod Res* 19(7):661–666
- Riederer M (2007) Carl Friedrich Philipp von Martius. Ein bayrischer Tropenforscher des 19. Jahrhunderts. Eine Vortrags- und Sendereihe der Bayrischen Akademie der Wissenschaften, der Landeshauptstadt München und des Bayrischen Rundfunks. 12. November 2007, TU München
- Rodrigues K, Ramos DF, Carrion LL, Cursino LMC, Jefreys MF, Pedroza LS, Osório MIC, Oliveira JL, Andrade JIA, Fernandes CC, Nunez CV, Silva PEA (2014) Antifungal activity of

- Brazilian amazon plants extracts against some species of candida spp. *Int J Phytopharmacol* 5(6):445–453
- Rodrigues DC, Vasconcellos Sobrinho M, Vasconcellos AMDA (2020) Capacidade de Inovação em Rede Interorganizacional na Amazônia Brasileira. *Rev Admin Contem* 24(1):100–118
- Santos RCV, Alves CFS, Schneider T, Lopes LQS, Aurich C, Giongo JL, Brandelli A, Vaucher RA (2012) Antimicrobial activity of Amazonian oils against *Paenibacillus* species. *J Invertebr Pathol* 109:265–268
- Sathishkumar T, Baskar R, Aravind M, Tilak S, Deepthi S, Bharathikumar VM (2012) Simultaneous extraction optimization and analysis of flavonoids from the flowers of *Tabernaemontana heyneana* by high performance liquid chromatography coupled to diode array detector and electron spray ionization/mass spectrometry. *ISRN Biotechnol* 2013:1–10
- Scherer R, Godoy HT (2014) Effects of extraction methods of phenolic compounds from *Xanthium strumarium* L. and their antioxidant activity. *Rev Bras Plantas* 16:1–10
- Seidel V (2012) Initial and bulk extraction of natural products isolation. In: *Natural products isolation. Methods in molecular biology*. Humana Press, Totowa, pp 27–42
- Shi J, Yu J, Pohorly J, Young C, Bryan M, Wu Y (2003) Optimization of the extraction of polyphenols from grape seed meal by aqueous ethanol solution. *J Food Agric Environ* 1(2):42–47
- Silva NCC, Fernandes Júnior A (2010) Biological properties of medicinal plants: a review of their antimicrobial activity. *J Venom Anim Toxins Incl Trop Dis* 16(3):402–413
- Silva BJM, Hage AAP, Silva EO, Rodrigues APD (2018) Medicinal plants from the Brazilian Amazonian region and their antileishmanial activity: a review. *J Integr Med* 16:211–222
- Souza GS et al (1938) *Tratado Descritivo do Brasil em 1587*, 3rd edn. Companhia Editora Nacional, São Paulo/Rio de Janeiro/Recife/Porto Alegre, p 493
- Suffredini IB, Sader HS, Gonçalves AG, Reis AO, Gales AC, Varella AD, Younes RN (2004) Screening of antibacterial extracts from plants native to the Brazilian Amazon Rain Forest and Atlantic Forest. *Braz J Med Biol Res* 37:379–384
- Suffredini IB, Paciencia MLB, Varella AD, Younes RN (2006) Antibacterial activity of Brazilian Amazon plant extracts. *Braz J Infect Dis* 10(6):400–402
- Taha FS, Mohamed GF, Mohamed SH, Mohamed SS, Kamil MM (2011) Optimization of the extraction of total phenolic compounds from sunflower meal and evaluation of the bioactives of chosen extract. *Am J Food Technol* 6:1002–1020
- Vongsak B, Sithisarn P, Mangmool S, Thongpraditchote S, Wongkrajang Y et al (2013) Maximizing total phenolics, total flavonoids contents and antioxidant activity of *Moringa oleifera* leaf extract by the appropriate extraction method. *Ind Crops Prod* 44:566–557
- Wong PY, Kitts DD (2006) Studies on the dual antioxidant and antibacterial properties of parsley (*Petroselinum crispum*) and cilantro (*Coriandrum sativum*) extracts. *Food Chem* 97(3):505–515
- World Health Organization (2017) Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. <https://www.who.int/news-room/detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed> 27-02-2017
- Wuschek E (1989) *Die Beiträge des Botanikers Carl Friedrich Philipp von Martius (1794–1868) zur Pharmakognosie und zur Erforschung tropischer Nutzpflanzen, mit einer Bearbeitung seiner Systema Metriae Vegetabilis Brasiliensis von 1834*. Dissertation der Fakultät für Chemie und Pharmazie der Ludwig-Maximilian—Universität München

Chapter 4

Assessment of Cinnamon as an Antimicrobial Agent



Merve Eylul Kiymaci and Banu Kaskatepe

Abstract Due to the lack of awareness and inappropriate use of antibiotics, as well as the ability of bacteria to cross-transmit antibiotic resistance, there is a significant increase in the number of multidrug-resistant strains worldwide. For this purpose, in order to be applied as an antimicrobial treatment approach in the coming years, there have been attempts to reduce the virulence of bacteria, as well as phage uses through alternative treatment methods based on aromatic and medicinal plants, in addition to new drug development studies. In this context, among the most important research is the usability of plants, plant-based herbs, spices, and essential oils as antimicrobial agents. Medicinal and aromatic plants are considered to be natural flora sources that are important in various fields, such as the pharmaceutical, food, cosmetics, chemical, and fragrance industries, and a large part of the current world population uses traditional plant-based alternative agents to treat different problems that threaten human health. One of the most researched alternative plant sources is cinnamon, which is a tropical spice in the genus *Cinnamomum*, in which over 200 species have been identified. Cinnamon is known to have antioxidant, antidiabetic, anti-inflammatory, anti-quorum sensing, insecticide, and anticancer effects in addition to its antibacterial and antifungal, that is, antimicrobial activities. Cinnamon has also been reported to inhibit allergen-specific immune responses, in addition to reducing the side effects of some anti-inflammatory drugs, such as gelofen, through its antioxidant properties.

In this chapter, the antimicrobial properties of cinnamon, together with its content and effectiveness, are discussed in light of the research and clinical studies on this subject.

M. E. Kiymaci

University of Health Sciences Turkey, Gulhane Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Ankara, Turkey

B. Kaskatepe (✉)

Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Ankara, Turkey

e-mail: bkaskatepe@ankara.edu.tr

Keywords Cinnamon · Antibacterial · Antifungal · Antiviral · Quorum sensing · Multidrug resistance

Abbreviations

A. baumannii	Acinetobacter baumannii
<i>C. albicans</i>	<i>Candida albicans</i>
<i>C. cassia</i>	<i>Cinnamomum cassia</i>
<i>C. zeylanicum</i>	<i>Cinnamomum zeylanicum</i>
CO	Cinnamon oil
<i>E. coli</i>	<i>Escherichia coli</i>
EO	Essential oil
HIV	Human immunodeficiency virus
IC ₅₀	The half maximal inhibitory concentration
<i>L. monocytogenes</i>	<i>Listeria monocytogenes</i>
MIC	Minimal inhibitory concentration
MRSA	<i>Methicillin-resistant Staphylococcus aureus</i>
<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
QS	Quorum sensing
<i>R. nigricans</i>	<i>Rhizopus nigricans</i>
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
<i>S. mutans</i>	<i>Streptococcus mutans</i>
SARS-CoV	Severe acute respiratory syndrome coronavirus

1 Introduction

Today, antimicrobial resistance is recognized by many countries as one of the greatest threats to global health. This has occurred due to the indiscriminate use of antibiotics, followed by antifungal, antiviral, and antiparasitic drugs and the emergence of highly virulent strains related to the overuse of newer and more potent antimicrobials. The use of antimicrobials is not limited to patients receiving hospital or home treatment, but also in animals and food; thus, resistance has become a major health problem. One Health is an approach that aims to design and implement programs and research in which multiple sectors, including human-animal-environment, communicate and work together to achieve better public health outcomes. Intervention with antimicrobial resistance is one of the most important research issues of this approach. Since most of the microorganisms share the environment in which they live with plants, animals, and people, they can infect them. Operating in a single health field cannot eliminate the current problem; therefore, all sectors need to act in coordination (WHO 2017). Bacteria have the ability to cross-transmit antibiotic resistance to each other, and this resistance indirectly spreads in the form a

chain, from human to human, animal to animal, animal to human, animal to food, food to human, etc., giving rise to a significant increase in the number of multidrug-resistant strains worldwide. In order to change this situation, an antimicrobial treatment approach has been adopted to reduce the virulence of microorganisms by developing alternative treatments, such as phage therapy, and methods based on aromatic and medicinal plants. In this context, the availability of plants, plant-based herbs, spices, and essential oils (EO) as antimicrobial agents is among the most important research. Medicinal and aromatic plants are natural flora sources that are important in various fields, such as the pharmaceutical, food, cosmetics, chemical, and fragrance industries, and a large part of the current world population uses traditional plant-based alternative agents to treat different problems that threaten human health (Akthar et al. 2014; Arumugam et al. 2016; Swamy et al. 2012; Swamy and Sinniah 2015).

In addition to infectious diseases, food products must also be protected against microbial degradation and oxidative degradation (WHO 2002), which is generally prevented using two types of synthetic preservatives in industry: antimicrobial agents and antioxidants. While the former stop the increase of microbial degradation, the latter slow down the auto-oxidation of fatty acids. However, as these food preservatives can be toxic and carcinogenic, there is a continuous search for new and natural food preservatives that are safer and effective from different sources, such as plant sources (Sharma 2015).

The therapeutic power of plants has been recognized by people over thousands of years, and humans have benefited from the medicinal properties to remain healthy. The plant-based treatment approach has decreased in recent years with the development of chemistry and technology, but continues today. The importance of plants in pharmacology is due to their having very different structures and carrying a wide variety of substances. There are various investigations on the use of aromatic and medicinal plants and their metabolites for the treatment of different types of diseases. A few of the natural products from these plants have been recorded in pharmacopoeias (Balijepalli et al. 2017). Among these plants, cinnamon is the most beneficial and researched spice, and studies on its different effectiveness and clinical uses still continue.

2 Types, Structure, and Content of Cinnamon

Cinnamomum, a genus belonging to *Lauraceae* family, has been extensively used as a traditional approach to a broad array of disorders (Balijepalli et al. 2017), and to date, over 250 species of this genus have been identified (Sangal 2011). Being a plant of tropical regions, this genus shows a natural spread mainly in South India and South Asia, but it is also widely cultivated in Indonesia, South America, parts of Africa, Malaysia, Madagascar, the Greater and Lesser Antilles, and the Seychelles, and Sri Lanka. It cannot be grown worldwide due to unfavorable climatic conditions (Blumenthal et al. 2000; Kitazuru et al. 2004; Willoughby and Mills 1996). These



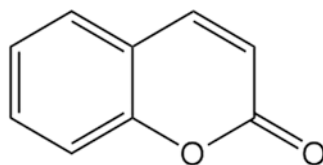
Fig. 4.1 *Cinnamomum cassia*



Fig. 4.2 *Cinnamomum zeylanicum*

small evergreen trees have angular branches, oval fine hairy green leaves, bluish fruit, and a reddish-brown bark. The inner bark of the tree is commonly used in stick or ground powder for culinary or medicinal purposes (Dugoua et al. 2007) (Figs. 4.1 and 4.2).

There are two main species of the genus *Cinnamomum*: true cinnamon (*Cinnamomum zeylanicum/verum*) or common cinnamon (vernacular name: dalchini) and Chinese cinnamon (*Cinnamomum cassialaromaticum*) (Sangal 2011). The bark of Chinese cinnamon is used in the form of dried flowers and young branches for medicinal purposes, while Ceylon cinnamon is used to produce cinnamon oil (CO), which is obtained from the barks and leaves of young branches

Fig. 4.3 Coumarin

(Çolak et al. 2018). The main difference between true cinnamon and Chinese cinnamon is the content of coumarin (1,2-benzopyrone), which is on the list of substances banned by the United States Food and Drug Administration as a direct additive/human food. The level of coumarin in Chinese cinnamon is much higher and can pose a variety of health risks when consumed regularly in large amounts (Ranasinghe et al. 2013, 2017) (Fig. 4.3).

There are studies suggesting that a daily coumarin intake of more than 0.1 mg/kg body weight may cause changes in the blood coagulation profile if the person is using warfarin and similar drugs, but their results are conflicting. Coumarin is also a highly hepatotoxic and is prohibited for use in food products. It is recommended that patients with liver disease avoid such products and cinnamon (Kawatra and Rajagopalan 2015). Apart from *Cinnamomum loureiroi* Nees (known as Vietnamese cinnamon) and *Cinnamomum burmanni* (Nees & T. Nees) Blume (known as Indonesian cinnamon), there are other species with widespread global use as a spice and great economic importance (Nabavi et al. 2015).

Cinnamon is a highly valuable spice that has been used worldwide since ancient times (Dugoua et al. 2007), and especially the oil of its bark is one of the most treasured flavors. It is used as a flavoring agent in various products, such as chewing gum, chocolate, sweets, confectionery, sauce-like foods, and some beverages, such as liquor and salty dishes, as well as in EO forms used in cosmetics (perfume, essence) and the pharmaceutical industry due to its homogeneous form, which makes it easy to adjust the amount, since it has a pleasant scent and sweet pungent taste (Huang et al. 2007).

The cortex of *Cinnamomum cassia* (*C. cassia*) is the dried bark of young shoots of the trees, 1–3-mm thick, in the form of a groove, tawny-colored, with a short break, partially or completely covered with a cork layer, and the inner surface of light brown or yellowish brown young shoots. Through water vapor distillation, 1–2% EO is extracted from the bark, and it carries 75–90% cinnamic aldehyde and a small amount of hydrocinnamic aldehyde. The cortex of *Cinnamomum zeylanicum* (*C. zeylanicum*) is the peeled bark of young branches. Firstly, the branches of the tree are cut, left to ferment for a day or two, and then the bark is peeled off and the cork layer is removed and dried at mild heat for 24 hours. The bark is 0.2–1-mm thick, with an outer surface that is light brown-tawny color, dull, in the form of longitudinal lines and dark brown inner face. By distillation of the bark with water vapor, an EO of light-yellow color and 0.5–1% is obtained. This EO contains 65–75% cinnamic aldehyde and hydrocinnamic aldehyde, as well as 4–10% eugenol. Ceylon cinnamon is used in pharmaceutical and cosmetic industries, especially in toothpastes, mouthwashes, various creams and lotions, soaps, and detergents (Tanker and Tanker 1990) (Fig. 4.4).

Fig. 4.4 Cinnamic aldehyde

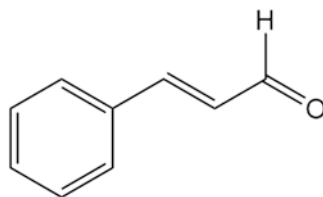
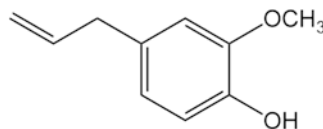


Fig. 4.5 Eugenol



An examination of the general composition of cinnamon barks in studies revealed that it consists of different proportions of moisture, crude fiber, carbohydrate, protein, fixed oil, cold alcohol extract, and EO. Among these, the main component of commercial importance is EO, generally classified as monoterpenes, sesquiterpenes, and phenylpropenes, found in all parts of cinnamon (Sangal 2011).

Cinnamon has two major chemical compounds including polyphenols, such as vanillic, caffeic, gallic, protocatechuic, *p*-coumaric, and ferulic acids and volatile phenols; for example, cinnamaldehyde, caryophyllene, benzyl benzoate, linalool, and eugenyl. The chemical composition, amount, and availability of compounds in COs vary depending on the species, the part of the plant from which they are extracted (bark, leaf, fruit, and root), the extraction method, and plant age (Muchuweti et al. 2007; Nabavi et al. 2015). While the main component of the cinnamon EO obtained from cinnamon bark was determined as cinnamaldehyde (range from 60% to 90%), it was obtained as eugenol (80%) in the oil obtained from the leaf and as (E) cinnamyl acetate and caryophyllene from fruits and flowers (Filoche et al. 2005; Jayaprakasha et al. 1997) (Fig. 4.5).

Across many different cultures, the use of cinnamon is known for its beneficial effects on oral health, such as removing oral infections, reducing toothache, and eliminating bad breath (Nabavi et al. 2015). Cinnamon is also known to have antioxidant (Mathew and Abraham 2006), antidiabetic (Lu et al. 2011), anti-inflammatory (Tung et al. 2008, 2010), insecticide (Cheng et al. 2009), antimalarial, hepatoprotective, antityrosinase (Anlar et al. 2018; Lee et al. 2002; Wiesner et al. 2001), and anticancer (Koppikar et al. 2010) effects in addition to its antibacterial (Melo et al. 2015), antifungal (Wang et al. 2005) and antiviral (Zhuang et al. 2009), that is, antimicrobial activities. Cinnamon (*Cinnamomi Ramulus*) has been promoted as a prescription ingredient recommended by traditional Chinese medicine and considered to help relieve the general symptoms of 2019-new coronavirus pneumonia (Ren et al. 2020). Cinnamon has also been reported to inhibit allergen-specific immune responses (Ose et al. 2020), in addition to reducing the side effects of some anti-inflammatory drugs, such as gelofen, through its antioxidant properties (Mohammad et al. 2014).

3 Cinnamon as an Antimicrobial Agent and Mechanism of Action

Antimicrobial activity is one of the most studied properties of cinnamon components. In addition to the primary metabolites necessary for survival, cinnamon has some secondary metabolites that show antimicrobial activity. Secondary metabolites, such as cinnamaldehyde, cinnamate, cinnamic acid and trans-cinnamaldehyde, cinnamyl acetate, linalool, α -cubebene, γ -amorphene, δ -cadinene, α -muurolene, eugenol, L-borneol, camphor, cariophyllene oxide, b-karyophilen, L-bornyl acetate, E-nerolidol, α -terpineol, and terpinolene, are involved and act as defense mechanisms against cinnamon rivals and pathogens (Deng et al. 2014; Kaskatepe et al. 2016; Vasconcelos et al. 2018). Trans-cinnamaldehyde has been shown to be mostly responsible for antimicrobial activity, and the acrolein group (α , β -unsaturated carbonyl moiety) is absolutely essential for activity in these molecules (Bae et al. 1992). Smith et al. (2000) reported that trans-cinnamic acid was easily oxidized to cinnamic acid and became unstable when exposed to air. If this decomposition occurs without bactericidal activity, trans-cinnamic acid can irreversibly become cinnamic acid through enzyme catalysis (Yuan et al. 1992). The most frequently reported adverse effects related to the use of cinnamon can be listed as gastrointestinal disorders, contact irritation, and allergic reactions due to cinnamaldehyde in its structure (Weibel and Hansen 1989). The literature review for this paper showed that the oil of cinnamon bark has very high antimicrobial activity and its effectiveness is on Gram-positive and Gram-negative bacteria, various yeasts, molds, and some type of viruses.

Although studies conducted with the mechanism of action of cinnamon show that the first target is the cell membrane, inhibition of ATPase enzyme, cell division and membrane porins in addition to inhibition of motility are among other mechanisms of action. (Vasconcelos et al. 2018). Studies on the mechanism of action in the literature have mostly focused on antibacterial activity. Cui et al. (2016) investigated the bacterial mechanism of cinnamon oil and indicated that microbial cell membrane damage was the major antibacterial mechanism, and also the loss of ATP concentration and DNA in the bacterial cell was detected. Zhang et al. (2016) determined the antibacterial mechanism of cinnamon EO against *E. coli* and *S. aureus* and reported that the bacterial cell membrane was destroyed after addition of cinnamon EO at the MIC level, whereas addition of cinnamon EO at the MBC levels resulted in the killing of the bacterial cell.

Nazzaro et al. (2013) indicated that cinnamaldehyde perturbs the membrane at a lethal concentration, it acts as an ATPase inhibitor at high concentrations, and at low concentrations, it inhibits enzymes involved in cytokine interactions or other less important cell functions at low concentrations. However, further investigations are needed to achieve a better understanding of the mechanisms of action. Also *in vivo* experiments, and clinical trials with CO and its active compounds, are necessary to clarify the pharmacodynamics and pharmacokinetics.

In addition to the extract, powder, and oil formulations of cinnamon, there are also studies on the nanoparticle form. Nanotechnology has a wide range of medical applications, including natural or polymer-based antimicrobial compounds that reduce toxicity with the use of small amounts of active ingredients (Edis et al. 2019; Jin and Jin 2019; Mamatha et al. 2019; Reda et al. 2019; Shah et al. 2019, 2020). In particular, obtaining metals with antimicrobial activity in nanosizes forms the basis of the carrier system for delivering antimicrobial agents to the target (Eleraky et al. 2020; Kanwar et al. 2019). It has also found application in many areas such as medical care products such as wound closure sponges, washing solutions, the textile industry, including masks, and the food and cosmetics industry (Lee and Jun 2019; Liao et al. 2019; Riau et al. 2019).

In pharmaceutical practice, studies on nanoparticles to reduce drug use have become widespread, but due to the increasing antimicrobial resistance, the use of especially plant compounds obtained from natural sources in combination with nanometals is among the topics studied with interest (Edis et al. 2020). The use of cinnamon as a bio-enhancing plant source due to its antimicrobial activity and to minimize toxicity is under investigation in some studies. Examples of these studies are given in related fields.

3.1 *Antibacterial Activity*

Gram-negative bacteria have a more complex cell wall than Gram-positive bacteria. Therefore, the former are known to be more resistant to plant extracts, oils, and components than Gram-positive bacteria. Porin proteins found in the Gram-negative cell wall make it difficult for hydrophobic compounds, such as antibiotics, to penetrate the cell (Hyldgaard et al. 2012). Gram-positive bacteria, in contrast, have a cell wall that allows hydrophobic molecules to easily affect the cell wall and cytoplasm (Nazzaro et al. 2013). Molecules rich in phenolics can pass through the phospholipid bilayer of bacterial cell walls and bind to proteins to inhibit their normal activities, and trans-cinnamaldehyde has a six-carbon aromatic phenol group in its structure (Juven et al. 1994). Some studies have reported that the antimicrobial mechanism of cinnamon can occur in the following ways: based on alterations in cell membrane (lipid profile), inhibition of ATPase enzyme, cell division and membrane porins in addition to inhibition of motility, biofilm formation, and quorum sensing (QS) effect (Vasconcelos et al. 2018).

The antimicrobial activity tests in the studies have been mostly performed using the disk diffusion method or agar well diffusion method as the inhibition zone. In these studies, the minimum inhibition concentration is usually determined with the liquid microdilution method for bacteria and fungi, and with a plaque reduction assay for viruses. Studies have shown that CO has a strong antimicrobial activity on different bacteria (Table 4.1). Hayatgheib et al. (2020) found that cinnamon bark oil showed strong inhibitor activity against *Aeromonas salmonicida* subsp. *salmonicida* strains. Tavares et al. (2020) indicated that cinnamon leaf oil (extracted from

Table 4.1 Antimicrobial activities of *Cinnamomum* species

Species	Part	Antimicrobial activity	References
<i>Cinnamomum zeylanicum</i>	Bark oil	<i>Aeromonas salmonicida</i> subsp. <i>salmonicida</i>	Hayatgheib et al. (2020)
<i>Cinnamomum zeylanicum</i>	Leaf oil	<i>Staphylococcus aureus</i> , <i>Staphylococcus epidermidis</i> , <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i>	Tavares et al. (2020)
<i>Cinnamomum verum</i>	Bark oil	<i>Agrobacterium tumefaciens</i>	Lee et al. (2020)
<i>Cinnamomum zeylanicum</i>	Bark oil	Methicillin-resistant <i>Staphylococcus aureus</i> , Vancomycin-resistant <i>Enterococcus faecium</i> , <i>Acinetobacter baumannii</i> , <i>Pseudomonas aeruginosa</i> , <i>Escherichia coli</i>	Saki et al. (2020)
<i>Cinnamomum verum</i>	Essential oil	Multidrug-resistant strains of <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , <i>Acinetobacter baumannii</i> , <i>Pseudomonas aeruginosa</i> , <i>Citrobacter freundii</i> , <i>Klebsiella oxytoca</i> , <i>Salmonella enteridis</i> , <i>Salmonella typhimurium</i> , <i>Salmonella zinzibar</i> , <i>Salmonella livingstone</i> , <i>Salmonella derby</i> , <i>Salmonella heidelberg</i>	Alibi et al. (2020)
<i>Cinnamomum verum</i>	Bark oil	<i>Enterococcus faecalis</i> , <i>Streptococcus mutans</i> , <i>Actinomyces israelii</i> , <i>Fusobacterium nucleatum</i> , <i>Porphyromonas endodontalis</i> , <i>Prevotella intermedia</i>	Marcoux et al. (2020)
<i>Cinnamomum zeylanicum</i>	Essential oil	<i>Streptococcus mutans</i> , <i>Enterococcus faecalis</i> , <i>Lactobacillus lactis</i> , <i>Staphylococcus aureus</i>	de Oliveira Carvalho et al. (2020)
<i>Cinnamomum zeylanicum</i>	Bark oil	<i>Bacillus cereus</i> , <i>Staphylococcus aureus</i> , <i>Listeria monocytogenes</i> , <i>Salmonella typhimurium</i> , <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i>	Purkait et al. (2020)
<i>Cinnamomum zeylanicum</i>	Bark oil	<i>Proteus mirabilis</i> , <i>Escherichia coli</i> , <i>Salmonella typhimurium</i> , <i>Yersinia enterocolitica</i> , <i>Klebsiella pneumoniae</i> , <i>Staphylococcus aureus</i> , Methicillin resistant <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> , <i>Escherichia coli</i> ESBL, <i>Streptococcus pyogenes</i> , <i>Streptococcus pneumoniae</i> , <i>Listeria monocytogenes</i> , <i>Bacillus fragilis</i> , <i>Bacillus catarrhalis</i> , <i>Candida albicans</i> , <i>Candida glabrata</i> , <i>Candida tropicalis</i>	Brochot et al. (2017)
<i>Cinnamomum cassia</i>	Essential oil	<i>Schizophyllum commune</i> , <i>Arthrinium phaeospermum</i> , <i>Rhizoctonia</i> sp., <i>Helminthosporium</i> sp., <i>Fusarium oxysporum</i> , <i>Cladosporium oxysporum</i> , <i>Fusarium solani</i> , <i>Alternaria alternata</i> , <i>Aureobasidium pullulans</i> , <i>Epicoccum nigrum</i> , <i>Aciculosporium take</i> , <i>Shiraia bambusicola</i>	Wang et al. (2020)
<i>Cinnamomum osmophloeum</i>	Leaf oil	<i>Coriolus versicolor</i> , <i>Laetiporus sulphureus</i>	Wang et al. (2005)

(continued)

Table 4.1 (continued)

Species	Part	Antimicrobial activity	References
<i>Cinnamomum zeylanicum</i>	Essential oil	<i>Rhizopus nigricans</i>	Li et al. (2014)
<i>Cinnamomum cassia</i>	Bark extract	H7N3 influenza A virus	Fatima et al. (2016)
Cinnamomi cortex	Butanol extract	HIV/SARS-CoV S pseudovirus	Zhuang et al. (2009)
<i>Cinnamomum cassia</i>	Extract	<i>Plasmodium falciparum</i>	Parvazi et al. (2016)
<i>Cinnamomum cassia</i>	Extract	<i>Dactylogyrus intermedius</i>	Ling et al. (2015)
Cinnamon	Bark oil	<i>Demodex folliculorum</i>	Li et al. (2015)
<i>Cinnamomum cassia</i>	Methanol extract	<i>Dactylogyrus intermedius</i>	Ji et al. (2012)
<i>Cinnamomum cassia</i> , <i>Cinnamomum zeylanicum</i>	Bark and leaf oil	<i>Bursaphelenchus xylophilus</i>	Kong et al. (2007)

C. zeylanicum) was effective against *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis*, *Escherichia coli* (*E. coli*), and *Pseudomonas aeruginosa* (*P. aeruginosa*). Lee et al. (2020) stated that *Cinnamomum verum* bark oil showed the most potent fumigant antibacterial activity against *Agrobacterium tumefaciens*. Antimicrobial resistance is emerging as an important public health problem. In infections caused by resistant strains, the rate of treatment decreases, the number of patient hospitalizations, duration of hospital stay, and the cost and extended risk increase. *P. aeruginosa*, *S. aureus* (especially methicillin resistant *S. aureus*, MRSA), *Enterococcus spp.* (especially vancomycin resistant), *Klebsiella spp.*, and *Enterobacter spp.* are among the leading causes of infections caused by antimicrobial resistance globally (Sakkas et al. 2019). Saki et al. (2020) found CO from *C. zeylanicum* to be effective against the extensively drug-resistant clinical isolates of MRSA, vancomycin-resistant *Enterococcus faecium*, *Acinetobacter baumannii* (*A. baumannii*), *P. aeruginosa*, and *E. coli*. Alibi et al. (2020) determined the antibacterial activity of cinnamon EO (from *Cinnamomum verum*) against 105 multidrug bacterial strains, including *E. coli*, *Klebsiella pneumoniae*, *A. baumannii*, *P. aeruginosa*, *Citrobacter freundii*, *Klebsiella oxytoca*, *Salmonella enteridis*, *Salmonella typhimurium*, *Salmonella zinzibar*, *Salmonella livingstone*, *Salmonella derby*, *Salmonella heidelberg* and reported remarkable activity at 1 minimal bactericidal concentration / minimal inhibition concentration values. Marcoux et al. (2020) tested CO from *Cinnamomum verum* bark (cinnamaldehyde 71.4%, purchased from Hunzaroma) against *Enterococcus faecalis* ATCC 19433, *Streptococcus mutans* (*S. mutans*) ATCC 25175, *Actinomyces israelii* 87.2, *Fusobacterium*

nucleatum ATCC 25586, *Porphyromonas endodontalis* ATCC 35406, and *Prevotella intermedia* ATCC 25611.

The strong inhibitory activity of cinnamon has led researchers to conduct synergy studies on different effectiveness trials on toothpastes and other biomedical applications. For example, de Oliveira Carvalho et al. (2020) highlighted that the inhibitory activity of CO from *C. zeylanicum*, alone and combination with toothpastes for *S. mutans*, *Enterococcus faecalis*, *Lactobacillus lactis*, and *S. aureus*. Purkait et al. (2020) tested *C. zeylanicum* bark oil combined with clove oil against *Bacillus cereus* (MTCC 1272), *S. aureus* (ATCC 6538P), *Listeria monocytogenes* (*L. monocytogenes*, MTCC 657), *Salmonella typhimurium* (MTCC 3224), *E. coli* (ATCC 8739), and *P. aeruginosa* (ATCC 9027). Felgueiras et al. (2020) loaded CO onto cellulose acetate/polycaprolactone wet spun microfibers. Edis et al. (2020) investigated the hydrophobic trans-cinnamic acid, natural *Cinnamomum zeylanicum* bark extract, and hydrophilic povidone iodine combinations to increase the antibacterial effect of silver nanoparticles against *S. pneumoniae* ATCC 49619, *S. aureus* ATCC 25923, and *E. faecalis* ATCC 29212, *P. aeruginosa* WDCM 00026, *E. coli* WDCM 00013, and was found effective. Nagarajan et al. (2020) determined that clove-cinnamon extract-mediated selenium nanoparticles had antibacterial activity against *Streptococcus mutans* and *Lactobacillus*. Kothari et al. (2020) found that silver nanoparticles with cinnamon extracts had antibacterial activity against *Lactobacillus* species. Potrč et al. (2020) indicated that chitosan nanoparticles with cinnamon extracts when used as surface coating of a plastic laminate showed antibacterial activity against *S. aureus* and *E. coli*. Letsididi et al. (2018) evaluated the antimicrobial activity of trans-cinnamic acid nanoemulsions (with a particle size of 46.7 ± 1 nm, polydispersity indice of 0.27 ± 0.01) against *S. aureus* and *S. typhimurium* and underlined that nanoemulsions of trans-cinnamic acid was more effective than the pure. Premkumar et al. (2018) found that cinnamon-loaded silver nanoparticles had antibacterial activity against *E. coli*, *P. aeruginosa*, *Bacillus cereus*, and *S. aureus* and indicated the higher susceptibility to silver nanoparticles with cinnamon extract in comparison silver nanoparticles alone. Brochot et al. (2017) tested the antimicrobial effectiveness of EO blends containing cinnamon and found it effective against *P. mirabilis* CIP 103181T, *E. coli* UTI 89, *S. typhimurium* CIP 6062T, *Y. enterocolitica* CIP 8027T, *K. pneumoniae* CIP 8291T, *S. aureus* MRSA ATCC 33591, *P. aeruginosa* CIP 103467, *E. coli* ESBL, *S. pyogenes* CIP 5641T, *S. pneumoniae* CIP 104471, *L. monocytogenes* CIP 82110T, *B. fragilis* ATCC 25285, and *B. catarrhalis* CIP 7321T.

Although the results of some studies showed that cinnamon may be effective in the supportive treatment of cancer, infectious diseases, and has antimicrobial, anti-inflammatory, antioxidant activity, and blood pressure-lowering effects, the clinical data in this area is still limited. One clinical trial on *Helicobacter pylori* infection (Nir et al. 2000) yielded negative results for cinnamon ingested at daily doses of 80 mg.

3.2 Antifungal Activity

Cinnamon EO and especially its active compound cinnamaldehyde is found to inhibit the growth of fungi and yeasts, as well as their mycotoxin production. Wang et al. (2020) showed that CO from *C. cassia* had antifungal activity (MIC was 0.005% to 10 fungi, 0.010% to 2 pathogen fungi) against *Schizophyllum commune*, *Arthrinium phaeospermum*, *Rhizoctonia sp.*, *Helminthosporium sp.*, *Fusarium oxysporum*, *Cladosporium oxysporum*, *Fusarium solani*, *Alternaria alternata*, *Aureobasidium pullulans*, *Epicoccum nigrum*, *Aciculosporium take*, and *Shiraia bambusicola*. Root canal or endodontic infections occur when microorganisms, such as Gram-positive or Gram-negative bacteria, as well as fungi, can enter the root canal cavity and adapt well to the selective conditions of this environment, causing trauma or tooth decay (Fouad 2017; Shin et al. 2018). Brochot et al. (2017) tested the antimicrobial effectiveness of EO blends containing cinnamon and found them to be effective against *C. albicans* DSM 1386, *C. albicans* F26, *C. albicans* F35, *C. albicans* F78, *Candida glabrata* DSM 11226, and *Candida tropicalis* IP 2148.93. Edis et al. (2020) investigated the antifungal activity of hydrophobic trans-cinnamic acid, natural *Cinnamomum zeylanicum* bark extract, and hydrophilic povidone iodine combinations and found that they increased the effect of silver nanoparticles against *C. albicans* and *C. albicans* WDCM 00054. Fathima et al. (2020) showed cinnamon-clove-mediated silver nanoparticles had antifungal activity against *C. albicans*. Kumar et al. (2020) evaluated the antifungal activity of cinnamon and clove-mediated selenium nanoparticles (150 µl) against *C. albicans*. Potrč et al. (2020) determined the antifungal activity of chitosan nanoparticles with cinnamon extracts, which was used as surface coating of a plastic laminate.

Generally, in the literature, studies indicated the antifungal activity of CO but there are only limited studies related to the mechanism of action. In a study investigating the action mechanisms of CO on the cell morphology, cell membrane, and the enzyme activity in tricarboxylic acid (TCA) cycle, the authors observed that CO (*C. zeylanicum*) inhibited the mycelia growth of *Rhizopus nigricans* (*R. nigricans*) and the ergosterol biosynthesis significantly, in addition to damaging the structure of cell membrane, causing the leakage of intracellular ions and protein, and affecting the energy metabolism of *R. nigricans* by reducing the activities of succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) enzymes in the TCA cycle (Li et al. 2014). As described by Shahina et al. (2018), cinnamaldehyde disrupts both the cell wall and tubulin polymerization. The authors indicated that the viability of strain was significantly compromised depending on the dose when exposed to cinnamon bark oil (62.5 µg/mL), and cinnamon might be an alternative as an effective antifungal, either by chemical modification to improve its specificity and efficacy or in combination with other antifungal drugs.

Clinical studies on antifungal effect are limited: in a pilot study conducted with 5 patients with human immunodeficiency virus (HIV) infection and oral candidiasis, the patients were given eight cinnamon preparations daily, and improvement

was observed in oral candidiasis in three of five patients. However, the sample size was very small; therefore, further clinical trials would be necessary to determine the effectiveness on candidiasis (Quale et al. 1996).

3.3 *Antiviral Activity*

The literature on the effect of cinnamon on viruses is limited; however, it has been shown to inhibit protein synthesis in mice following infection with influenza A/PR/8 virus (Hayashi et al. 2007). Polansky and Lori (2020) noted that cinnamon extract had an antiviral activity against RNA viruses. Fabra et al. (2016) found that cinnamaldehyde was effective in reducing the titers of norovirus surrogates in a dose-dependent manner, while Hepatitis A virus titers were reduced by 1 log₁₀ after treatment with 1% cinnamaldehyde. Azizkhani and Tooryan (2016) determined the reduction on the infectivity titers for the tested norovirus by an EO combination with 3% cinnamon. Cinnamon bark extract and its nanoparticles were tested against H7N3 influenza A virus in vero cells and the viability of cells was determined by Fatima et al. (2016). The silver nanoparticles of cinnamon extract enhanced the antiviral activity and were found to be effective in both treatments when incubated with the virus prior to infection and introduced to cells after infection. In addition, nanoparticles were tested for its cytotoxic effects in vero cells and found nontoxic. Zhuang et al. (2009) evaluated the dose-dependent inhibitory activity of CO against HIV/SARS-CoV (severe acute respiratory syndrome coronavirus) S pseudovirus (the half-maximal inhibitory concentration [IC₅₀] of 30.3 µg/mL), and wild-type SARS-CoV (IC₅₀ of 43 µg/mL). Prasanth et al. (2020) showed that the nine phytochemicals of cinnamon were very likely active against the main protease enzyme of COVID-19 according to the results of the docking analysis. Fauvelle et al. (2017) demonstrated that a procyanidin type A molecule isolated from cinnamon inhibited Hepatitis C virus cell entry in a dose-dependent manner.

3.4 *Antiparasitic Activity*

Although there are no clinical studies determining antiparasitic efficacy, some *in vitro* studies are available in the literature. The inhibitory activity of cinnamon or cinnamaldehyde on various parasites, such as *Plasmodium falciparum* (Parvazi et al. 2016), *Dactylogyrus intermedius* (Ling et al. 2015), *Demodex folliculorum* (Liu et al. 2015), *Dactylogyrus intermedius* (Ji et al. 2012), and *Bursaphelenchus xylophilus* (Kong et al. 2007), has been determined.

3.5 *Antibiofilm Activity*

In addition to antimicrobial activity, the antibiofilm and anti-QS activities of CO were also determined. Mishra et al. (2021) showed that gellan/polyvinyl alcohol-based electrospun nanofibers with cinnamon EO were effective against *Candida glabrata* and *C. albicans* biofilm formation. Somrani et al. (2020) determined that there was antibiofilm activity of CO against *L. monocytogenes*. de Oliveira Carvalho et al. (2020) reported that CO had antibiofilm activity on *S. mutans* and *S. aureus*. Qi et al. (2018) detected that cinnamon extract under MIC had an inhibitory effect on the biofilm formation of *P. aeruginosa* PAO1. Letsididi et al. (2018) underlined that trans-cinnamic acid nanoemulsions (0.78, 6.25, and 6.25 mg/mL concentrations needed for $\geq 80\%$ biofilm eradication, respectively) reduced the biofilm formation of *S. aureus*, *S. typhimurium*, and *P. aeruginosa*. Alibi et al. (2020) evaluated the anti-QS and antibiofilm activity of CO from *Cinnamomum verum*. Kavyani et al. (2019) found that the aqueous extracts of cinnamon had strongest inhibition activity in the QS gene expression against *P. aeruginosa* PAO1. Sheng et al. (2016) evaluated the inhibitory effect of CO against *E. coli* O157:H7 Shiga toxin production. Yap et al. (2015) underlined the potential of cinnamon bark oil to reverse *E. coli* J53 R1 resistance to piperacillin by modifying the permeability of the outer membrane or bacterial QS inhibition.

3.6 *Synergistic Effect of Cinnamon with Antibacterial Agents*

Combination therapy is used for extending the antimicrobial spectrum, reducing toxicity, and decreasing or preventing the antimicrobial resistance during treatment. In parallel with the increasing antimicrobial resistance in recent years, studies investigating the effectiveness of combinations of antibiotics with natural products particularly against multidrug resistance bacteria have accelerated. Cinnamon is one of the candidates studied on this subject. The effectiveness of cinnamon EO against different bacteria when used in combination with different antibiotics including piperacillin and amoxicillin was determined in the literature (Uzair et al. 2017; Yap et al. 2013). Likewise, cinnamon's efficacy on multidrug-resistant bacteria has also been determined. The study conducted on combination *C. zeylanicum* EO with amikacin and gentamicin against multidrug resistance *A. baumannii* showed synergistic effect with amikacin and additive effect with gentamicin (Guerra et al. 2012). Cinnamon bark oil and cinnamaldehyde combined with colistin demonstrated synergistic activity at 16.7% and 10% rates on multidrug-resistant *P. aeruginosa* clinical isolates in Utcharyyakiat et al. (2016)'s study.

4 Conclusion

Various studies have focused on the effectiveness of the active ingredients in the EO or extracts obtained from the bark or leaves of the most known cinnamon species and determined that they have an inhibitory effect on many bacteria, viruses, fungi, and parasites in the *in vitro* environments, but clinical studies are still limited. Although the results published in the literature are promising, research on *in vivo* efficacy, toxicity, and bioavailability studies and determination of molecular targets must be undertaken and supported by clinical studies.

References

- Akthar M, Degaga B, Azam T (2014) Antimicrobial activity of essential oils extracted from medicinal plants against the pathogenic microorganisms: a review. *Issues Biol Sci Pharm Res* 2(1):1–7
- Alibi S, Ben Selma W, Ramos-Vivas J, Smach MA, Touati R, Boukadida J, Navas J, Ben Mansour H (2020) Anti-oxidant, antibacterial, anti-biofilm, and anti-quorum sensing activities of four essential oils against multidrug-resistant bacterial clinical isolates. *Curr Res Transl Med* 68(2):59–66. <https://doi.org/10.1016/j.retram.2020.01.001>
- Anlar HG, Bacanlı M, Çal T, Aydın S, Ari N, Ündeğer Bucurgat Ü, Başaran AA, Başaran AN (2018) Effects of cinnamic acid on complications of diabetes. *Turk J Med Sci* 48(1):168–177. <https://doi.org/10.3906/sag-1708-8>
- Arumugam G, Swamy MK, Sinniah UR (2016) *Plectranthus amboinicus* (Lour.) Spreng: botanical, phytochemical, pharmacological and nutritional significance. *Molecules* 21(4):369. <https://doi.org/10.3390/molecules21040369>
- Azizkhani M, Tooryan F (2016) Effect of cinnamon, rosemary and zataria essential oils on food-borne gastroenteritis viruses. *J Maz Univ Med Sci* 26(141):8–15
- Bae KH, Ji JM, Park KL (1992) The antibacterial component from *Cinnamomi* Cortex against a cariogenic bacterium *Streptococcus mutans* OMZ 176. *Arch Pharm Res* 15(3):239–241. <https://doi.org/10.1007/BF02974062>
- Balijepalli MK, Buru AS, Sakirolla R, Pichika MR (2017) *Cinnamomum* genus: a review on its biological activities. *Cinnamomum* genus: A review on its biological activities. *Int J Pharm Pharm Sci* 9(2):1–11. <https://doi.org/10.22159/ijpps.2017v9i2.11156>
- Blumenthal M, Goldberg A, Brinckmann J (2000) Herbal medicine: expanded commission E monographs. Integrative Medicine Communications, Boston
- Brochot A, Guilbot A, Haddioui L, Roques C (2017) Antibacterial, antifungal, and antiviral effects of three essential oil blends. *MicrobiologyOpen* 6(4):1–6. <https://doi.org/10.1002/mbo3.459>
- Cheng SS, Liu JY, Huang CG, Hsui YR, Chen WJ, Chang ST (2009) Insecticidal activities of leaf essential oils from *Cinnamomum osmophloeum* against three mosquito species. *Bioresour Technol* 100(1):457–464. <https://doi.org/10.1016/j.biortech.2008.02.030>
- Çolak Ç, Elibol E, Demir T, Kivçak B (2018) Overdose of cinnamon barks is the cause of poisoning in the pediatric patient: case report. *J US China Med Sci* 15(1):1–3. <https://doi.org/10.17265/1548-6648/2018.01.004>
- Cui H, Zhou H, Lin L, Zhao CT, Zhang XJ, Xiao ZH, Li C (2016) Antibacterial activity and mechanism of cinnamon essential oil and its application in milk. *J Anim Plant Sci* 26(2):532–541
- de Oliveira Carvalho I, Purgato GA, Piccolo MS, Pizzolo VR, Coelho RR, Diaz-Munoz G, Alves Nogueira Diaz M (2020) In vitro anticariogenic and antibiofilm activities of toothpastes

- formulated with essential oils. *Arch Oral Biol* 117:104834. <https://doi.org/10.1016/j.archoralbio.2020.104834>
- Deng X, Liao Q, Xu X, Yao M, Zhou Y, Lin M, Zhang P, Xie Z (2014) Analysis of essential oils from cassia bark and cassia twig samples by GC-MS combined with multivariate data analysis. *Food Anal Methods* 7:1840–1847. <https://doi.org/10.1007/s12161-014-9821-y>
- Dugoua JJ, Seely D, Perri D, Cooley K, Forelli T, Mills E, Koren G (2007) From type 2 diabetes to antioxidant activity: a systematic review of the safety and efficacy of common and cassia cinnamon bark. *Can J Physiol Pharmacol* 85(9):837–847. <https://doi.org/10.1139/Y07-080>
- Edis Z, Haj BS, Ashames A, Ibrahim M (2019) Copper-based nanoparticles, their chemistry and antibacterial properties: a review. In: Ramasami P, Gupta Bhowon M, Jhaumeer Laulloo S, Li Kam Wah H (eds) *Chemistry for a clean and healthy planet*. Springer, Cham, pp 401–428
- Edis Z, Haj Bloukh S, Ibrahim MR, Abu Sara H (2020) “Smart” antimicrobial nanocomplexes with potential to decrease surgical site infections (SSI). *Pharmaceutics* 12(4). <https://doi.org/10.3390/pharmaceutics12040361>
- Eleraky NE, Allam A, Hassan SB, Omar MM (2020) Nanomedicine fight against antibacterial resistance: an overview of the recent pharmaceutical innovations. *Pharmaceutics* 12(2). <https://doi.org/10.3390/pharmaceutics12020142>
- Fabra MJ, Castro-Mayorga JL, Randazzo W, Lagaron JM, Lopez-Rubio A, Aznar R, Sanchez G (2016) Efficacy of cinnamaldehyde against enteric viruses and its activity after incorporation into biodegradable multilayer systems of interest in food packaging. *Food Environ Virol* 8(2):125–132. <https://doi.org/10.1007/s12560-016-9235-7>
- Fathima HM, Rajasekar A, Rajeshkumar S (2020) Antifungal activity of cinnamon – clove mediated silver nanoparticles against *Candida albicans*. *Plant Cell Biotechnol Mol Biol* 21(27–28):21–27
- Fatima M, Zaidi NU, Amraiz D, Afzal F (2016) In vitro antiviral activity of *Cinnamomum cassia* and its nanoparticles against H7N3 influenza a virus. *J Microbiol Biotechnol* 26(1):151–159. <https://doi.org/10.4014/jmb.1508.08024>
- Fauvelle C, Lambotin M, Heydmann L, Prakash E, Bhaskaran S, Vishwaraman M, Baumert TF, Moog C (2017) A cinnamon-derived procyanidin type a compound inhibits hepatitis C virus cell entry. *Hepato Int* 11(5):440–445. <https://doi.org/10.1007/s12072-017-9809-y>
- Felgueiras HP, Homem NC, Teixeira MA, Ribeiro ARM, Antunes JC, Amorim MTP (2020) Physical, thermal, and antibacterial effects of active essential oils with potential for biomedical applications loaded onto cellulose acetate/polycaprolactone wet-spun microfibers. *Biomol Ther* 10(8). <https://doi.org/10.3390/biom10081129>
- Filoche SK, Soma K, Sissons CH (2005) Antimicrobial effects of essential oils in combination with chlorhexidine digluconate. *Oral Microbiol Immunol* 20(4):221–225. <https://doi.org/10.1111/j.1399-302X.2005.00216.x>
- Fouad AF (2017) Endodontic microbiology and pathobiology: current state of knowledge. *Dent Clin N Am* 61(1):1–15. <https://doi.org/10.1016/j.cden.2016.08.001>
- Guerra FQ, Mendes JM, Sousa JP, Morais-Braga MF, Santos BH, Melo Coutinho HD, Lima Ede O (2012) Increasing antibiotic activity against a multidrug-resistant *Acinetobacter spp* by essential oils of *Citrus Limon* and *Cinnamomum zeylanicum*. *Nat Prod Res* 26(23):2235–2238. <https://doi.org/10.1080/14786419.2011.647019>
- Hayashi K, Imanishi N, Kashiwayama Y, Kawano A, Terasawa K, Shimada Y, Ochiai H (2007) Inhibitory effect of cinnamaldehyde, derived from *Cinnamomi* cortex, on the growth of influenza A/PR/8 virus in vitro and in vivo. *Antivir Res* 74(1):1–8. <https://doi.org/10.1016/j.antiviral.2007.01.003>
- Hayatgheib N, Fournel C, Calvez S, Pouliquen H, Moreau E (2020) In vitro antimicrobial effect of various commercial essential oils and their chemical constituents on *Aeromonas salmonicida* subsp. *salmonicida*. *J Appl Microbiol* 129(1):137–145. <https://doi.org/10.1111/jam.14622>
- Huang TC, Fu HY, Ho CT, Tan D, Huang YT, Pan MH (2007) Induction of apoptosis by cinnamaldehyde from indigenous cinnamon *Cinnamomum osmophloeum* Kaneh through reactive

- oxygen species production, glutathione depletion, and caspase activation in human leukemia K562 cells. *Food Chem* 103(2):434–443. <https://doi.org/10.1016/j.foodchem.2006.08.018>
- Hyldgaard M, Mygind T, Meyer RL (2012) Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. *Front Microbiol* 3:12. <https://doi.org/10.3389/fmicb.2012.00012>
- Jayaprakasha GK, Singh RP, Pereira J, Sakariah KK (1997) Limonoids from *Citrus reticulata* and their moult inhibiting activity in mosquito *Culex quinquefasciatus* larvae. *Phytochemistry* 44(5):843–846. [https://doi.org/10.1016/s0031-9422\(96\)00589-4](https://doi.org/10.1016/s0031-9422(96)00589-4)
- Ji J, Lu C, Kang Y, Wang GX, Chen P (2012) Screening of 42 medicinal plants for in vivo anthelmintic activity against *Dactylogyrus intermedius* (Monogenea) in goldfish (*Carassius auratus*). *Parasitol Res* 111(1):97–104. <https://doi.org/10.1007/s00436-011-2805-6>
- Jin SE, Jin HE (2019) Synthesis, characterization, and three-dimensional structure generation of zinc oxide-based nanomedicine for biomedical applications. *Pharmaceutics* 11(11). <https://doi.org/10.3390/pharmaceutics11110575>
- Juven BJ, Kanner J, Schved F, Weisslowicz H (1994) Factors that interact with the antibacterial action of thyme essential oil and its active constituents. *J Appl Bacteriol* 76(6):626–631. <https://doi.org/10.1111/j.1365-2672.1994.tb01661.x>
- Kanwar R, Rathee J, Salunke DB, Mehta SK (2019) Green nanotechnology-driven drug delivery assemblies. *ACS Omega* 4(5):8804–8815. <https://doi.org/10.1021/acsomega.9b00304>
- Kaskatepe B, Kiymaci ME, Simsek D, Erol HB, Erdem SA (2016) Comparison of the contents and antimicrobial activities of commercial and natural cinnamon oils. *Indian J Pharm Sci* 78(4):541–548
- Kavyani B, Arabestani MR, Nejad ASM, Moradkhani S, Kamarehei F, Taheri M, Alikhani MY (2019) The effect of cinnamon bark on the expression of quorum sensing system and virulence genes in *Pseudomonas aeruginosa* strain PAO1. *Res J Biotechnol* 14(2):42–48. <Go to ISI>://WOS:000456824600006
- Kawatra P, Rajagopalan R (2015) Cinnamon: mystic powers of a minute ingredient. *Pharm Res* 7(Suppl 1):S1–S6. <https://doi.org/10.4103/0974-8490.157990>
- Kitazuru ER, Moreira AVB, Mancini-Filho J, Delincée H, Villavicencio ALCH (2004) Effects of irradiation on natural antioxidants of cinnamon (*Cinnamomum zeylanicum* N.). *Radiat Phys Chem* 71(1–2):39–41
- Kong JO, Lee SM, Moon YS, Lee SG, Ahn YJ (2007) Nematicidal activity of cassia and cinnamon oil compounds and related compounds toward *Bursaphelenchus xylophilus* (Nematoda: *Parasitaphelenchidae*). *J Nematol* 39(1):31–36. <https://www.ncbi.nlm.nih.gov/pubmed/19259472>
- Koppikar SJ, Choudhari AS, Suryavanshi SA, Kumari S, Chattopadhyay S, Kaul-Ghanekar R (2010) Aqueous cinnamon extract (ACE-c) from the bark of *Cinnamomum cassia* causes apoptosis in human cervical cancer cell line (SiHa) through loss of mitochondrial membrane potential. *BMC Cancer* 10:210. <https://doi.org/10.1186/1471-2407-10-210>
- Kothari S, Rajasekar A, Rajeshkumar S (2020) Antimicrobial activity of clove and cinnamon mediated silver nanoparticles against *Lactobacillus* species - an in vitro study. *Plant Cell Biotechnol Mol Biol* 21(59–60):1–7
- Kumar A, Arvina R, Rajeshkumar S (2020) Antifungal activity of clove and cinnamon mediated selenium nanoparticles: an in vitro study. *Plant Cell Biotechnol Mol Biol* 21(23–24):18–23
- Lee SH, Jun BH (2019) Silver nanoparticles: synthesis and application for nanomedicine. *Int J Mol Sci* 20(4). <https://doi.org/10.3390/ijms20040865>
- Lee EJ, Kim SR, Kim J, Kim YC (2002) Hepatoprotective phenylpropanoids from *Scrophularia buergeriana* roots against CCl₄-induced toxicity: action mechanism and structure-activity relationship. *Planta Med* 68(5):407–411. <https://doi.org/10.1055/s-2002-32081>
- Lee JE, Jung M, Lee SC, Huh MJ, Seo SM, Park IK (2020) Antibacterial mode of action of trans-cinnamaldehyde derived from cinnamon bark (*Cinnamomum verum*) essential oil against *Agrobacterium tumefaciens*. *Pestic Biochem Physiol* 165:104546. <https://doi.org/10.1016/j.pestbp.2020.02.012>

- Letsididi KS, Lou Z, Letsididi R, Mohammed K, Maguy BL (2018) Antimicrobial and antibiofilm effects of trans-cinnamic acid nanoemulsion and its potential application on lettuce. *LWT-Food Sci Technol* 94:25–32
- Li Y, Nie Y, Zhou L, Li S, Tang X, Ding Y (2014) The possible mechanism of antifungal activity of cinnamon oil against *Rhizopus nigricans*. *J Chem Pharm Res* 6(5):12–20
- Li J, Sun Y, Li, C (2015) Volatile oils of Chinese crude medicines exhibit antiparasitic activity against human *Demodex* with no adverse effects in vivo. *Exp Ther Med* 9(4):1304–1308
- Liao C, Li Y, Tjong SC (2019) Bactericidal and cytotoxic properties of silver nanoparticles. *Int J Mol Sci* 20(2). <https://doi.org/10.3390/ijms20020449>
- Ling F, Jiang C, Liu G, Li M, Wang G (2015) Anthelmintic efficacy of cinnamaldehyde and cinnamic acid from cortex cinnamon essential oil against *Dactylogyrus intermedius*. *Parasitology* 142(14):1744–1750. <https://doi.org/10.1017/S0031182015001031>
- Liu JX, Sun YH, Li CP (2015) Volatile oils of Chinese crude medicines exhibit antiparasitic activity against human *Demodex* with no adverse effects in vivo. *Exp Ther Med* 9(4):1304–1308. <https://doi.org/10.3892/etm.2015.2272>
- Lu Z, Jia Q, Wang R, Wu X, Wu Y, Huang C, Li Y (2011) Hypoglycemic activities of A- and B-type procyanidin oligomer-rich extracts from different cinnamon barks. *Phytomedicine* 18(4):298–302. <https://doi.org/10.1016/j.phymed.2010.08.008>
- Mamatha G, Varada Rajulu A, Madhukar K (2019) Development and analysis of cellulose nanocomposite films with in situ generated silver nanoparticles using tamarind nut powder as a reducing agent. *Int J Polym Anal Charact* 24(3):219–226. <https://doi.org/10.1080/1023666X.2018.1564572>
- Marcoux E, Lagha AB, Gauthier P, Grenier D (2020) Antimicrobial activities of natural plant compounds against endodontic pathogens and biocompatibility with human gingival fibroblasts. *Arch Oral Biol* 116:104734. <https://doi.org/10.1016/j.archoralbio.2020.104734>
- Mathew S, Abraham TE (2006) Studies on the antioxidant activities of cinnamon (*Cinnamomum verum*) bark extracts, through various in vitro models. *Food Chem* 94(4):520–528. <https://doi.org/10.1016/j.foodchem.2004.11.043>
- Melo AD, Amaral AF, Schaefer G, Luciano FB, de Andrade C, Costa LB, Rostagno MH (2015) Antimicrobial effect against different bacterial strains and bacterial adaptation to essential oils used as feed additives. *Can J Vet Res* 79(4):285–289. <https://www.ncbi.nlm.nih.gov/pubmed/26424908>
- Mishra P, Gupta P, Pruthi V (2021) Cinnamaldehyde incorporated gellan/PVA electrospun nanofibers for eradicating *Candida* biofilm. *Mater Sci Eng C Mater Biol Appl* 119:111450. <https://doi.org/10.1016/j.msec.2020.111450>
- Mohammad P, Zienab B, Hossein KJ (2014) The effect of cinnamon extract on gonadotropin changes (FSH & LH) in rats treated with gelophen. *Biomed Pharmacol J* 7(1):363–367. <https://doi.org/10.13005/bpj/499>
- Muchuweti M, Kativu E, Mupure CH, Chidewe C, Ndhkala AR, Benhura MAN (2007) Phenolic composition and antioxidant properties of some spices. *Am J Food Technol* 2(5):414–420
- Nabavi SF, Di Lorenzo A, Izadi M, Sobarzo-Sanchez E, Daglia M, Nabavi SM (2015) Antibacterial effects of cinnamon: from farm to food, cosmetic and pharmaceutical industries. *Nutrients* 7(9):7729–7748. <https://doi.org/10.3390/nu7095359>
- Nagarajan K, Rajasekar A, Rajeshkumar S (2020) Antimicrobial activity of clove and cinnamon formulation mediated selenium nanoparticles against oral pathogens. *Plant Cell Biotechnol Mol Biol* 21(31–32):27–35
- Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V (2013) Effect of essential oils on pathogenic bacteria. *Pharmaceuticals (Basel)* 6(12):1451–1474. <https://doi.org/10.3390/ph6121451>
- Nir Y, Potasman I, Stermer E, Tabak M, Neeman I (2000) Controlled trial of the effect of cinnamon extract on *Helicobacter pylori*. *Helicobacter* 5(2):94–97. <https://doi.org/10.1046/j.1523-5378.2000.00014.x>

- Ose R, Tu J, Schink A, Maxeiner J, Schuster P, Lucas K, Saloga J, Bellinghausen I (2020) Cinnamon extract inhibits allergen-specific immune responses in human and murine allergy models. *Clin Exp Allergy* 50(1):41–50. <https://doi.org/10.1111/cea.13507>
- Parvazi S, Sadeghi S, Azadi M, Mohammadi M, Arjmand M, Vahabi F, Sadeghzadeh S, Zamani Z (2016) The effect of aqueous extract of cinnamon on the metabolome of *Plasmodium falciparum* using (1)H NMR spectroscopy. *J Trop Med* 2016:3174841. <https://doi.org/10.1155/2016/3174841>
- Polansky H, Lori G (2020) Coronavirus disease 2019 (COVID-19): first indication of efficacy of Gene-Eden-VIR/Novirin in SARS-CoV-2 infection. *Int J Antimicrob Agents* 55(6):105971. <https://doi.org/10.1016/j.ijantimicag.2020.105971>
- Potrč S, Fras Zemljč L, Sterniša M, Smole Možina S, Plohl O (2020) Development of biodegradable whey-based laminate functionalised by chitosan–natural extract formulations. *Int J Mol Sci* 21(10):3668. <https://www.mdpi.com/1422-0067/21/10/3668>
- Prasanth D, Murahari M, Chandramohan V, Panda SP, Atmakuri LR, Guntupalli C (2020) In silico identification of potential inhibitors from cinnamon against main protease and spike glycoprotein of SARS CoV-2. *J Biomol Struct Dyn*:1–15. <https://doi.org/10.1080/07391102.2020.1779129>
- Premkumar J, Sudhakar T, Dhakal A, Shrestha JB, Krishnakumar S, Balashanmugam P (2018) Synthesis of silver nanoparticles (AgNPs) from cinnamon against bacterial pathogens. *Biocatal Agric Biotechnol* 15:311–316
- Purkait S, Bhattacharya A, Bag A, Chattopadhyay RR (2020) Synergistic antibacterial, antifungal and antioxidant efficacy of cinnamon and clove essential oils in combination. *Arch Microbiol* 202(6):1439–1448. <https://doi.org/10.1007/s00203-020-01858-3>
- Qi G, Chen J, Chen G, Yang Z, Wang H, Wang S (2018) Inhibition of biofilm formation in *Pseudomonas aeruginosa* by cinnamon extract. *J Food Sci Biotechnol* 37(8):807–811. <https://doi.org/10.3969/j.issn.1673-1689.2018.08.004>
- Quale JM, Landman D, Zaman MM, Burney S, Sathe SS (1996) In vitro activity of *Cinnamomum zeylanicum* against azole resistant and sensitive *Candida* species and a pilot study of cinnamon for oral candidiasis. *Am J Chin Med* 24(2):103–109. <https://doi.org/10.1142/S0192415X96000153>
- Ranasinghe P, Jayawardena R, Galappaththy P, Constantine GR, de Vas Gunawardana N, Katulanda P (2013) Response to Akilen et al. Efficacy and safety of 'true' cinnamon (*Cinnamomum zeylanicum*) as a pharmaceutical agent in diabetes: a systematic review and meta-analysis. *Diabet Med* 30(4):506–507. <https://doi.org/10.1111/dme.12141>
- Ranasinghe P, Jayawardena R, Pigera S, Wathurapatha WS, Weeratunga HD, Premakumara GAS, Katulanda P, Constantine GR, Galappaththy P (2017) Evaluation of pharmacodynamic properties and safety of *Cinnamomum zeylanicum* (Ceylon cinnamon) in healthy adults: a phase I clinical trial. *BMC Complement Altern Med* 17(1):550. <https://doi.org/10.1186/s12906-017-2067-7>
- Reda M, Ashames A, Edis Z, Bloukh S, Bhandare R, Abu Sara H (2019) Green synthesis of potent antimicrobial silver nanoparticles using different plant extracts and their mixtures. *PRO* 7(8):510. <https://www.mdpi.com/2227-9717/7/8/510>
- Ren JL, Zhang AH, Wang XJ (2020) Traditional Chinese medicine for COVID-19 treatment. *Pharmacol Res* 155:104743. <https://doi.org/10.1016/j.phrs.2020.104743>
- Riau AK, Aung TT, Setiawan M, Yang L, Yam GHF, Beuerman RW, Venkatraman SS, Mehta JS (2019) Surface immobilization of nano-silver on polymeric medical devices to prevent bacterial biofilm formation. *Pathogens* 8(3). <https://doi.org/10.3390/pathogens8030093>
- Saki M, Seyed Mohammadi S, Montazeri EA, Siahpoosh A, Moosavian M, Latifi SM (2020) In vitro antibacterial properties of *Cinnamomum zeylanicum* essential oil against clinical extensively drug-resistant bacteria. *Eur J Integr Med* 37:101146
- Sakkas H, Bozidis P, Ilia A, Mpekoulis G, Papadopoulou C (2019) Antimicrobial resistance in bacterial pathogens and detection of carbapenemases in klebsiella pneumoniae isolates from hospital wastewater. *Antibiotics* 8(3). <https://doi.org/10.3390/antibiotics8030085>

- Sangal A (2011) Role of cinnamon as beneficial antidiabetic food adjunct : a review. *Adv Appl Sci Res* 2(4):440–450
- Shah A, Ali Buabeid M, Arafa EA, Hussain I, Li L, Murtaza G (2019) The wound healing and antibacterial potential of triple-component nanocomposite (chitosan-silver-sericin) films loaded with moxifloxacin. *Int J Pharm* 564:22–38. <https://doi.org/10.1016/j.ijpharm.2019.04.046>
- Shah A, Ashames AA, Buabeid MA, Murtaza G (2020) Synthesis, in vitro characterization and antibacterial efficacy of moxifloxacin-loaded chitosan-pullulan-silver-nanocomposite films. *J Drug Deliv Sci Technol* 55:101366
- Shahina Z, El-Ganiny AM, Minion J, Whiteway M, Sultana T, Dahms TES (2018) *Cinnamomum zeylanicum* bark essential oil induces cell wall remodelling and spindle defects in *Candida albicans*. *Fungal Biol Biotechnol* 5:3. <https://doi.org/10.1186/s40694-018-0046-5>
- Sharma S (2015) Food preservatives and their harmful effect. *Int J Sci Res Publ* 5(4):5–6. <http://www.ijsrp.org/research-paper-0415/ijsrp-p4014.pdf>
- Sheng L, Rasco B, Zhu MJ (2016) Cinnamon oil inhibits Shiga toxin type 2 phage induction and Shiga toxin type 2 production in *Escherichia coli* O157:H7. *Appl Environ Microbiol* 82(22):6531–6540. <https://doi.org/10.1128/AEM.01702-16>
- Shin JM, Luo T, Lee KH, Guerreiro D, Botero TM, McDonald NJ, Rickard AH (2018) Deciphering endodontic microbial communities by next-generation sequencing. *J Endod* 44(7):1080–1087. <https://doi.org/10.1016/j.joen.2018.04.003>
- Smith CK, Moore CA, Elahi EN, Smart AT, Hotchkiss SA (2000) Human skin absorption and metabolism of the contact allergens, cinnamic aldehyde, and cinnamic alcohol. *Toxicol Appl Pharmacol* 168(3):189–199. <https://doi.org/10.1006/taap.2000.9025>
- Somrani M, Ingles MC, Debbabi H, Abidi F, Palop A (2020) Garlic, onion, and cinnamon essential oil anti-biofilms' effect against *Listeria monocytogenes*. *Foods* 9(5). <https://doi.org/10.3390/foods9050567>
- Swamy MK, Sinniah UR (2015) A comprehensive review on the phytochemical constituents and pharmacological activities of *Pogostemon cablin* Benth.: an aromatic medicinal plant of industrial importance. *Molecules* 20(5):8521–8547. <https://doi.org/10.3390/molecules20058521>
- Swamy KM, Neeki MA, Bhaumik SH, Darshil SH, Vijay R, Kashyap S (2012) Phytochemical screening and in vitro antimicrobial activity of Bougainvillea spectabilis flower extracts. *Int J Phytomed* 4:375–379
- Tanker M, Tanker N (1990) *Pharmacognosy*. Ankara University, Ankara
- Tavares TD, Antunes JC, Padrao J, Ribeiro AI, Zille A, Amorim MTP, Ferreira F, Felgueiras HP (2020) Activity of specialized biomolecules against gram-positive and gram-negative bacteria. *Antibiotics* (Basel) 9(6). <https://doi.org/10.3390/antibiotics9060314>
- Tung YT, Chua MT, Wang SY, Chang ST (2008) Anti-inflammation activities of essential oil and its constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) twigs. *Bioresour Technol* 99(9):3908–3913. <https://doi.org/10.1016/j.biortech.2007.07.050>
- Tung YT, Yen PL, Lin CY, Chang ST (2010) Anti-inflammatory activities of essential oils and their constituents from different provenances of indigenous cinnamon (*Cinnamomum osmophloeum*) leaves. *Pharm Biol* 48(10):1130–1136. <https://doi.org/10.3109/13880200903527728>
- Utchariyakiat I, Surassmo S, Jaturanpinyo M, Khuntayaporn P, Chomnawang MT (2016) Efficacy of cinnamon bark oil and cinnamaldehyde on anti-multidrug resistant *Pseudomonas aeruginosa* and the synergistic effects in combination with other antimicrobial agents. *BMC Complement Altern Med* 16:158. <https://doi.org/10.1186/s12906-016-1134-9>
- Uzair B, Niaz N, Bano A, Khan BA, Zafar N, Iqbal M, Tahira R, Fasim F (2017) Essential oils showing in vitro anti MRSA and synergistic activity with penicillin group of antibiotics. *Pak J Pharm Sci* 30(5(Supplementary)):1997–2002. <https://www.ncbi.nlm.nih.gov/pubmed/29105634>
- Vasconcelos NG, Croda J, Simionatto S (2018) Antibacterial mechanisms of cinnamon and its constituents: a review. *Microb Pathog* 120:198–203. <https://doi.org/10.1016/j.micpath.2018.04.036>
- Wang SY, Chen PF, Chang ST (2005) Antifungal activities of essential oils and their constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) leaves against wood decay fungi. *Bioresour Technol* 96(7):813–818. <https://doi.org/10.1016/j.biortech.2004.07.010>

- Wang A, Bi Y, Wen X, Wang Y, Cai H (2020) Antifungal activity of 4 kinds of aromatic essential oil derived from plants to pathogenic fungi of bamboo. *Sci Silvae Sin* 56(6):59–67. <https://doi.org/10.11707/j.1001-7488.20200606>
- Weibel H, Hansen J (1989) Interaction of cinnamaldehyde (a sensitizer in fragrance) with protein. *Contact Dermatitis* 20(3):161–166. <https://doi.org/10.1111/j.1600-0536.1989.tb04650.x>
- WHO (2002) Evaluation of certain food additives and contaminants (World Health Organization Technical Report Series). Issue
- WHO (2017) One health. <https://www.who.int/news-room/q-a-detail/one-health>
- Wiesner J, Mitsch A, Wissner P, Jomaa H, Schlitzer M (2001) Structure-activity relationships of novel anti-malarial agents. Part 2: cinnamic acid derivatives. *Bioorg Med Chem Lett* 11(3):423–424. [https://doi.org/10.1016/s0960-894x\(00\)00684-3](https://doi.org/10.1016/s0960-894x(00)00684-3)
- Willoughby MJ, Mills S (1996) *British herbal pharmacopoeia*, 4th edn. British Herbal Medicine Association, London
- Yap PSX, Lim SHE, Hu CP, Yiap BC (2013) Combination of essential oils and antibiotics reduce antibiotic resistance in plasmid-conferred multidrug resistant bacteria. *Phytomedicine* 20(8–9):710–713. <https://doi.org/10.1016/j.phymed.2013.02.013>
- Yap PS, Krishnan T, Chan KG, Lim SH (2015) Antibacterial mode of action of *Cinnamomum verum* bark essential oil, alone and in combination with Piperacillin, against a multi-drug-resistant *Escherichia coli* strain. *J Microbiol Biotechnol* 25(8):1299–1306. <https://doi.org/10.4014/jmb.1407.07054>
- Yuan JH, Dieter MP, Bucher JR, Jameson CW (1992) Toxicokinetics of cinnamaldehyde in F344 rats. *Food Chem Toxicol* 30(12):997–1004. [https://doi.org/10.1016/0278-6915\(92\)90109-x](https://doi.org/10.1016/0278-6915(92)90109-x)
- Zhang Y, Liu X, Wang Y, Jiang P, Quek S (2016) Antibacterial activity and mechanism of cinnamon essential oil against *Escherichia coli* and *Staphylococcus aureus*. *Food Control* 59:282–289. <https://doi.org/10.1016/j.foodcont.2015.05.032>
- Zhuang M, Jiang H, Suzuki Y, Li X, Xiao P, Tanaka T, Ling H, Yang B, Saitoh H, Zhang L, Qin C, Sugamura K, Hattori T (2009) Procyanidins and butanol extract of *Cinnamomi* Cortex inhibit SARS-CoV infection. *Antivir Res* 82(1):73–81. <https://doi.org/10.1016/j.antiviral.2009.02.001>

Chapter 5

The Antimicrobial Activities of Oleuropein and Hydroxytyrosol



Nataša Zorić and Ivan Kosalec

Abstract Several studies have reported that olive leaf extract and its constituents, particularly oleuropein and hydroxytyrosol, have health benefits including antioxidant and antimicrobial properties. Oleuropein and hydroxytyrosol have significant *in vitro* activity against fungi including opportunistic pathogen *Candida albicans*. Both compounds target virulence factors essential for the establishment of *C. albicans* infection. Both biomolecules express wide antibacterial activity *in vitro*. On the bacterial model *Staphylococcus aureus*, different targets have been detected. Oleuropein and hydroxytyrosol also interact with biofilm formation and could potentiate the activity of ampicillin. Considering the growing resistance to existing therapeutics has triggered the need for the development of new antimicrobial drugs, based on the presented results in this chapter, it seems that oleuropein and its derivative hydroxytyrosol could be considered as promising candidates for the treatment and/or prevention of candidiasis, and local infections caused by bacteria.

Keywords Antimicrobial activity · Oleuropein · Hydroxytyrosol · *Olea europaea* · Olive leaf extract

Abbreviations

ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
BC	Bactericidal concentration
CSH	Cell surface hydrophobicity
DMPG	Dimyristoylphosphatidylglycerol
DNA	Deoxyribonucleic acid

N. Zorić
Agency for Medicinal Products and Medical Devices of Croatia (HALMED), Zagreb, Croatia

I. Kosalec (✉)
University of Zagreb, Faculty of Pharmacy and Biochemistry, Institute for Microbiology,
Zagreb, Croatia
e-mail: ivan.kosalec@pharma.hr

EUCAST	European Committee on Antimicrobial Susceptibility Testing
EVOO	Extra virgin olive oil
GAE	Gallic acid equivalent
HPLC-DAD	High-performance liquid chromatography with a diode-array detector
IC	Inhibitory concentration
MFC	Minimum fungicidal concentration
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
SAP	Secreted aspartyl proteinases

1 Introduction

Vegetation native to Mediterranean region due to its substantial sun exposure has been correlated to the high antioxidant content in plants. Epidemiologic studies have established an inverse relationship between intake of fruit- and vegetable-based antioxidants and mortality rates from chronic diseases (Huang and Sumpio 2008). Olive oil and olive leaf extract have been used in folk medicine within European Mediterranean countries and islands since ancient times and are well known for their broad health benefits associated with high levels of antioxidants (Medina et al. 2007; Khan et al. 2007; Zorić et al. 2021). The most widespread species of olive is the *Olea europaea* L. and its genus includes 35 species of evergreen shrubs and trees (Boskou 1996). It is scientifically accepted that *O. europaea* products, such as fruits and virgin olive oil, have beneficial health effects when they are a regular part of the human diet (Thielmann et al. 2017). The biological and pharmacological properties of olive oil and olive leaf extract have been attributed to its high content of biophenols (oleuropein, hydroxytyrosol, and their derivatives) (Ortega-Garcia and Peragon 2010). Previous studies on the composition of olive (*Olea europaea* L.) varieties, organs, and olive products have led to the identification of a plethora of phenolic compounds, including phenolic alcohols, secoiridoid derivatives, phenolic acids, lignans, and flavonoids (Suarez et al. 2010). The phenolic profiles of olive leaves and fruits are dominated by phenolic acids (e.g., ferulic, vaillic, coumaric acid), phenolic alcohols (e.g., hydroxytyrosol and tyrosol), flavonoids (e.g., luteolin-7-glucoside, cyanidin-3-glucoside, cyanidin-3-rutinoside, rutin, apigenin-7-glucoside, quercetin-3-rhamnoside, luteolin), and secoiridoids (e.g., oleuropein, ligstroside) (Thielmann et al. 2017). The content of phenolic compounds varies depending on environmental conditions including region, climate and is also affected by the variety, organ, olive product, ripeness of the olives at harvesting, and the processing system employed (Hassen et al. 2015). The bitter tasting secoiridoid oleuropein (Fig. 5.1) is one of the most abundant bioactive components contained in the *Olea europaea* L. and is exclusive to the plants of *Oleaceae* family (Servili et al. 2004). Oleuropein consists of a polyphenol, namely, 4-(2-hydroxyethyl) benzene-1,2-diol, commonly known

Fig. 5.1 Chemical structure of oleuropein

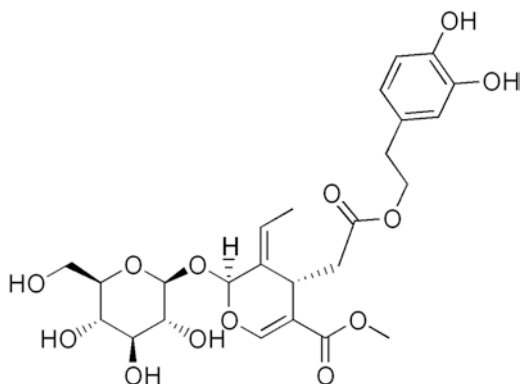
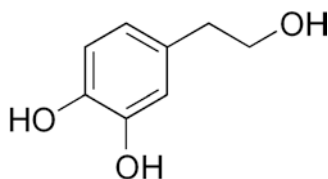


Fig. 5.2 Chemical structure of hydroxytyrosol



as hydroxytyrosol, a secoiridoid called oelenolic acid and a glucose molecule (Fig. 5.2). Phytoalexins and their precursors such as oleuropein, accumulated during fruit and leaf maturation, act as defense molecules against herbivores and microbial pathogens (Thielmann et al. 2017; Kubo et al. 1985). Although the main biological activities demonstrated so far are antioxidant and anti-inflammatory effects of oleuropein and its derivative hydroxytyrosol including their ability to treat oxidant and inflammatory-related diseases (i.e., cancer, cardiovascular disease, diabetes, etc.) (Hassen et al. 2015), in this chapter, available scientific data on their antimicrobial activities will be discussed.

2 Antifungal Activity

Natural compounds are potential source of antimycotic agents either in their nascent form or in the form of their more effective derivatives (Jacob and Walker 2005). Often these natural compounds are phenolics found in edible plants and are innately safe for humans (Faria et al. 2011). Interest in medicinal plants and their isolated constituents has increased due to the efficacy of new plant-derived drugs and in general the growing interest in natural products. Also, because of the concerns about the side effects of conventional medicine, the use of natural products as an alternative to conventional therapy in the healing and treatment of various diseases has been on the rise in the last few decades (Zuzarte et al. 2011). Additionally, the progression of drug resistance to conventional therapeutics, partially as a consequence of rising overprescription and overuse of conventional antifungals, triggered a need for more

effective treatment. As an interim solution, antibiotic resistance could be “broken” by coadministering appropriate nonantibiotic drugs with failing antibiotics. Some of these compounds can either directly kill microorganisms, reduce the antibiotic minimum inhibitory concentration when used in combination with existing antibiotics, and/or modulate host defense through effects on host innate immunity (Brown 2015). There has also been an increase in serious human infections in immunocompromised patients caused by fungi. The range of severity of these infections is a consequence of the host reaction to the metabolic products produced by fungi, the virulence of the infecting strain, the site of infection, and environmental factors (Zuzarte et al. 2011). Available antifungals predominantly include azoles, echinocandins, polyenes, and allylamines. They have a distinct mode of action; for example, azoles target heme protein, cytochrome P450 lanosterol-14- α -demethylase, thereby impeding conversion of lanosterol to fecosterol and subsequently blocking ergosterol biosynthesis. Echinocandins interfere with cell wall synthesis by inhibiting $\beta(1-3)$ -glucan-synthase. Polyenes have an affinity to bind membrane sterols that results in the formation of aqueous pores, ensuring the leakage of crucial cellular components and subsequent cell death. Allylamines are a relatively newer class of antifungals that also inhibit ergosterol biosynthesis but by specifically targeting squalene epoxidase (Dhamgaye et al. 2014).

The prime requirement at the moment would be finding an agent with a broad-spectrum of activity against susceptible species (Odds et al. 2003). This chapter summarizes the current knowledge on oleuropein and hydroxytyrosol activities against fungi as potentially promising targets and inhibitors in continuous research for effective antifungal therapy. Mainly, available literature on the effects against fungi for these compounds is directed toward members of genus *Candida* including *C. albicans* as one of the most important human pathogens (Kosalec et al. 2011). The outgrowth of *C. albicans* results in superficial mycoses of the skin, nails, and mucous membranes. However, in individuals with immune deficiencies caused by underlying disease, chemotherapy treatment, or immunosuppression following transplantation, *C. albicans* can cause severe, life-threatening invasive candidiasis. Recently, targeting virulence rather than essential process, as with conventional drugs, has been postulated as a new paradigm for the development of antifungal agents, following the successful development of drugs targeting bacterial virulence in antimicrobial therapy. So, instead of being killed, a pathogen is maintained in a harmless form by blocking virulence attributes that contribute to its pathogenicity. In addition, resistance to drugs that target virulence instead of growth is less likely to develop, given that selective pressure is reduced on nonessential targets that are required only to colonize host environments (Shareck and Belhumeur 2011). The majority of published reports are describing antimicrobial properties of olive leaf extracts with identified composition and with quantified phenolic compounds present in the extract. Pereira et al. (2007) showed *in vitro* activity of olive leaf aqueous extracts against several microorganisms including fungi *C. albicans* and *Cryptococcus neoformans*. Seven phenolics were identified and quantified by HPLC-DAD analysis of olive leaf extract: caffeic acid, verbascoside, oleuropein, luteolin, 7-*O*-glucoside, rutin, apigenin 7-*O*-glucoside, and luteolin 4'-*O*-glucoside.

Oleuropein was a compound present in the extract in the highest amount, representing approximately 73% of total identified compounds. The extract showed antimicrobial activity of olive leaf extracts in a concentration-dependent manner and *C. albicans* was found to be one of the most sensitive microorganisms with IC_{25} value lower than 1 mg/ml ($IC_{25} = 0.85$ mg/ml). *C. neoformans* was found to be less susceptible to olive leaf extract activity with $IC_{25} = 3.00$ mg/ml. Previously, also Pereira et al. (2006) performed comparative studies using extracts from olives that did not show activity against *C. albicans* (up to 100 mg/ml). Authors have concluded that cultivar and processing technology-dependent changes in phenolic composition have a considerable impact on the antimicrobial potential of crude olive extracts. Also, Medina et al. (2006) assessed the antimicrobial activity of virgin olive oil (50% v/v) where prevalent phenolic compounds were oleuropein aglycone, hydroxytyrosol, and tyrosol and they observed that *C. albicans* was unaffected. In 2014, Karygianni et al. (2014) examined dried extract from *Olea europaea* obtained by extraction with acetone (containing 60% oleuropein), table olive processing wastewater extract (contained as its main compound, the degradation product of oleuropein, hydroxytyrosol, in a percentage around 15%) against bacterial and one *Candida albicans* strain. In general, table olive extract was more active than olive leaf extract. Olive leaf extract showed a milder inhibitory effect against investigated oral pathogens. Although the extract was found to be active against each of the tested microorganisms; however, it was less active against *C. albicans* than against bacterial strains (minimum inhibitory concentration (MIC) value for *C. albicans* strain was 10.00 mg/ml). The authors concluded that the conflicting outcomes of investigated activity of olive leaf extract against *C. albicans* could be attributed to different extraction methods, which result in different chemical profiles, so which phenolic or other compound was responsible for this favorable effect remains unknown. In that study, two main compounds of the extracts were oleuropein in olive leaves and hydroxytyrosol in table olive processing wastewater. Halawi et al. (2015) performed a comparative study to evaluate the antifungal activity of olive leaves and cake samples extracted differently to obtain three categories of extracts: ethanolic extract, cold aqueous, and hot aqueous against five strains of *C. albicans* isolated in hospital. The antifungal activity was tested using well-diffusion method. Cold aqueous extract of olive cake (total phenolic content was 91.76 GAE mg/g dry matter) and ethanolic extract from leaves (total phenolic content was 98.03 GAE mg/g dry matter) showed antifungal activity against the growth of all isolates with the lowest recorded MIC and minimum fungicidal concentration (MFC) of 2.5 and 15 mg/ml, respectively, for both extracts. Also, the time-kill assay showed that fungal cells died within 6 hours after their treatment with both selected extracts. The ultrastructure of treated *C. albicans* with the two selected extracts revealed the presence of deformed cells with disintegrated protoplasm and even ruptured cell wall and cell membrane. An additional study was performed by Zorić et al. (2016b) to evaluate activity of olive leaf water extract against *C. albicans* and *C. dubliniensis*. MIC values of the extract were determined by several *in vitro* assays. The water extract showed concentration-dependent effect on the viability of *C. albicans* with MIC value of 46.875 mg/ml and *C. dubliniensis* with MIC value of 62.5 mg/ml. The

most sensitive methods for testing the antifungal effect of the extract were trypan blue exclusion method and fluorescent dye exclusion method, while MIC could not be determined according to the EUCAST recommendation, suggesting that herbal preparations contain compounds that may interfere with this susceptibility testing. The fluorescent dye exclusion method was also used for the assessment of morphological changes in the nuclei of treated cells. Necrosis predominated over apoptosis (1 h and 18 h of incubation) in the *C. albicans* sample treated with the highest concentration of olive leaf extract (46.875 mg/ml) and in the *C. dubliniensis* sample treated also with the highest concentration of olive leaf extract (187.5 mg/ml). In other samples, apoptosis was the predominated type of cell death. It has to be mentioned that 46.875 mg/ml to *C. albicans* and 187.5 mg/ml to *C. dubliniensis* were highly cytotoxic after 18 h of incubation. Induction of apoptosis in that sample was comparable to positive controls (amphotericin B and H₂O₂). Even though there are research studies dealing with antimicrobial including antifungal effects of olive leaf extract, there is far less studies dealing with oleuropein and hydroxytyrosol activities against fungi. There are reports published in 1998 (Aziz et al. 1998; Koutsoumanis et al. 1998) regarding antimicrobial activity of oleuropein against yeasts, fungi, molds, and other parasites. According to Bisignano et al. (1999) and later Khan et al. (2007), hydroxytyrosol demonstrated broader antimicrobial activity than oleuropein and is comparable to ampicillin and erythromycin in spectrum and potency. In 2009, Rahioui et al. (2009) have shown that polyphenols, hydroxycinnamic derivatives, oleuropein derivatives, tyrosol derivatives, and flavonol monoglucosides, were responsible for olive tree resistance to the leaf-spot disease caused by *Fusicladium oleagineum*. Resistance to *F. oleagineum* was related positively to tyrosol derivatives, oleuropein and rutin contents and negatively to verbascoside and apigenin contents. Recently, Khan and Murphy (2021) performed study with *Cunninghamella elegans*, a filamentous fungus that is of biotechnological interest as it catabolizes drugs and other xenobiotics in an analogous manner to animals. The authors reported that 3-hydroxytyrosol is a novel signaling molecule that regulates fungal biofilm growth. The cultures were grown planktonically and as biofilms for 72 h. Planktonic cultures have higher concentrations of the metabolite. In the presence of exogenous hydroxytyrosol (at concentrations 0.3, 0.5, and 0.8 mg/ml), the growth of aerial mycelium was inhibited and there was selective inhibition of biofilm when it was added to culture medium. The compound was not biotransformed by the fungus, when it was added to 72-h-old cultures. In the presence of 0.8 mg/ml, biofilm of *C. elegans* was approximately 75% less than the control, but planktonic growth was 30% lower.

Karygianni et al. (2019) have reported that MIC value of 1.25 mg/ml has been observed for the strain of *C. albicans*, while 99.9% of *C. albicans* was eradicated with 2.5 mg/ml. In 2013, a study was performed (Zorić et al. 2013) to test antifungal activity of hydroxytyrosol against medically important yeasts and dermatophyte strains using several *in vitro* approaches. MIC values were as follows: 6.25 mg/ml for *C. albicans*, *C. dubliniensis*, *C. tropicalis* and *Saccharomyces cerevisiae*, 1.5625 mg/ml for *C. parapsilosis* and *C. kefyr*, 0.1953 for mg/ml *Blastoschizomyces capitatum* and 0.0976 mg/ml for *C. curvata*, while for dermatophyte strains, they were 1.5625 mg/

ml for *Trichophyton mentagrophytes* var. *mentagrophytes* and 0.7812 mg/ml for *Trichophyton mentagrophytes* var. *interdigitale*. It was also observed that below MIC value, hydroxytyrosol showed potent damage of *C. albicans* cell wall using the fluorescent dye exclusion method. At subinhibitory concentrations (sub-MIC), hydroxytyrosol caused disturbances in cell surface hydrophobicity (CSH) of *C. albicans* and influenced dimorphic transition of the same strain, which is considered as one of the most important virulence factors of *C. albicans* (Ishida et al. 2006). Also, in 2016, additional *in vitro* study was performed (Zorić et al. 2016a), in which authors have investigated antifungal activity against *C. albicans*. Oleuropein was found to have antifungal activity with MIC value of 12.5 mg/ml. Morphological changes in the nuclei after staining with fluorescent DNA-binding dyes revealed apoptosis as a primary mode of cell death in the analyzed samples treated with sub-MIC concentrations of oleuropein. Results suggest that this antifungal agent targets virulence factors an essential for establishment of the fungal infection. It was noticed that oleuropein modulates morphogenetic conversion and inhibits filamentation of *C. albicans*. The hydrophobicity assay showed that oleuropein in sub-MIC values has significantly decreased, in both aerobic and anaerobic conditions, the CSH of *C. albicans*, a factor associated with adhesion to epithelial cells. It was also demonstrated that the tested compound inhibits the activity of SAPs, cellular enzymes secreted by *C. albicans*, which are reported to be related to the pathogenicity of fungi (Costa et al. 2010). Additionally, it was noted that oleuropein accomplishes its antifungal activity by altering total sterol content and subsequently affecting the membrane of *C. albicans* cells. Based on these findings, authors report that oleuropein *Candida*-cidal activity involves mechanisms at the level of the cell membrane, so this compound could potentially serve in treatment and/or prevention of candidiasis.

3 Antibacterial Activity

Since ancient years, olive products such as oil and different extracts prepared from leaves were used as remedies against many maladies, especially in Mediterranean area. Chemical composition analysis of olive product has confirmed presence of phytochemicals in extracts with antibacterial activity. The abundant group of chemicals present in olive products (such as cake) are biophenolics, and antibacterial activities against Gram-positive bacterial *Staphylococcus aureus*, *Bacillus cereus*, Gram-negative bacteria *Klebsiella pneumoniae*, and *Escherichia coli* were performed with MIC values up to 0.4 mg/ml for oleuropein (Aziz et al. 1998; Korukluoglu et al. 2010). Different authors found higher MIC values of commercially available olive leaf extracts (main active compound oleuropein 12–16 mg/capsule) against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922, 100 and 400 mg/mL, respectively (Lim et al. 2016).

Ethanol-obtained extracts from olive leaves showed antibacterial activity against Group B *Streptococcus* (*Streptococcus agalactiae* from vaginal swabs) isolated

from woman with inhibition zones 28 mm (concentration of olive extract 0.5 mg/ml) and MIC values 0.02 mg/ml in microdilution assay (Mukesi et al. 2019).

Antimicrobial susceptibility of tested bacterial strains largely depends on method of extraction performed. Aqueous extract of olive leaf expresses antibacterial activity using agar well diffusion assay with dose-dependent inhibition zones using from 10 to 50 mg/ml extracts (Aliabadi 2012). In more extensive study Korukluoglu et al. (2010) using ethyl alcohol, acetone, and diethyl ether extracts of olive leaf showed that all extracts have potent antibacterial activity against Gram-positive pathogens (*B. cereus*, *Enterococcus faecalis*, *S. aureus*), with MIC ranging from 50 to 105 µg/ml; and against Gram-negative pathogens (*Salmonella typhimurium*, *S. enteritidis*, *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*), with MICs ranging from 25 to 178 µg/ml.

Authors noted differences in antimicrobial activity using microdilution assay depending on the type of solvents used. Another study showed that 0.6% (w/v) olive leaf extract after 3 hours of exposure in “kill-time” assay expressed bactericidal activity against *E. coli*, *K. pneumoniae* and *S. aureus* with MBC of 0.3%, 0.3%, and 0.6%, respectively (Markin et al. 2003). Contrary to the findings of Aliabadi (2012), the results of Korukluoglu et al. (2010) were negative using aqueous extract of olive leaf. However, due to differences in phytochemical profiles, pH of water for extraction, and sample preparation, these variables could change the outcomes of *in vitro* susceptibility testing (Korukluoglu et al. 2010). Olive leaf products are commercially available, and health claims include cardioprotective, antioxidative, anti-inflammatory, and antimicrobial activities (Romani et al. 2019). Sudjana et al. (2009) conducted extensive *in vitro* survey of antimicrobial activities by broth microdilution assay on 122 microbial strains (both Gram-positive, Gram-negative bacterial pathogens and yeasts as well). The results showed big differences between MIC values and strains tested, and interestingly, the most susceptible were *Campylobacter jejuni* (MIC_{range} 0.3–2.5% v/v), *Helicobacter pylori* (MIC_{range} 0.6–1.2% v/v), and MRSA strains (MIC_{range} 0.8–12.5 V) (Sudjana et al. 2009). Data presented indicate that olive leaf extracts do not possess broad-spectrum activity, but only potent activity was in the case of *C. jejuni*, *H. pylori*, and MRSA. Since the products derived from olive were widely used in diet and as food-supplements, after ingestion there may be a direct or local activity of bioactive compounds from oil or leaf that have antimicrobial activity against *H. pylori*. Olive oil reduces the gastric acid production, and it suppresses the serum gastrin level and higher levels of peptide YY in cholecystectomized patients (Serrano et al. 1997). Olive oil significantly reduces the size of ulcers (Tait 1986), the role of small-molecule compounds presents in olive oil, besides the fatty acid content could be a key role in pharmacological effect (Romero et al. 2007). Furthermore, dialdehydic form of decarboxymethyl ligstroside aglycon is the most potent polyphenol from olive oil, and after diffusion into gastric juice could inhibit the growth of *H. pylori* at concentrations, which are bactericidal (Romero et al. 2007). The pilot clinical study with virgin olive oil on 60 *H. pylori*-positive patients showed that *H. pylori* was eradicated in 27–40% of individuals, and 23% after 1 month of intervention, which is promising result and good base for future studies (Castro et al. 2012). Some of the

phenolics present in olive oil could easily penetrate in acid phase of gastric juice, such as hydroxytyrosol but not in the case of oleuropein, which cannot hydrolyze in lower pH (Romero et al. 2007). Due to its presence in hydrophilic phase and in acid environment of gastric juice, anti- *H. pylori* activity of hydroxytyrosol could be predicted.

The antimicrobial activity of olive oil's compounds, namely, maslinic and oleanic acid, exhibited more potent antibacterial activity against oral pathogens *Streptococcus mutans*, *S. sobrinus*, *S. oralis*, *Prevotella intermedia*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Parvimonas micra* than oleuropein, hydroxytyrosol, olocanthal, and oleacin (Karygianni et al. 2019).

Furthermore, olive mill waste waters rich in biophenols showed antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa* using disk diffusion assay at 5 mg/ml (Obied et al. 2007), which imply the use of waste waters as by-products as source of possible bioactive compounds.

The extracts of olive leaves present in the market as nutraceuticals can decrease the count of food-borne bacteria, such as *E. coli* O157:H7, *Salmonella enterica*, *Listeria monocytogenes*, and *S. aureus* with different range of quantitative bactericidal 50% value (BC₅₀) (Friedman et al. 2013). The authors presented data of BC₅₀ value, suggested a broad spectrum of antibacterial activity of olive juice powder (12% olive polyphenolics of which 4-hydroxytyrosol is approximately 6% total) against *E. coli* O157:H7 (0.829 ± 0.019%), *Salmonella enterica* (0.318 ± 0.006%), *Listeria monocytogenes* (0.284 ± 0.031%), and *S. aureus* (0.252 ± 0.014%). The olive pomace also possesses antibacterial activity, expressed as BC₅₀ as follows against *E. coli* O157:H7 (0.178 ± 0.006%), *S. enterica* (0.070 ± 0.001%), *L. monocytogenes* (0.039 ± 0.001%), and *S. aureus* (0.008 ± 0.001%) (Friedman et al. 2013).

In 2020, Menchetti et al. (2020) published study about the influence of phenolic extract from olive vegetation water (PEOVW) on the survival of *Salmonella enteritidis* on mayonnaise. Phenolic extract from olive vegetation water has antibacterial effect on mayonnaise. The most abundant phenolic compound identified in PEOVW was the dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol. *S. enteritidis* is reduced by 9.5%/h in mayonnaise added with polyphenols at 4 °C, while lower elimination rate of *S. enteritidis* was found at room temperature.

Additional study by Shiry et al. (Shiry et al. 2020) evaluated changes in cutaneous mucosal immunity in the intestine of rainbow trout (*Oncorhynchus mykiss*) orally administrated florfenicol (FFC) and/or olive leaf extract (obtained by methanol), experimentally infected with *Streptococcus iniae*. The most obvious active component of olive leaf extract, found by HPLC analysis, is oleuropein (0.496 mg/l). The juvenile fish (55 ± 7.6 g) were divided into different groups according to the use of added olive leaf extract (80 g/kg food), the presence/absence of FFC (15 mg/kg body weight for 10 consecutive days), and the streptococcal infectivity (2.87 × 10⁷ CFU/ml as 30% of LD₅₀-96 h). Authors report that the combined use of olive leaf extract and FFC could lower some skin mucous immunological indices (e.g., TP, TIg, and ALP) and the gene expression of inflammatory cytokines (e.g.,

Table 5.1 Spectrum of antibacterial activity of oleuropein and hydroxytyrosol

Microbes	Oleuropein	Hydroxytyrosol	References
	MIC (µg/ml)		
<i>S. aureus</i>	62.5–3200	7.85–400	Bisignano et al. (1999) Furneri et al. (2002) Tuck and Hayball (2002) Tafesh et al. (2011) Medina-Martínez et al. (2015) Lim et al. (2016) Karygianni et al. (2019)
<i>Streptococcus mutans</i>	625	312	Tafesh et al. (2011)
<i>S. sobrinus</i>	625	625	Karygianni et al. (2019)
<i>S. oralis</i>	1250	1250	
<i>S. pyogenes</i>		400	
<i>Enterococcus faecalis</i>	1250	1250	Karygianni et al. (2019)
<i>Pseudomonas aeruginosa</i>	ND	1000–>1000	Furneri et al. (2002) Medina-Martínez et al. (2015)
<i>E. coli</i>	ND	400	Bisignano et al. (1999) Medina-Martínez et al. (2015)
<i>Klebsiella pneumoniae</i>	ND	400–1000	Bisignano et al. (1999) Tafesh et al. (2011) Medina-Martínez et al. (2015)
<i>Haemophilus influenzae</i>	500	0.97	Bisignano et al. (1999) Tuck and Hayball (2002)
<i>Moraxella catarrhalis</i>	>500	1.92	Bisignano et al. (1999) Tuck and Hayball (2002)
<i>Salmonella typhi</i>	125	3.94	Tassou and Nychas (1995)
<i>S. typhimurium</i>	ND	>1000	Bisignano et al. (1999) Tuck and Hayball (2002) Medina-Martínez et al. (2015)
<i>Vibrio</i> spp.	62.5–125	0.97–7.8	Bisignano et al. (1999) Tuck and Hayball (2002)
<i>Mycoplasma hominis</i>	20	0.03–0.12	Furneri et al. (2002)
<i>M. fermentans</i>	20	0.25	Furneri et al. (2004)
<i>M. pneumoniae</i>	160	0.5	Furneri and Bisignano (2010)

Legend: ND not determined

TNF- α and IL- 1β) of rainbow trout. Furthermore, lysozyme and protease activities were invigorated by the FFC and olive leaf extract treatment. Use of olive leaf extract induced the gene expression of hepcidin-like antimicrobial peptides.

As stated in the introduction section, olive oil, leaves, and other products are source of bioactive polar components in EVOO or leaf extracts, and oleuropein (as secoiridoids) and hydroxytyrosol (as phenolic) were scientifically explored in more details than other compounds belonging to non-fatty fraction of the olive oil. As it can be seen in Table 5.1, both oleuropein and hydroxytyrosol exhibited wide spectrum of antimicrobial activity against food-borne, respiratory, and nosocomial bacterial pathogens. The oleuropein and 4-hydroxytyrosol possess more potent bactericidal activity than olive pomace or olive juice powder, suggesting that some

Table 5.2 Effects of oleuropein and hydroxytyrosol on *Staphylococcus aureus* cells

Target sites	Results of interaction	References
Inhibition of enterotoxin B production	0.6% of oleuropein inhibited growth of <i>S. aureus</i> and toxin production <i>in vitro</i> Low pH and low inoculum enhance anti- <i>S. aureus</i> activity	Tassou and Nychas (1994)
Cell membrane integrity	Disruption caused leakage of glutamate, potassium, and inorganic phosphate (on <i>E. coli</i> as a model) Oleuropein interacts with phosphatidylglycerol (PG) as a model membrane for <i>S. aureus</i> Oleuropein possibly promotes pores and disruption in membranes in interaction with dimyristoylphosphatidylglycerol (DMPG) Leakage of intracellular proteins with irreversible damage of <i>S. aureus</i> cells with oleuropein at conc. 20 mg/ml	Juven et al. (1972) Tranter et al. (1993) Caturla et al. (2005) Cinar (2009)
Interaction with antibiotics	Oleuropein with ampicillin decreases MIC values against <i>S. aureus</i> Additive effects of oleuropein and hydroxytyrosol according to FIC index Strong synergistic activity in combination ampicillin with hydroxytyrosol	Lim et al. (2016)
Interaction with biofilm formation	Hydroxytyrosol-polymer (polyacrylate) nanoparticles significantly reduce adhesion of <i>S. epidermidis</i> Reduction of ROS production in biofilm	Crisante et al. (2015)
Interaction with H ₂ O ₂	Hydrogen peroxide triggers antibacterial activity of oleuropein against <i>Staphylococcus aureus</i> , <i>S. epidermidis</i> , and <i>S. saprophyticus</i>	Zanichelli et al. (2005)

antagonistic effect could be seen in extracts (Friedman et al. 2013). For example, in case of *S. aureus*, 4-hydroxytyrosol has more potent bactericidal activity with BC₅₀ 0.057 ± 0.004% than oleuropein BC₅₀ 0.141 ± 0.013%, respectively (Friedman et al. 2013). For the same pathogen, BC₅₀ value of olive juice powder is 0.252 ± 0.014% (Friedman et al. 2013).

The activity is not exclusively connected within Gram-positive or Gram-negative bacterial species. Important nosocomial pathogen *S. aureus* is more susceptible to hydroxytyrosol than oleuropein (Table 5.1), which encourage to elucidate mechanism of action on different target sites inside bacterial cells, which include cell wall and membrane structure, toxin production, and biofilm formation. Early works on mechanisms reveal that oleuropein has surface-active properties and could disrupt the structure of bacterial cell membranes (Juven et al. 1972). Furthermore, the effect of interaction of oleuropein with membrane structures in bacteria has been provided by leakage of cytoplasmic molecules potassium and inorganic phosphate together with decreased level of ATP at a concentration 2 mg/ml (Juven et al. 1972). Leakage of potassium outside the bacterial cell was induced with damage of membrane physical structure, and the leaked of potassium is good marker of damage of lipophilic structures in lipid bilayer of membranes. More precise research in integrity of bacterial cells found that oleuropein has affinity to membrane-based phosphatidylglycerol and promotes pores, which lead to leakage of intracellular

molecules and consequently lead to cell death (Caturla et al. 2005; Cinar 2009). Based on early works on mechanisms of bactericidal activity, new research data revealed more complex activity of oleuropein and hydroxytyrosol. As shown in Table 5.2, there are several targets in planktonic and biofilm cells of *S. aureus*. Both compounds from olive leaf, olive oil and extracts, interact with enterotoxin production, and could potentiate the antibacterial activity of antibiotics.

These data, demonstrated *in vitro*, could lead to more extensive research into translation of data from *in vitro* to the *in vivo* conditions. Data of antibacterial activity of hydroxytyrosol and oleuropein are in favor of local treatment of infections with products rich in hydroxytyrosol and oleuropein.

4 Conclusions

In conclusion, findings of so far conducted studies have shown that both oleuropein and hydroxytyrosol have promising *in vitro* antifungal activity including activity against opportunistic fungal pathogen *C. albicans*. These antifungal agents target virulence factors of *C. albicans*, which are essential for the establishment of opportunistic infection. However, additional studies are necessary to further investigate the mechanism of action of oleuropein and hydroxytyrosol and the possible development of new antifungal therapeutics. Both oleuropein and hydroxytyrosol possess a wide range of antibacterial activity, as well. Based on bacterial models (such as *S. aureus*), targets of bactericidal activity of oleuropein and hydroxytyrosol include several sites. Both compounds interact also with bacterial biofilm formation and enterotoxin production. To potentiate activity of some antibiotics is also positive outcome of interaction of oleuropein and hydroxytyrosol with bacterial cells. As very interesting biomolecules, the research on antimicrobial activities of both oleuropein and hydroxytyrosol could lead to translation of data to *in vivo* conditions.

References

- Aliabadi MA (2012) Antimicrobial activity of olive leaf aqueous extract. *Ann Biol Res* 3:4189–4191
- Aziz NH, Farag SE, Mousa LA, Abo-Zaid MA (1998) Comparative antibacterial and antifungal effects of some phenolic compounds. *Microbios* 93:43–54
- Bisignano G, Tomaino A, Lo Cascio R, Crisafi G, Uccella N, Saija A (1999) On the *in-vitro* antimicrobial activity of oleuropein and hydroxytyrosol. *J Pharm Pharmacol* 51:971–974
- Boskou D (1996) Olive oil: chemistry and technology. AOCS Press, Champaign
- Brown D (2015) Antibiotic resistance breakers: can repurposed drugs fill the antibiotic discovery void? *Nat Rev Drug Discov* 14:821–832
- Castro M, Romero C, de Castro A, Vargas J, Medina E, Millán R, Brenes M (2012) Assessment of *Helicobacter pylori* eradication by virgin olive oil. *Helicobacter* 17:305–311

- Caturla N, Perez Fons L, Estepa A, Micol V (2005) Differential effects of oleuropein, a biophenol from *Olea europea*, on anionic and zwitterionic phospholipid model membranes. *Chem Phys Lipids* 137:2–17
- Cinar D (2009) Purification and antimicrobial properties of Oleuropein. Thames Valley University, PhD Thesis. Accessed 19 Nov 2020. <http://repository.uwl.ac.uk/id/eprint/381>
- Costa CR, Jesuino RSA, Lemos J dA, Fernandes O d FL, Hasimoto e Souza LK, Passos XS, Silva M d RR (2010) Effects of antifungal agents in sap activity of *Candida albicans* isolates. *Mycopathologia* 169:91–98
- Crisante F, Tafesco V, Donelli FD, Vuotto C, Martinelli A, D’Ilario L, Pietrelli L, Francolini I, Piozzi A (2015) Antioxidant hydroxytyrosol-based polyacrylate with antimicrobial and antiadhesive activity versus *Staphylococcus epidermidis*. *Adv Exp Med Biol* 901:25–36
- Dhamgaye S, Devaux F, Vandeputte P, Khandelwal NK, Sanglard D, Mukhopadhyay G, Prasad R (2014) Molecular mechanisms of action of herbal antifungal alkaloid berberine, in *Candida albicans*. *PLoS One* 9(8):e104554
- Faria NCG, Kim JH, Gonçalves LAP, Martins ML, Chan KL, Campbell BC (2011) Enhanced activity of antifungal drugs using natural phenolics against yeast strains of *Candida* and *Cryptococcus*. *Lett Appl Microbiol* 52:506–513
- Friedman M, Henika PR, Levin CE (2013) Bactericidal activities of health-promoting, food-derived powders against the foodborne pathogens *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica*, and *Staphylococcus aureus*. *Food Microbiol Saf* 78:270–275
- Furneri PM, Bisignano G (2010) Usage of hydroxytyrosol for antimycoplasmal activity. *Olive and olive oil in health and disease prevention* 1283–1288
- Furneri PM, Marino A, Saija A, Uccella N, Bisignio G (2002) *In vitro* antimycoplasmal activity of oleuropein. *Int J Antimicrob Agents* 20:293–296
- Furneri PM, Piperno A, Saija A, Bisignano G (2004) Antimycoplasmal activity of hydroxytyrosol. *Antimicrob Agents Chemother* 48(12):4892–4894
- Halawi MH, Rahman SMA, Yusef H (2015) Comparative study of the antifungal activity of *Olea europaea* L. against some pathogenic *Candida albicans* isolates in Lebanon. *Int J Curr Microbiol App Sci* 4:970–984
- Hassen I, Casabianca H, Hosni K (2015) Biological activities of the natural antioxidant oleuropein: exceeding the expectation—a mini-review. *J Funct Foods* 18:926–940
- Huang CL, Sumpio BE (2008) Olive oil, the Mediterranean diet, and cardiovascular health. *J Am Coll Surg* 207:407–416
- Ishida K, de Mello JC, Cortez DA, Filho BP, Ueda-Nakamura T, Nakamura CV (2006) Influence of tannins from *Stryphnodendron adstringens* on growth and virulence factors of *Candida albicans*. *J Antimicrob Chemother* 58:942–949
- Jacob M, Walker LA (2005) Natural products and antifungal drug discovery. *Methods Mol Med* 118:83–109
- Juven B, Henis Y, Jacoby B (1972) Studies of the antimicrobial action of oleuropein. *J Appl Bacteriol* 35:559–567
- Karygianni L, Cecere M, Skaltsounis AL, Argyropoulou A, Hellwig E, Aligiannis N, Wittmer A, Al-Ahmad A (2014) High-level antimicrobial efficacy of representative Mediterranean natural plant extracts against oral microorganisms. *Biomed Res Int* 2014:839019
- Karygianni L, Cecere M, Argyropoulou A, Hellwig E, Skaltsounis AL, Wittmer A, Tchorz JP, Al-Ahmad A (2019) Compounds from *Olea europaea* and *Pistacia lentiscus* inhibit oral microbial growth. *BMC Complement Altern Med* 16:51
- Khan MF, Murphy CD (2021) 3-Hydroxytyrosol regulates biofilm growth in *Cunninghamella elegans*. *Fungal Biol* 125(3):211–217
- Khan Y, Panchal S, Vyas N, Butani A, Kumar V (2007) *Olea europaea*: a phyto-pharmacological review. *Pharmacogn Rev* 1:114–118
- Korukluoglu M, Sahan Y, Yigit A, Ozer ET, Gücer S (2010) Antibacterial activity and chemical constitutions of *Olea europaea* L. leaf extracts. *J Food Process Preserv* 34:383–396

- Kosalec I, Ramić S, Jelić D, Antolović R, Pepeljnjak S, Kopjar N (2011) Assessment of tryptophol genotoxicity in four cell lines *in vitro*: a pilot study with alkaline comet assay. *Arh Hig Rada Toksikol* 62:41–49
- Koutsoumanis K, Tassou CC, Taoukis PS, Nychas GJE (1998) Modelling the effectiveness of a natural antimicrobial on *Salmonella enteritidis* as a function of concentration, temperature, and pH, using conductance measurements. *J Appl Microbiol* 84:981–987
- Kubo I, Matsumoto A, Takase I (1985) A multichemical defence mechanism of bitter olive *Olea europaea* (Oleaceae): is oleuropein a phytoalexin precursor? *J Chem Ecol* 11:251–263
- Lim A, Subhan N, Jazayeri JA, John G, Vanniasinkam T, Obied HK (2016) Plant phenols as antibiotic boosters: *in vitro* interaction of olive leaf phenols with ampicillin. *Phytother Res* 30:503–509
- Markin D, Duek L, Berdicevsky I (2003) *In vitro* antimicrobial activity of olive leaves. *Mycoses* 46:132–136
- Medina E, de Castro A, Romero C, Brenes M (2006) Comparison of the concentrations of phenolic compounds in olive oils and other plant oils: correlation with antimicrobial activity. *J Agric Food Chem* 54:4954–4961
- Medina E, Brenes M, Romero C, Garcia A, de Castro A (2007) Main antimicrobial compounds in table olives. *J Agric Food Chem* 55:4954–4961
- Medina-Martínez MS, Truchado P, Castro-Ibáñez I, Allende A (2015) Antimicrobial activity of hydroxytyrosol: a current controversy. *Biosci Biotechnol Biochem* 80:810–810
- Menchetti L, Tattichi A, Esposito S, Servili M, Ranucci D, Branciarri R, Miraglia D (2020) The influence of phenolic extract from olive vegetation water and storage temperature on the survival of *Salmonella Enteritidis* inoculated on mayonnaise. *Food Sci Technol* 129:109648
- Mukesi M, Iweriebor BC, Obi LC, Nwodo UU, Moyo SR, Okoh AI (2019) The activity of commercial antimicrobials, and essential oils and ethanolic extracts of *Olea europaea* on *Streptococcus agalactiae* isolated from pregnant women. *BMC Complement Altern Med* 19:34
- Obied HK, Bedgood DR, Prenzler PD, Robards K (2007) Bioscreening of Australian olive mill waste extracts: biophenol content, antioxidant, antimicrobial and molluscicidal activities. *Food Chem Toxicol* 45:1238–1248
- Odds FC, Brown AJ, Gow NA (2003) Antifungal agents: mechanisms of action. *Trends Microbiol* 11:272–279
- Ortega-García F, Peragon J (2010) Phenol metabolism in the leaves of the olive tree (*Olea europaea* L.) cv. Picual, Verdial, Arbequina, and Frantoio during ripening. *J Agric Food Chem* 58:12440–12448
- Pereira JA, Pereira APG, Ferreira ICFR, Valentão P, Andrade PB, Seabra R, Estevinho L, Bento A (2006) Table olives from Portugal: phenolic compounds, antioxidant potential, and antimicrobial activity. *J Agric Food Chem* 54 (22):8425–8431
- Pereira AP, Ferreira IC, Marcelino F, Valentão P, Andrade PB, Seabra R, Estevinho L, Bento A, Pereira JA (2007) Phenolic compounds and antimicrobial activity of olive (*Olea europaea* L. Cv. Cobrançosa) leaves. *Molecules* 12:1153–1162
- Rahioui B, Zine El Aabidine A, Baissac Y, El Boustani E, Khadari B, Jay-Allemand C, El Modafar C (2009) Phenolic compounds of olive tree leaves and their relationship with the resistance to the leaf-spot disease caused by *Spilocaea oleaginea*. *Am Euroasian J Agric Environ Sci* 5:204–214
- Romani A, Ieri F, Urciuoli S, Noce A, Marrone G, Nedlani C, Bernini R (2019) Health effects of phenolic compounds found in extra-virgin olive oil, by-products, and leaf of *Olea europaea* L. *Nutrients* 11:1776
- Romero C, Medina E, Vargas J, Brenes M, DeCastro A (2007) *In vitro* activity of olive oil polyphenols against *Helicobacter pylori*. *J Agric Food Chem* 55:680–686
- Serrano P, Yago MD, Mañas M, Calpena R, Mataix J, Martínez-Victoria E (1997) Influence of type of dietary fat (olive and sunflower oil) upon gastric acid secretion and release of gastrin, somatostatin, and peptide YY in man. *Dig Dis Sci* 42:626–633

- Servili M, Selvaggini R, Esposto S, Taticchi A, Montedoro G, Morozzi G (2004) Health and sensory properties of virgin olive oil hydrophilic phenols: agronomic and technological aspect of production that affect their occurrence in the oil. *J Chromatogr* 1504:113–127
- Shareck J, Belhumeur P (2011) Modulation of morphogenesis in *Candida albicans* by various small molecules. *Eukaryot Cell* 10:1004–1012
- Shiry N, Khoshnoodifar K, Alavinia SJ (2020) Cutaneous mucosal immune-parameters and intestinal immune-relevant genes expression in streptococcal-infected rainbow trout (*Oncorhynchus mykiss*): a comparative study with the administration of florfenicol and olive leaf extract. *Fish Shellfish Immunol* 107:403–410
- Suarez M, Romero MP, Motilva MJ (2010) Development of a phenol-enriched olive oil with phenolic compounds from olive cake. *J Agric Food Chem* 58:10396–10403
- Sudjana AN, D’Orazio C, Ryan V, Rasool N, Ng J, Islam N, Riley TV, Hammer KA (2009) Antimicrobial activity of commercial *Olea europaea* (olive) leaf extract. *Int J Antimicrob Agents* 33:461–463
- Tafesh A, Najami N, Jadoun J, Halahlil F, Riepl H, Azaizeh (2011) Synergistic antibacterial effects of polyphenolic compounds from olive mill wastewater. *Evid Based Complement Alternat Med*:ID431021
- Taits NS (1986) Use of olive oil in the treatment of ulcer patients. *Urach Delo* 7:67–70
- Tassou CC, Nychas GJE (1995) Inhibition of Salmonella enteritidis by oleuropein in broth and in a model Food system. *Lett Appl Microbiol* 20:120–124
- Tassou CC, Nychas GJE (1994) Inhibition of *Staphylococcus aureus* by olive phenolics in broth and in a model food system. *J Food Prot* 57:120–124
- Thielmann J, Kohnen S, Hauser C (2017) Antimicrobial activity of *Olea europaea* Linné extracts and their applicability as natural food preservative agents. *Int J Food Microbiol* 251:48–66
- Tranter HS, Tassou CC, Nychas GJ (1993) The effect of the olive phenolic compound, oleuropein, on growth and enterotoxin B production by *Staphylococcus aureus*. *J Appl Bacteriol* 4:253–259
- Tuck KL, Hayball PJ (2002) Major phenolic compounds in olive oil: metabolism and health effects. *J Nutr Biochem* 13:636–644
- Zanichelli D, Baker TA, Clifford MN, Adams MR (2005) Inhibition of *Staphylococcus aureus* by Oleuropein is mediated by hydrogen peroxide. *J Food Prot* 68:1492–1496
- Zorić N, Horvat I, Kopjar N, Vučemilović A, Kremer D, Tomić S, Kosalec I (2013) Hydroxytyrosol expresses antifungal activity *in vitro*. *Curr Drug Targets* 14:992–998
- Zorić N, Kopjar N, Bobnjarić I, Horvat I, Tomić S, Kosalec I (2016a) Antifungal activity of oleuropein against *Candida albicans* – the *in vitro* study. *Molecules* 21:1631
- Zorić N, Kopjar N, Oršolić N, Tomić S, Kosalec I (2016b) Olive leaf extract activity against *Candida albicans* and *C. dubliniensis* – the *in vitro* viability study. *Acta Pharma* 66:411–421
- Zorić N, Kopjar N, Vuković Rodriguez J, Tomić S, Kosalec I (2021) Protective effects of olive oil phenolics oleuropein and hydroxytyrosol against hydrogen peroxide-induced DNA damage in human peripheral lymphocytes. *Acta Pharma* 71:131–141
- Zuzarte M, Gonçalves MJ, Cavaleiro C, Canhoto J, Vale-Silva L, Silva MJ, Pinto E, Salgueiro L (2011) Chemical composition and antifungal activity of the essential oils of *Lavandula viridis* L’Hér. *J Med Microbiol* 60:612–618

Chapter 6

Antimicrobial Properties of Selected Native Greek Aromatic Plants: An Ethnopharmacological Overview



Christos G. Ganos, Olga Gortzi, Efrossini B. Chinou, Gioacchino Calapai,
and Ioanna B. Chinou

Abstract Aromatic plants and their essential oils have been used therapeutically for centuries. Many published scientific studies have described their remarkable healing properties. The antimicrobial activity of plant species, such as *Origanum dictamnus*, commonly known as “cretan dittany”, *Sideritis* sp. (Lamiaceae), *Cistus creticus* (Cistaceae), and *Pistacia lentiscus* (Anacardiaceae), has been reported by several researchers. These plants are used since antiquity and in this chapter, the authors attempt to present comprehensive information of their ethnopharmacological uses and chemical composition, together with data extracted from published antimicrobial studies (*in vitro*, *in vivo*, and clinical ones). Novel information and reports of medicinal uses not previously described in relevant ethnobotanical and pharmacological literature are highlighted. It is also noteworthy, that except *Cistus*, all selected species have been approved as traditional herbal medicines, based on their longstanding medicinal use in European Union, by the European Medicines Agency, and Herbal Monographs on them have been developed recently by Herbal Medicinal Products Committee (HMPC).

Keywords Lamiaceae · *Origanum dictamnus*; cretan dittany · *Sideritis* species · Cistaceae *Cistus creticus* · Anacardiaceae *Pistacia lentiscus* · Mastic

C. G. Ganos · I. B. Chinou (✉)

Lab of Pharmacognosy and Chemistry of Natural Products, Department of Pharmacy,
National and Kapodistrian University of Athens, Athens, Greece
e-mail: ichinou@pharm.uoa.gr

O. Gortzi

Food Chemistry, School of Agricultural Sciences, Department of Agriculture Crop Production
and Rural Environment, University of Thessaly, Volos, Greece

E. B. Chinou

Lab of Clinical Microbiology, “St Savvas” Anticancer Hospital of Athens, Athens, Greece

G. Calapai

Department of Biomedical and Dental Sciences and Morphological and Functional Imaging,
University of Messina, Messina, Italy

Abbreviations

CAPeo	Cretan Aromatic Plant Essential Oil
CIM	Complementary and Integrative Medicine
CMG	Chios Mastic Gum
EDQM	European Directorate of Quality of Medicines
EMA	European Medicines Agency
EO	Essential Oil
EU	European Union
HMPC	Herbal Medicinal Products Committee
MBC	Minimum Bactericidal Concentration
MIC	Minimal Inhibitory Concentration
PDO	Product of Protected Designation of Origin
THMP	Traditional Herbal Medicinal Product

1 Introduction

Ethnopharmacology, as a specifically designated field of research, has a relatively short history. The term was first described in 1967 as the title of a book on hallucinogens. It considers the pharmacological activity of plants, fungi, and other organisms used in traditional medicine used locally or traditionally as a medicine or to improve health. It applies a unique approach in pharmacology: it also considers the traditional, and therefore anthropological, context of the drug's origin. Ethnopharmacology represents a multidimensional approach, shaped by tradition and science that improves knowledge of plant uses and local meaning of health and disease (Heinrich 2000; World Health Organization [WHO] 2002).

The Balkan Peninsula and regions around the South East Mediterranean Sea are characterized by high biodiversity endemism and long tradition in folk medicine. Parts of the areas belong to one of the thirty-six world's biodiversity hotspots due to geology, climate, and geographical location (high mountains and water near the coast). Moreover, the inhabitation and organization of societies, the development of trade among autochthonous populations along with the constant observation of nature and human health, have led to the established use of various natural products as therapeutics. Especially Greece is noted for its high plant species diversity (5800 species and 1893 subspecies) and endemism (22.2% of all species present with 1278 species and 452 subspecies) (Strid 1986, 2016; Strid and Tan 1991, 1997, 2002; Kougioumoutzis et al. 2021).

The ethnopharmacological knowledge found in the historical texts of these regions (from the fifth century BC to the nineteenth century AD) can be considered as the basis of Western pharmacopoeias. Traces for this development can be found

in ancient Greece in the Corpus of Hippocrates “*The Father of Medicine*,” in Roman empire in the book of “*De Materia Medica*” of Dioscorides, in Byzantine manuscripts, and in several manuscripts of the early modern periods found in monasteries and libraries. Besides historical texts, the significant ethnopharmacological knowledge and the experiences of people traditionally using folk medicinal practices, especially in remote places of these regions, are transmitted through generations orally. However, oral traditions on which much of this medical knowledge rests are imperiled, may eventually fade away and hence need to be recorded, assessed, and preserved (Hanlidou et al. 2004).

Aromatic plants and their essential oils have a long-standing tradition of medicinal use dating back to ancient times. Thus, essential oils are commonly applied to the skin; inhaled; gargled and ingested as well as being used as bath additives. Essential oils have also been proved to exert antimicrobial activity against a large number of bacteria and fungi. Pharmacology, medical and clinical microbiology, dermatology, veterinary medicine, phytopathology, food industry, food preservation, and cosmetology are some fields in which essential oils can be applied effectively (Koutelidakis et al. 2016).

In light of the recent growth in antibiotic resistance, the usage of plant-derived antimicrobial agents could serve as an effective alternative treatment against infections from human-, zoo-, and phyto-pathogenic microorganisms and/or as safe natural food preservatives, effective in the storage process. The antimicrobial activity of certain Greek endemic aromatic plants has played a very important role ethnopharmacologically, in the Mediterranean basin as well as in Greece since antiquity (Hanlidou et al. 2004; Baser and Buchbauer 2010; Tisserand and Young 2014; Koutelidakis et al. 2016).

This chapter aims to perform a comprehensive assessment of the antimicrobial potential of selected Greek native aromatic plants (the aerial parts of *Origanum dictamnus* L., the four most widely cultivated and used *Sideritis* species, *Cistus creticus* as well as *Pistacia lentiscus*, resin so called mastic). This chapter is based on information collected from scientific journals, books, and electronic search. These sources include Scopus, Pubmed, Web of Science, and Google scholar as well as local books on ethnopharmacology, and botany of these plants. The reported data on phytochemical studies, biological activity, and traditional uses have been reviewed. Issues discussed comprise the following:

1. Ethnopharmacological uses of the plants through an extensive review of relevant literature.
2. Systematic analysis of their phytochemical constituents.
3. An evaluation of these species’ antimicrobial effects as a basis for supporting potential therapeutic applications.

2 *Origanum dictamnus* EMA/HMPC/200429/2012 Corr

2.1 *History and Ethnobotany*

Origanum dictamnus (Lamiaceae) known as Dittany of Crete consists of the dried flowering aerial parts of the herb (Liolios et al. 2010). The plant is native and endemic to Crete, widely distributed along the Cretan island. Dittany is a short green-white lanate shrub with a pink corolla. It grows wild inside fissures of calcareous cliffs – up to 1900 m above sea level. The name of the genus “*Origanum*,” according to researchers, has its roots in the Greek words “oros” (mountain) + “ganos” (which means “becoming bright”). This name probably originates and is closely associated with the high mountains, which form the natural growing environment of *Origanum* plants in the Mediterranean (Liolios et al. 2010). The species name *dictamnus* probably derived from “*Dicti*” + “*thamnos*” [*Dicti*” is the name of Cretan mountain where Zeus (Jupiter) was raised up by the goat Amalthea, dedicated to him, and “*thamnos*” means shrub in Greek] (Liolios et al. 2010).

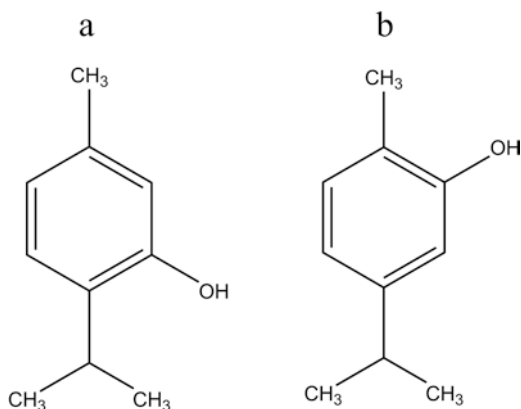
Seeds of the plant have been excavated in the Minoic palaces Knossos, and Zacros. Since antiquity, dittany was considered as a drug against many illnesses such as gastric ulcers, to facilitate childbirth and against stomach and gynecological disorders. Hippocrates, father of Medicine, has used it on Cos island, against gastric complaints, tuberculosis, and in poultices for wounds. The most famous reference to the medicinal potential of dittany comes from Aristotle, who claimed that when wild goats living on the Cretan Mountains were struck by poisoned arrows, they ate aerial parts of *O. dictamnus*. This had the effect of causing the arrows to leave their bodies and heal their wounds (Historia Animalium 9.16.1). Theophrastus (Liolios et al. 2010) has repeated the above statement of his mentor. The Latin writers, Cicero, Virgil, followed by Pliny and Celsus and later Dioscorides in “De Materia Medica,” have also attributed multiple therapeutic effects to *O. dictamnus* (Liolios et al. 2010). During the middle ages, the plant was recorded in the Codex of Charlemagne in about 795 A.D. “...*dictamnnum, sinape, satureiam, sisimbrium, mentam, mentastrum...*” (chapter LXX.). Around this time, the plant started being used in monasteries, as a constituent of the renowned liqueurs Benedictine and Trappistine by Benedictines and Trappistine monks, respectively. Even in our day, dittany is being used in distilleries. Vermouth, for example, is flavored with this highly aromatic species with significant commercial interest for herbal teas (Liolios et al. 2010). Since the last century, dittany has been cultivated widely in Crete reaching an annual production of 50 tons/year: 85% of the product is exported (mainly to EU and Japan), while 15% of the total production is absorbed by the Greek market (Liolios et al. 2010).

2.2 Chemical Constituents

The lipid composition and a variety of nonpolar components (fatty acids, lipids, sterols and essential oil) have been fully identified (EMA 2012). Major components of *O. dictamnus* essential oil are monoterpenes: carvacrol (major constituent) and its isomer thymol, γ -terpinene, and p-cymene (Liolios et al. 2009, 2010). Although the composition of EOs can differ between harvesting seasons and between geographical sources, the sum of the amounts of these four compounds present has been found to be comparable in almost all the specimens.

Polyphenolic components, flavonoids, and coumarins have also been isolated and identified from methanol and water extracts of the aerial parts of the plant (Liolios et al. 2010; EMA 2012; Varsani et al. 2017) such as: coumaric acid, ferulic acid, hydrated catechin, or catechin (Proestos et al. 2006). Depsides have been isolated from polar extracts of the aerial parts of *O. dictamnus* such as salvianolic acid P, rosmarinic acid and rosmarinic acid methyl ester, along with monoterpenes (thymoquinone and thymoquinol 2-*O*- β -glucopyranoside), phenolic acids (oresbuisin A and caffeic acid), flavonoids (apigenin, kaempferol, quercetin, eriodictyol, taxifolin, narigenin), and alicyclic derivatives (12-hydroxy jasmonic acid and its 12-*O*- β -D-glucoside) (Chatzopoulou et al. 2010). Triterpenes, such as oleanolic acid, the rare 21 α -OH oleanolic acid, ursolic acid, 21 α -OH ursolic acid, and ouvaol, have also been structurally determined (Fig. 6.1).

Fig. 6.1 (a) Thymol and (b) carvacrol



2.3 Antimicrobial Activity

In Vitro

The water extract of *O. dictamnus* has been tested against the yeast *Yarrowia lipolytica*. Dittany extracts (concentration of 5 g/L – presented the greater lag time and the greater inhibition activity against *Y. lipolytica* of all tested extracts (Karanika et al. 2001). The traditionally known use of the plant against gastric ulcers was supported *in vitro* as an aqueous 70% methanol extract was tested against one reference strain of *Helicobacter pylori*. Additionally, 15 clinical isolates of *H. pylori* (from clinical biopsies) and *O. dictamnus* proved very active against *Helicobacter* strains (MIC approximately 2.50 mg/ml) (Stamatis et al. 2003). The methanol extract and isolated polar compounds from *O. dictamnus* (salvianolic acid, rosmarinic acid methyl ester, thymoquinone, thymoquinol 2-O- β -glucopyranoside; oresbiusin A, caffeic acid; eriodictyol, taxifolin, naringenin, and 12-hydroxyjasmonic acid) have showed significant MIC values of 0.012–0.22 mmol/ml, showing activity against Gram-negative clinical strains *Acinetobacter haemolyticus*, *Empedobacter brevis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* (Chatzopoulou et al. 2010). The EOs from the leaves and bracts of dittany of Crete have exerted strong antimicrobial effect versus *Staphylococcus aureus*, *S. epidermidis*, and *S. hominis*, (Economakis et al. 2002).

Moreover, the essential oil of dittany exerted antimicrobial action against the phytopathogenic bacteria for potato tubers *Erwinia carotovora*, also due to carvacrol (Vokou et al. 1993).

A dose-dependent inhibition against the mycelial growth of *P. digitatum* and conidial germination was observed by *O. dictamnus*, EO. Mycelial growth was totally inhibited at 300 μ g/mL, while complete inhibition of germination was observed at the concentration of 250 μ g/mL. In another study (Liolios et al. 2009), the antimicrobial activities of the oils from the plant as well as the pure substances carvacrol, thymol, and their mixtures carvacrol/thymol (6:1) carvacrol/c-terpinene, have been determined before and after liposomal encapsulation. The tests were conducted against the following: four Gram-positive bacteria – *S. aureus*, *S. epidermidis*, *S. mutans*, and *S. viridans*; four Gram-negative bacteria – *P. aeruginosa*, *E. coli*, *E. cloacae*, and *K. pneumoniae*; three human pathogenic fungi – *Candida albicans*, *C. tropicalis*, and *C. glabrata*; as well as against the food-pathogen *Listeria monocytogenes*. The EOs showed comparable activities against all tested microbial strains. Pure compounds (carvacrol, thymol) are proved to be more active than oil, and their antimicrobial activities significantly increased after their encapsulation in liposomes.

Carvacrol and thymol, the isomeric phenol of carvacrol, seem to have strong antimicrobial activities, whereas their biosynthetic precursors, γ -terpinene and p-cymene, are inactive. Carvacrol in particular seems to possess strong antibacterial activity against *Bacillus cereus*, a spore-forming food-borne pathogenic bacteria, and it has also shown antifungal activity versus *Rhizopus* (9 species), *Mucor*

(4 species), and *Aspergillus* (7 species). A dose-dependent inhibition of the mycelial growth of *Penicillium digitatum* was observed for carvacrol and thymol with MIC of 125 µg/mL and 160 µg/mL, respectively (Liolios et al. 2010).

Furthermore, dittany decoctions have also been revealed to exert promising antibacterial effects against Gram-positive and Gram-negative human pathogens, as well as against a panel of isolated clinical *Malassezia* strains (Varsani et al. 2017; Paloukopoulou et al. 2021).

2.4 Antiviral Activity

Clinical Data

An EO combination based on three aromatic plants (*Coridothymus capitatus*= syn *Thymbra capitata*, *O dictamnus* and *Salvia fruticosa*), in extra-virgin olive oil, denoted as CAPEo (Cretan Aromatic Plant essential oil) has been shown to reduce the duration and severity of symptoms of patients with upper respiratory tract viral infections. Pretreatment with the EO combination demonstrated that CAPEo exerts its antiviral activity after A/H1N1 or HRV14 entry in host cells, whereas it confers a preventive reactivity against RSV. The combination further resulted in a defective trafficking of influenza A Nucleoprotein (NP), suggesting NP as a valid target of this mixture. Moreover, the combination has been concluded to possess antiviral effects and has potential as an herbal agent against influenza viruses and rhinovirus (Tselioui et al. 2019), confirming the longstanding traditional use of Cretan dittany against cough and cold (Pirintsos et al. 2020).

2.5 Food Preservative Action

A range of fungal species are associated with postharvest spoilage of grapes. However, *Aspergillus carbonarius* is the primary fungus responsible for the contamination of grapes with ochratoxin A, a mycotoxin causing several confirmed negative health effects in humans and animals. Aiming to find a method, safe for consumers, to prevent postharvest decay and ochratoxin A contamination of grapes, the potential use of essential oils as preservatives was investigated. EOs of dittany, together with other oreganos' EOs, were tested after they have been studied by GC-MS and the results showed that EO of dittany was most effective in causing total inhibition of fungal growth with a minimum concentration of 100 µL L⁻¹, after having been tested on Sultana grapes during postharvest storage. The exhibited activity has been attributed mostly to high carvacrol content (Kontaxakis et al. 2020).

Among *Origanum* species, *O. dictamnus*, known as Cretan dittany, is a well-known Greek plant characterized by strong antimicrobial activities, while it is also reported to be effective against fungal pathogens of humans and plants. The above

effects are supported by limited *in vitro* and clinical data as well as a long-standing traditional use in the Mediterranean region. Based to these data the medicinal use of the infusion/decoction of the plant has been approved, as a Traditional Herbal Medicine (THM) by EMA against cough and cold. Moreover, the plant could be further used in food area as a potential natural preservative.

3 Greek Mountain Tea *Sideritis* sps. (*S. scardica*, *S. raeseri*, *S. clandestina*, *S. syriaca*) (EMA/HMPC/39455/2017)

3.1 History and Ethnobotany

Sideritis (Lamiaceae), also known as iron wort and shepherd's tea, is a genus of flowering plants known for their traditional use as aromatic herbal teas. *Sideritis* plants are abundant in the Mediterranean, the Balkans, and the Iberian Peninsula. The *Sideritis* genus comprises of certain Greek species (*S. scardica*, *S. raeseri*, *S. clandestina*, *S. syriaca*) commonly known as "Greek mountain tea" or "iron wort" and has been widely used in traditional medicine of the region, for their antimicrobial and anti-inflammatory properties.

Sideritis plants are hardy flowering perennials, found on rocky slopes at elevations over 1000 m. Only some of the species are cultivated, among which *Sideritis scardica* Griseb., *Sideritis clandestina* (Bory & Chaub) Hayek, *Sideritis raeseri* Boiss & Heldr., and *Sideritis syriaca* L. are cultivated mainly in Greece and Bulgaria.

Sideritis is known to ancient Greeks as described by Dioscorides in "*De Materia Medica*". Since antiquity, *Sideritis* was typically referenced for being capable of healing wounds caused by iron weapons during battle. The name "sideritis" (iron wort) derives from the Greek word for iron, "σίδηρος" literally translated as "he who is or has the iron," because it has been considered a great "remedy against trauma from iron weapons," that is to say wounds of war in ancient times. Dioscorides recommended its herbal infusion to soldiers as a rejuvenating, regenerating aid to help them heal quicker (González-Burgos et al. 2011).

In Crete, under Venetian rule, iron wort (*Sideritis syriaca*) earned another name, popular to this day on the island and throughout the world: *malotira* (μαλοτήρα). This name derives from the Italian: *male* means ailment/illness, while *tirare* means to pull, to draw out. *Malotira* draws out the illness.

The most common English name other than Mountain Tea is "Shepherd's Tea," because Greek shepherds would use the plant to brew tea while tending their flocks high in the mountains. Indeed, *sideritis* (or *malotira*) is a pleasant herbal remedy for sore throat, a great aid for diseases of the respiratory system, possessing soothing and healing properties,

3.2 Chemical Constituents

Terpenes, flavonoids, essential oils, iridoids, coumarins, lignans, and sterols have been identified in the *Sideritis* genus. Differences in chemical composition are observed between the *Sideritis* sp. and between the geographic regions where they grow (Bojovic et al. 2011). According to existing references, the following secondary metabolites have been isolated from Mediterranean *Sideritis* species: (Petreska et al. 2011; Todorova and Trendafilova 2014; Papaefstathiou et al. 2014; Vassilopoulou et al. 2013).

Monoterpenes, Diterpenes Many diterpenes (ent- kaurene derivatives) have been identified (Fraga 2012) such as isolinearol, leucanthol 18-monoacetate, siderol, sideroxol, epoxysiderol, and eubol.

Sesquiterpenes Verbascoside, leucosceptoside, martynoside and lavandulifolioside, ajugol, ajugoside, and melittoside have been isolated from *S. scardica* (Fraga 2012).

Flavonoids Flavonoid 7-O-diglycosides, two types of flavones, 8-OH (hypolaetin and isoscutellarein and their methoxy derivatives) and 5,7-OH (apigenin and luteolin), 8-OH (hypolaetin and isoscutellarein and their methoxy derivatives) and 5,7-OH isomers of apigenin 7-O-(coumaroyl) glucopyranoside together with apigenin 7-O-acetylcoumaroyl- allosylglucoside (Petreska et al. 2011; Bojovic et al. 2011; Yaneva and Balabanski 2013; Fraga 2012; Vassilopoulou et al. 2013; Papaefstathiou et al. 2014). **Triterpenes, Coumarins, Sterols**, campesterol (7.6%), stigmasterol (28.4%) and β -sitosterol, **Phenylpropanoids** hydroxycinnamic acids, phenylethanoid glycosides.

Essential Oil of Sideritis Species

The essential oil of *S. scardica* (0.03%) consists mainly of beta-pinene (17.9%), carvacrol (14.8%), and α -pinene (Fraga 2012). Another study (Kostadinova et al. 2008) has reported that the EOs of *S. scardica* and *S. raeseri* from Bulgaria and North Macedonia contain mainly diterpenes, while the EO of *S. raeseri* had higher concentrations of sesquiterpenes with germacrone (25%) and elemol acetate (15.9%) as main constituents (Bankova et al. 1996; Kostadinova et al. 2008; Bojovic et al. 2011).

3.3 Antimicrobial Activity

Sideritis scardica, *Sideritis clandestina*, *S. raeseri*, and *Sideritis syriaca* L. are botanically very closely related species of the same genus and have been described as endemic species of the Balkan Peninsula growing wild and also cultivated since the last century in Central Balkan and has been traditionally used as a healing

aromatic herbal tea in folk medicine of this geographic area since centuries. “Mountain tea” species (“Pirin tea” or “Mursalski tea”) (*S. scardica*; *S. clandestina*, *S. raeseri* and *S. syriaca*) are widely used mainly for the relief of cough associated with cold and mild gastrointestinal disorder.

Todorova and Trendafilova (2014) have summarized the available data on the antimicrobial activity of *S. scardica* (ethanol extract and its ethyl-ether, ethyl-acetate, and n-butanol fractions). Antimicrobial activity of varying degrees against *S. epidermidis*, *Micrococcus luteus*, *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and the yeast *C. albicans* has been demonstrated. Stronger activity was observed against *S. epidermidis*, *M. luteus*, *E. coli*, and *P. aeruginosa*, with moderate activity against *K. pneumoniae*. The MIC values of the tested extracts ranged from 40 µg/mL. The investigators concluded that the different types of terpenoids could contribute to this antibacterial activity (Tadic et al. 2012). Extracts from *S. scardica*, *S. syriaca*, and *S. montana*, /extracted with organic solvents, have exhibited strong activity against *S. aureus*, and butanol extract of *S. syriaca* exhibited antiyeast activity toward *C. albicans* (Yaneva and Balabanski 2013). Very recently, the activity of the essential oil from *Sideritis raeseri* susp *raeseri* was studied against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *E. coli*, *Listeria monocytogenes*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Pseudomonas fragi*, *Saccharomyces cerevisiae*, and *Aspergillus niger*. Growth inhibition of all microorganisms tested was documented, although it was significantly lower compared to gentamycin, ciproxin, and voriconazole, which were used as positive controls (Mitropoulou et al. 2020).

Traditionally, Iron wort has widely been used for wound healing and the treatment of respiratory conditions. These alleged effects are supported mainly by its essential oil’s antimicrobial effects, based on multiple *in vitro* studies. *Sideritis* species have been used by local people as herbal tea and in traditional medicine since centuries and people are taking advantage of the high species diversity and are aware of their useful properties through their traditional uses demonstrated biological activities and pharmacological properties (Aneva et al. 2019).

4 *Cistus creticus* L. Pink Rock-Rose

Cistus L. (from the Greek word *kistos-κίστος*) or rock-rose is a genus of perennial herbaceous plants among the Cistaceae family, that have hard leaves and grow in open areas of stony, infertile soils and they are indigenous to the Mediterranean region. *C. creticus* (pink rock-rose) is a small, woody plant, distributed along the coast of the Central-Eastern Mediterranean including the islands of Corsica, Sardinia, and Crete, as well as N Africa, and W Asia. Its distribution extends from sea level to 800 m in arid and warm areas. The plant has five violet petals on its flowers and numerous stamens; its fruits are capsules containing small seeds, covered by a hard water-impermeable coating (Papaefthimiou et al. 2014). *C creticus* has been traditionally known as a natural remedy due to its pharmacological

properties. It comprises of three identified subspecies, subsp. *corsicus* limited to the islands of Corsica (France) and Sardinia (Italy), subsp. *creticus*, which is endemic to the coastal areas of Crete (Greece), while subsp. *eriocephalus* is exclusively located on all three mentioned islands (Corsica, Sardinia, and Crete) (Demetzos et al. 1997).

4.1 History: Ethnopharmacology

Most *Cistus* species have aromatic foliage while *C. creticus*, in particular, also exudes a highly aromatic gum or resin, called “*ladano*” “*ladanum*” or “*labdanum*,” which has been used in incenses since ancient times and is now a valuable ingredient of perfumes.

The oleoresin ‘ladano’ has been mentioned by Dioscorides, Plinius the younger, and Herodotus (Papaefthimiou et al. 2014) and has been established as an anti-infective agent since antiquity in Mediterranean traditional medicine.

C. creticus has been used during the Middle Ages, as an expectorant against catarrh, externally in plasters for treating ulcerating wounds and as an ingredient for wound ointments. Traditionally, the herbal tea of the plant is drunk in cases of bronchitis or colds. Available literature suggests that *Cistus* plants are grown and collected mainly in the island of Crete, in Turkey, Spain, and Italy. Therefore, it is mostly used and preferred in these countries.

4.2 Chemical Constituents

Labdane-Type Diterpenes

In *C. creticus*, several terpenes (monoterpenes, sesquiterpenes, labdane-type diterpenes) have been reported (Papaefthimiou et al. 2014; Chinou et al. 1994; Demetzos et al. 1997). The labdane derivatives labd-13(E)-ene, 8a-15-diol, labd-7,13-(E)-dien-15-ol, labd-13(E)-en-8a-ol-15-yl acetate, labd-7,13(E)-dien-15-yl acetate, and ent-manoyl oxide mixture of the isomers (ent-13-epi-manoyl oxide; ent-manoyl oxide; ent-8-epi-manoyl oxide) were identified and characterized from nonpolar extracts and of its essential oils (leaves, fruits and resin) (Demetzos et al. 1990) (Fig. 6.2).

Polyphenols, Catechins

Aerial parts: catechin, gallo catechin, protocatechuic acid, shikimic acid, and gallo catechin-3-gallate, as well as trimeric and dimeric proanthocyanins have been isolated (Demetzos et al. 1990; Danne et al. 1993; Peterleit et al. 1991). Several

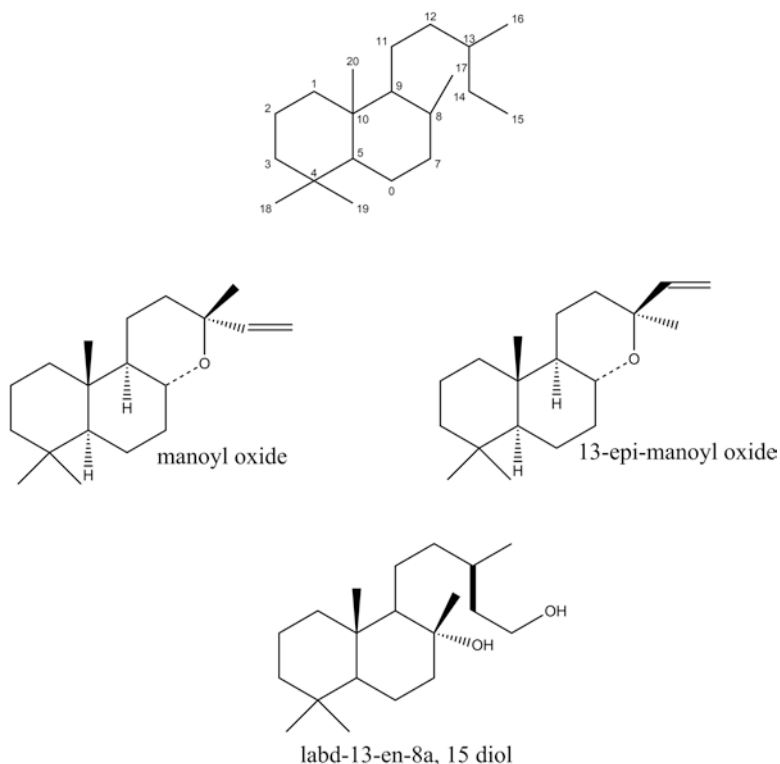


Fig. 6.2 Basic structure of labdane-type diterpene (a), (b) manoyl oxide, 13-epi-manoyl oxide, and (c) labd-13-en-8a,15 diol, in *C. creticus*

flavonoid derivatives have been identified including kaempferol, quercetin, apigenin, naringenin myricetin, coumarins (aesculin, scopoletin (6-O-methyl-7-hydroxycoumarin)) (Demetzos et al. 1990; Danne et al. 1993; Petereit et al. 1991). The polyphenolic composition of aqueous extracts from *C. creticus* has been performed using HPLC-DAD-ESI-MS/MS methodology. Major compounds of three main groups have been identified, i.e., ellagitannins, flavonoids, and phenolic acids derivatives (Barrajón-Catalán et al. 2011). Moreover, the polyphenols from four different commercial *C. creticus* (*C. incanus*) herbal teas have been extracted and all extracts have been characterized qualitatively and quantitatively by HPLC. Twenty nine polyphenols, including ellagitannins, flavanols, and glycosylated flavonols, were identified (Wittpahl et al. 2015) (Fig. 6.3).

Essential Oil

Monoterpenes, sesquiterpenes, diterpene esters, and alcohols are the main components identified (α -cadinene, δ -cadinene, viridiflorol, bulnesol, ledol, α -copaene, β -selinene, cubenene, manoyloxide and 13-epi-manoyloxide) (Demetzos et al.

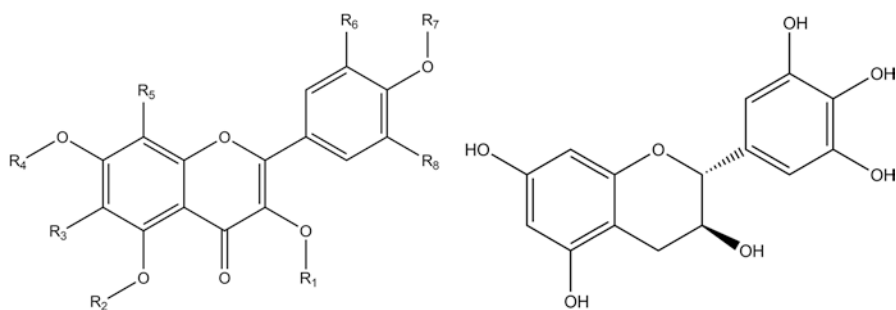


Fig. 6.3 (a) Flavonols, (b) gallocatechin from *Cistus creticus* L

1997). A comparison between the essential oil composition of *C. creticus* subsp. *corsicus* against *riocephalus* has been reported (Paolini et al. 2009). It has been documented that the two species are differentiated by botanical characteristics linked with essential oil production and composition of the volatile fraction (limonene or 13-epi-manoyl oxide, respectively), as the major components. Recently, it has been also affirmed that some differences are evident between the chemical profile of polyphenols in subsp. *creticus* and the other two subspecies (*eorsicus* and *eriocephallus*). However, it appears to be clear that the secondary metabolites are similarly comparable (quantitative than qualitative differences) (Mastino et al. 2018).

4.3 Antimicrobial and Antifungal Activity

In Vitro

The antimicrobial and antifungal activity of seven labdane-type diterpenoids isolated from the leaves of *Cistus incanus* subsp. *creticus* was assessed *in vitro* (Chinou et al. 1994) showing strong antimicrobial effects against *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *C. albicans*.

Bouamama et al. (2006) examined the antimicrobial activity of leaf extracts obtained from *C. villosus* (= *C. incanus*) and *C. monspeliensis* L. against ten strains of bacteria and fungi. All extracts showed inhibitory activity against most of them (*S. aureus*, *P. aeruginosa*, *C. albicans*, *C. krusei*, *C. glabrata* and *Aspergillus fumigatus* were investigated). The most susceptible ones were *S. aureus* and *C. glabrata* (MIC values 0.78 mg/ml and 0.19 mg/ml respectively).

Diterpenes of the plant have proved to be the driving force behind the antimicrobial activity of pink rock-rose infusions.

In situ studies revealed that *C. creticus* (syn *C. incanus*) infusions reduce the initial bacterial adhesion in the oral cavity, potentially due to contained polyphenols. Furthermore, the *in vitro* antibacterial activity of the methanolic extracts against *Streptococcus mutans* has been examined using a live/dead assay (BacLight®). With this approach, it has yielded antibacterial properties. Antibacterial

studies of the essential oil of *C. creticus subsp. eriocephalus* leaves have been carried out against Gram-positive, Gram-negative organisms and fungi *B. cereus*, *B. subtilis*, *C. albicans*, *S. faecalis*, *S. aureus*, *S. epidermidis*, *P. aeruginosa*, and *E. coli*. Gram-negative bacteria (*P. aeruginosa*, *E. coli*) were more resistant than the Gram-positive bacteria and the yeast (Wittpahl et al. 2015).

The aqueous extracts of *C. creticus* (= *C. incanus*) has revealed antibacterial activities more effective against Gram-positive bacteria, particularly *S. aureus* (MIC values 0.5–32 mg/mL) and *S. epidermidis* (MIC 0.25–8 mg/mL) than Gram-negative bacteria. They were also weak inhibitors of *C. albicans* and *C. glabrata* growth (MIC values over 8 mg/mL) (Viapiana et al. 2017).

Moreover, the antibacterial activities of phenolics derived from 14 *C. incanus* samples of different origin (Turkey, Albania, Greece, etc.), obtained as herbal teas, were compared. The antimicrobial activity was assessed with the use of thin-layer chromatography–direct bioautography (TLC-DB) against the Gram-negative naturally luminescent marine bacterium *Aliivibrio fischeri* and the Gram-positive soil bacterium *Bacillus subtilis*. It was established that in spite of the different origin of the investigated herbal samples, in qualitative terms, their antibacterial activity was closely comparable and more strongly pronounced against the Gram-positive than the Gram-negative bacteria (Szeremeta et al. 2018). Moreover, the antibacterial profile from 11 among previously referred commercial *C. incanus* herbal teas against the same microorganisms (*A. fischeri* and *B. subtilis*) proved apigenin, kaempferide, and acylated kaempferol glycosides (cis- and trans-tiliroside and their conjugates with p-coumaric acid) to be most effective antibacterial components (Móricz et al. 2018).

Very recently, the essential oil from *Cistus* leaves and crude extracts from *Tafraout, Morocco, has also been examined for antibacterial and antifungal activities* (Ait Lahcen et al. 2020).

4.4 Antiviral Activity

In Vitro

A special branded plant extract from *Cistus incanus*, rich in polyphenols (CYSTUS052), exhibited antiviral activity against the avian influenza A virus (H7N7) in cell (Droebner et al. 2007). In MDCK cells, a 90% reduction of plaque numbers on cells preincubated with the plant extract was achieved. The same extract demonstrated to exert an anti-influenza virus activity in A549 or MDCK cell cultures infected with prototype avian and human influenza strains of different subtypes (Ehrhardt et al. 2007). Viruses did not develop resistance to the extract when compared to amantadine that resulted in the generation of resistant variants after only a few passages. The authors have suggested that the effect appears to be mainly due to binding of the polymeric polyphenol components of the extract to the virus surface, thereby inhibiting binding of the haemagglutinin to cellular receptors.

Cistus creticus (= *C. incanus*) had also been shown to inhibit human immunodeficiency virus (HIV) infections *in vitro*. Antiviral activity seems highly selective for virus particles, preventing primary attachment of the virus to the cell surface and viral envelope proteins from binding to heparin. Rockrose extracts also inhibited infection by virus particles pseudotyped with Ebola and Marburg virus envelope proteins, indicating that antiviral activity of the extract extends to emerging viral pathogens showing potent and broad *in vitro* antiviral activity against viruses that cause life-threatening diseases in humans as promising sources of antiviral agents targeting virus particles (Rebensburg et al. 2016).

The hemorrhagic dengue fever affects up to 500 million patients, annually causing 20.000 deaths, with no chemotherapeutic agent available. Several extracts and fractions of *Cistus creticus* resin (labdanum) – standardized on labdane-type diterpenes via GC-MS – have been studied on their activity against the dengue virus (DENV-2 strain 00st-22A) using *in vitro* Vero cell cultures. Preliminary experiments with a labdanum diethyl ether raw extract have not yielded measurable results due to cytotoxic effects against Vero cells; cell viability was constantly checked using the MTT-test. In the most active fraction at 30 µg/ml, dengue virus proliferation was 100% suppressed and cell viability was over 90%. Such antiviral activity of a diethyl ether extract of labdanum against a virulent hemorrhagic fever like dengue is described for the first time (Kuchta et al. 2020).

***In Vitro* Effects**

The same branded *Cistus* extract as mentioned before (Droebner et al. 2007) in a form of aerosol has been assayed for the treatment of mice infected with a mouse-adapted highly pathogenic avian influenza virus (FPV, H7N7). Inbred female Balb/c and C57Bl/6 mice were infected. The treatment was performed for 5 days and monitored for 15 days after infection. In conclusion, the mice treated with the extract did not develop disease, showed neither differences in their body temperature nor in their gross motor activity, and exhibited no histological alterations of the bronchioles epithelial cells (Droebner et al. 2007). From the reported data, a potential mechanism of action was proposed. This suggests that polyphenolic ingredients of the extract block virus infection by a direct (physical) interaction with the virus particles.

Clinical Studies

A double-blind, placebo-controlled clinical study (Kalus et al. 2009) was developed, where 160 patients (7–81 years old), suffering from an infection of the upper respiratory tract, participated. The product (in lozenges) investigated consisted of a special branded aqueous *Cistus creticus* extract as explained before (Droebner et al. 2007). Of the 160 participants in this study (56 men and 104 women), 129 patients completed the study, 82.5% of the *Cistus* group, and 80.0% of the placebo group.

Based on the findings 92 (57.5%), volunteers had a viral infection (11 patients with Influenza A and 7 patients with Influenza B) and 67 (41.8%) a bacterial infection. Patients were observed and treated up to 7 days. All participants of the trial were asked to judge the maximum severity of the following symptoms on a questionnaire by their physician: pain, cough intensity, cough frequency, sputum and sniffles. Each symptom was determined as follows: 0 = not present, 2 = slight, 4 = mild, and 6 = severe. The total score was calculated by adding the five symptom scores and therefore ranged from 0 to 30. The score of subjective symptoms decreased significantly during observation ($p < 0.001$). The decrease was more pronounced in the group treated with *Cistus* compared to placebo ($p < 0.001$). The time period, in which the drug achieved clinical symptom improvement, was shorter for *Cistus* than for placebo. It is still unclear how *Cistus* extracts in the mouth help to reduce the spread of influenza virus in the lung via a binding effect. According to previous studies (both *in vitro* and *in vivo*), nonspecific pharmacological interactions could be involved (Ehrhardt et al. 2007; Droebner et al. 2007) Many different metabolites (mostly polyphenols) may be responsible for the antiviral and antibacterial properties of this medicinal plant. In conclusion, *Cistus* was more effective in reducing the average duration and severity of symptoms in patients with infection of the upper respiratory tract than placebo.

In a second trial (Kalus et al. 2010), three hundred volunteers (age 5–85 years) were recruited in this randomized, open trial study. All suffered from an infection of the upper respiratory tract: 131 (43.7%) volunteers had a viral infection, 163 (54.3%) a bacterial infection, and six patients (2%) both types or a mixed infection. Two hundred and seventy-seven (277) patients completed the study. One hundred and forty-one (141) patients were randomly placed into the *Cistus* group, and 136 into the green tea placebo group. Patients were observed and treated for 7 days. The subjects were asked to judge the maximum severity of their symptoms as previously described (Kalus et al. 2009). An improvement of the symptoms in the *Cistus* group was observed in 57.8% (160/277) of the patients after an average of 3.2 days. A comparison of the single parameters shows that in the *Cistus* branded group, the severity level decreased in all the parameters [pain cough (strength), cough (frequency), rhinorrhoea, and sputum] for the 277 examined patients with the exception of rhinorrhoea, whereas in the green tea group, the pain intensity only slightly decreased. Approximately 24.9% (69/277) of the patients complained of adverse effects, observed more significantly in the green tea group with 35.3% (48/136) than in the *Cistus* group with 14.9% (21/141) ($p < 0.001$). More frequent adverse effects were nausea and dizziness.

4.5 Activities Against SARS-CoV-2

During the COVID-19 pandemic, people are facing risks of adverse health effects due to the restrictions implemented such as quarantine measures, reduced social contact, and self-isolation. In several review articles, data has been collected on

potential preventive and therapeutic health benefits of Complementary and Integrative Medicine (CIM) that might be useful during the COVID-19 pandemic. In several among them, the safe and supportive use of *Cistus creticus* herbal teas has been proposed due to its potentially beneficial antiviral effects (Seifert et al. 2020; Şener 2020; Stange and Uehleke 2020).

4.6 Food Supplementation

In the search for new functional foods and novel products to develop new prohealth high-quality foods, *C. creticus* has been proposed as an innovative functional supplement, added in wheat as a flour supplement. Supplementation of bread or pasta with 3% water extract yielded products with desirable characteristics, which were favored by consumers (Cacak-Pietrzak et al. 2019; Lisiecka et al. 2019). The use of such water extracts in low concentrations (up to 3%) as flavoring agents could be helpful, while further safety assessment studies must be conducted in order to approve the use of pink rockrose for food preservation, brought about through its expressed antimicrobial activities (Barrajón-Catalán et al. 2011).

Conclusively, *Cistus* genus is widespread in the Mediterranean regions with several species traditionally known as natural remedies, among which *C. creticus* (= *Cistus × incanus* L.) (pink rock-rose), rich in bioactive metabolites, has been used by the pharmaceutical and food industries. The aerial parts of the plant have been used as a healing herbal tea, and consumption has been proposed mainly for the relief of cough associated with cold. *Cistus* water leaf extracts have showed *in vitro* antimicrobial activity against a range of human pathogenic bacteria and fungi, while a special branded extract of *Cistus* rich in polyphenols has exhibited antiviral activity against the avian influenza A virus (H7N7) *in vitro* and *In vivo* (Ehrhardt et al. 2007; Droebner et al. 2007), while in COVID-19 pandemic, it had been also proposed due to its beneficial antiviral effects. All scientific data are strongly supportive to the ethnopharmacological relevance of the wide continuous use of pink rock-rose establishing it as an anti-infective agent since antiquity in Mediterranean ethnopharmacology.

5 *Pistacia lentiscus* (EMA/HMPC/46756/2015)

Pistacia lentiscus (Anacardiaceae), cultivated in the island of Chios in Greece, is the source of unique resin called “mastic” – a well-known natural antimicrobial and healing agent all around the world. The mastic tree (*P. lentiscus* L.) is naturally distributed in coastal regions of the Mediterranean, (Gruenwald et al. 2007). It is an evergreen shrub, 2–3 meters high that grows slowly, and becomes ready for cultivation after 40–50 years. The plant is renowned for its production of a resin, known as Mastic gum, which is obtained as an exudate after carving the trunk and branches

(Paraschos et al. 2007, 2012). The Southern area (Mastihohoria) of the Greek island of Chios is among the major global commercial sources of mastic resin [Chios Mastic Gum (CMG)], while, since 1997, it has been registered as a Product of Protected Designation of Origin (PDO) in the EU (EMA 2015a, b).

A well-documented botanical study by Browicz has proposed that instead of *Pistacia lentiscus* var. *Chia* (Desf. Ex Poiret) DC, the name *Pistacia lentiscus* cv. *Chia* should be used as cv, means cultivated clone. Recently, botanical classification of *Pistacia lentiscus* L. without any further variety and/or cultivar has been accepted in the monograph of European Pharmacopoeia (Browicz 1987; EMA 2015a, b).

5.1 History and Ethnopharmacology

Preparations of *P. lentiscus* resin have been used traditionally for stomach disorders like dyspepsia and peptic ulcer, as well as for dermatological problems and oral hygiene for more than 2500 years. A series of reports in international medical journals refer to these historically recorded properties of mastic, which are based on the results of scientific studies as well as on clinical trials carried out by researchers, and have revealed that metabolites of the mastic tree possess interesting bioactive properties (Al-Habbal et al. 1986; Huwez and Al-Habbal 1986; Huwez et al. 1998; EMA 2015a, b).

Ancient Greek physicians, such as Hippocrates, Dioscorides, Theophrastus, and Galen, first mentioned its properties and recommended its use for its distinctive flavor and its therapeutic properties (Paraschos et al. 2007). In the last century, mastic gum is being used as a flavoring agent in Mediterranean cuisine, in the production of chewing gum, in the cosmetics industry, in dentistry and traditionally, by the local population of Chios Island, for the relief of epigastric pain and protection against peptic ulcer disease. Mastic gum has been demonstrated to possess anti-inflammatory, antioxidant as well as antimicrobial activities.

5.2 Chemical Constituents

The resin of the tree appears to consist of a variety of secondary metabolites, some of which have been isolated and determined in nature for the first time in this plant. A major of these components is the natural polymer, which was identified as *cis*-1,4-poly- β -myrcene (EMA 2015a, b) (Fig. 6.4).

The resin is also rich in nonvolatile natural products such as triterpenes, phytosterols, and polyphenolic molecules. Resin's triterpenes are mainly composed of the tetracyclic euphane and dammarane skeleton type and of the pentacyclic oleanane and lupane skeleton type such as mastic acid, isomastic acid, oleanolic acid, and tirucallol etc. (Gruenwald et al. 2007; EMA 2015a, b) (Fig. 6.5).

Fig. 6.4 Natural polymer of *P. lentiscus* resin, *cis*-1,4-poly- β -myrcene

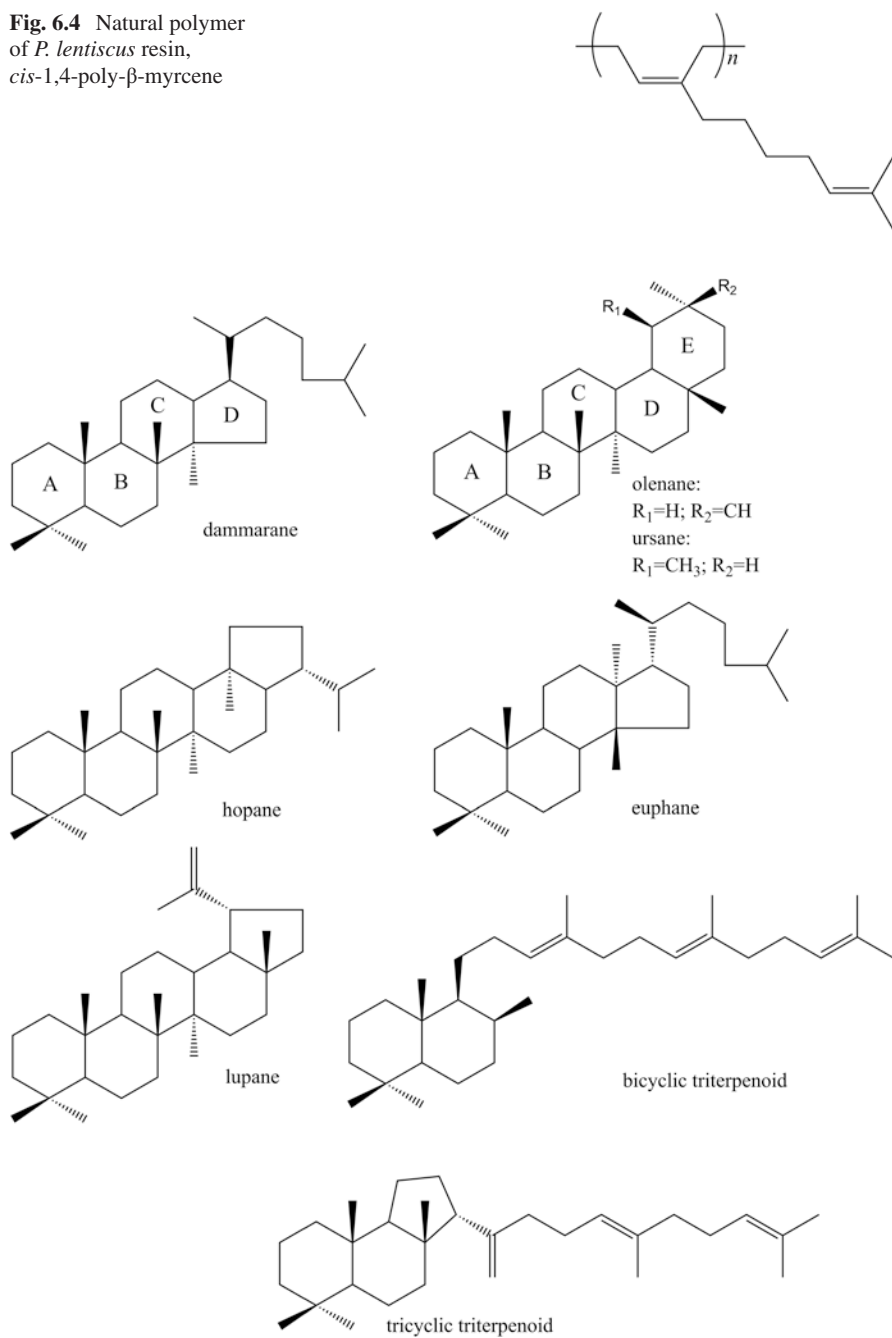


Fig. 6.5 Triterpenoid types in mastic

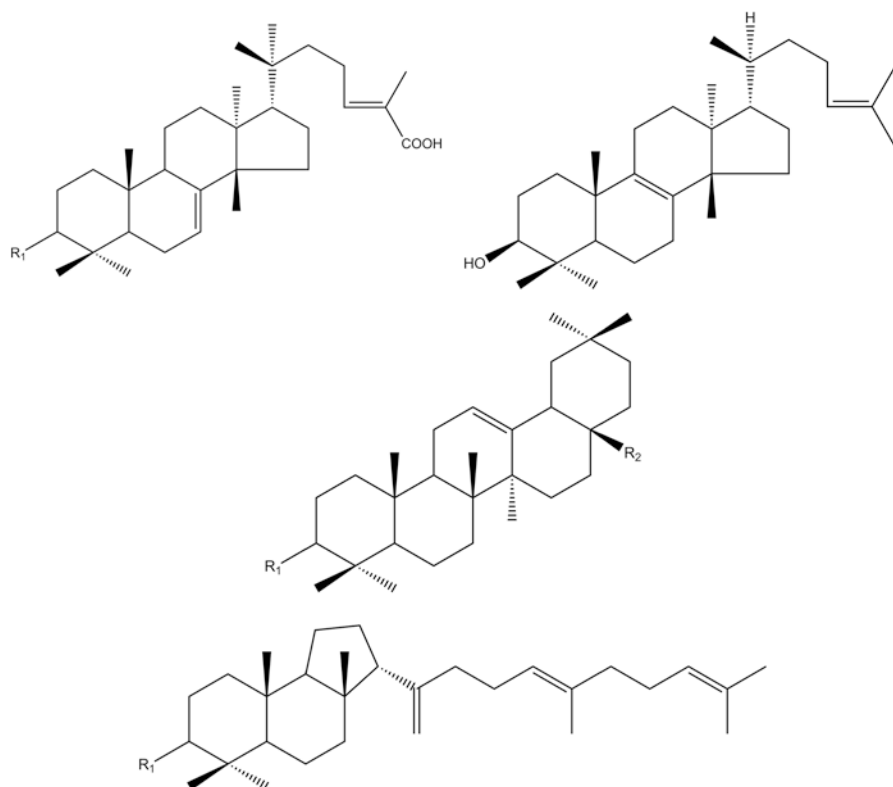


Fig. 6.6 (a) Masticadienonic acid, (b) tirucallol, (c) oleanolic acid, and (d) 3 β -hydroxymalabarica-14(26),17E,21-triene

The main such nonvolatile natural products reported in the literature are masticadienonic acid, tirucallol, oleanolic acid, masticadienonic acid, 3-oxo-dammara-20(21),24-diene, 3 β -hydroxymalabarica-14(26),17E,21-triene, 3-oxo-malabarica-14(26),17E,21-triene, etc., together with 1,4-poly- β -myrcene (EMA 2015a, b; Paraschos et al. 2007) (Fig. 6.6).

Mastic gum also contains a small fraction (approximately 2%) of essential oil, thoroughly analyzed (Magiatis et al. 1999; EMA 2015a, b). This essential oil contains volatile and aromatic ingredients (monoterpene hydrocarbons, oxygenated monoterpenes, and sesquiterpenes). The chemical composition of essential oils of resin, leaves and unripe and ripe fruits of mastic has been studied comparatively, identifying a total of 90 components (50% monoterpene hydrocarbons, 20% oxygenated monoterpenes, and 25% sesquiterpenes) with α -pinene (79%) and the myrcene (3%) being the major components (EMA 2015a, b). In a very recent study, α -pinene and β -myrcene were found in abundance in the fresh oils of mastic gum; however, in the oil of the aged collection, oxygenated monoterpenes and benzenoids, such as verbenone, pinocarveol, and α -campholenal, were found at the highest rates (Pachi et al. 2021).

5.3 Mastic Gum: Antimicrobial Activities

In Vitro

Several studies have been conducted to investigate mastic antimicrobial properties. Different *P. lentiscus* L. leaves preparations (10% decoction, petroleum ether extract, ethanol extract, infusion, and maceration) have tested against selected Gram-positive (+) and Gram-negative (–) bacteria, but only the leaves decoction showed activity (MIC 312 mg/ml), while all other preparations were practically inactive against *S. lutea*, *S. aureus* and *E. coli* (Aksoy et al. 2006; EMA 2015a, b).

Daifas et al. (2004) have investigated the effect of mastic and its essential oil, alone and in conjunction with ethanol, on the growth of proteolytic strains of *Clostridium botulinum*. They proved that mastic and mastic oil in ethanol can effectively be used as factors against the appearance of *botulinum* neurotoxin in nutrition goods. More specifically, the results of the laboratory tests showed that the addition of only 0.3% mastic oil was required for the inhibition of proteolytic strains of *Clostridium botulinum*. The authors concluded that mastic and mastic oil could potentially be used as natural preservative in bakery products.

The antibacterial activity of mastic oil can be attributed to the combination of several components rather than to one particular compound. It is also interesting to note that different bacteria are susceptible or not to different compounds of the essential oil (Magiatis et al. 1999; EMA 2015a, b).

A number of studies have shown that mastic and mastic oil exhibit actions on gastrointestinal lesions. After discovery of *Helicobacter pylori* and correlation with gastrointestinal disease in 1983, the interest for the determination of mechanism of action of mastic and mastic oil for these disorders focused on the exploration and eventual finding of anti-*H. pylori* properties.

The first study that proves mastic anti-*H. pylori* activity is published in the New England Journal of Medicine in 1998 (Huwez et al. 1998). Mastic proved to kill the *H. pylori* NCTC 11637 strain and the six clinical isolates. This action was due to the fact that mastic exterminated *H. pylori*, which is liable for the majority of the digestive ulcer cases. In the specific study, fresh samples were used with the presence of *H. pylori*, which were isolated from patients and the minimum bactericidal concentration (MBC) of mastic was searched, which means the minimum concentration required in order to exterminate 99.9% of the bacterium within 24 hours. Mastic exterminated the bacterium in all the examined samples, regardless of the size of the population. The MBC of mastic was 60 µg/ml, but even in smaller concentrations, the antibacterial action has been observed.

Marone et al. (2001) and Bona et al. (2001) assessed the antibacterial effect of mastic on the clinical isolates of *H. pylori* at concentrations of 2000 to 1.9 µg/ml. Specifically, the inhibition of growth of *H. pylori* in the presence of aqueous mastic extracts was studied. The results showed that the extracts of at least 1.4 g resin affect the viability of bacterium, preventing cell growth. (Bona et al. 2001).

Paraschos et al. (2007) utilized an established *H. pylori* infection model to evaluate the potential therapeutic effect of total mastic extract administration on *H. pylori* colonization and development of associated gastritis. The antimicrobial activities of different fractions as well as that of mastic total extract, against a panel of 10 clinical isolates of *H. pylori* and the CCUG 38771 reference strain, were tested in a period of 3 months. Mastic extracts exhibited concentration- and strain-dependent bactericidal activities. The experiments showed that the mastic total extract could moderately reduce *H. pylori* colonization in the antrum and corpus of the stomach. According to the authors, the results also suggest that habitual long-term mastic consumption may be effective in moderating *H. pylori* colonization.

A recent study (Miyamoto et al. 2014) examined the components of mastic responsible for anti-*H. pylori* activity. GC–MS analysis of the essential oil of mastic led to the identification of 20 components among which α -pinene (82.26%) was the most abundant. Then, the authors examined which component inhibits the growth of *H. pylori* testing commercially available compounds against *H. pylori* strains that were established from patients with gastritis, gastric ulcer, and gastric cancer. Some of them showed antibacterial activity against clarithromycin-resistant and/or metronidazole-resistant strains. α -Terpineol and (E)-methyl isoeugenol showed anti-*H. pylori* activity not only against drug-sensitive strains but also against drug-resistant strains. These 10 compounds also showed antibacterial activity against three different strains (*Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*). The authors have concluded that these components could be useful to overcome the drug-resistance *H. pylori* growth in stomach.

Mastic has been also used widely, as a traditional remedy, since antiquity for oral malodour, and oral hygiene and this knowledge has been assessed also in recent studies. Mastic has showed selective antibacterial action against oral bacteria *Porphyromonas gingivalis* and *Prevotella melaninogenica* (EMA 2015a, b). Additionally, the antimicrobial activity of Chios Mastic Gums with their respective Chios Mastic Oils was evaluated, with growth tests against the fungi *Aspergillus nidulans*, *Aspergillus fumigatus*, *Candida albicans*, *Mucor circinelloides*, and *Rhizopus oryzae*, and the bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*, with the samples exhibiting a moderate activity (Pachi et al. 2021).

Clinical Data

Sixty volunteers (60) with symptoms and endoscopic confirmation of duodenal ulcer participated in a first clinical study (Al-Habbal et al. 1986). The results showed that in the group that consumed CMG, there was an alleviation of the symptoms in 80% of the cases, while the endoscopic examination has confirmed that duodenal ulcer was cured in 70% of the cases. The authors have concluded that mastic was more active than placebo for the alleviation and the treatment of ulcer symptoms, with no undesirable side effects. The same research team has published (Huwez and Al-Habbal 1986) the findings of another clinical study in patients who suffered from gastric ulcers but of benign nature. CMG was also administered in the dosage of 2 g

per day for 4 weeks to 6 patients with diagnosed gastric ulcer by means of gastroscopy. The results of the study have shown that the administration of CMG caused symptom relief in all patients.

The *in vitro* studies showed antimicrobial activity of mastic and mastic fractions and preparations against a panel of Gram-positive and Gram-negative bacteria as well as its particular strong activity against *H. pylori*, with MBC, at 60 µg/ml. These findings gave a positive signal to the therapeutic indication of mastic against mild dyspeptic disorders. Overall, the results of this study signify the prospects of *Pistacia lentiscus* resin for the development of herbal formulations against infections as well as for its applications in oral hygiene products.

6 Conclusions

The chemistry of aromatic plants exhibits an amazing assortment of biologically active compounds such as essential oils mixtures, terpenoids, phenolic acids, and flavonoids that are widely employed as bioactive sources. This chapter aims to provide a critical evaluation of current knowledge about four selected aromatic plant genera (*O. dictamnus*, *Sideritis* species, *C. creticus* and *P. lentiscus* resin), all of them native and/or endemic in Greece, to be used as potential source of natural antimicrobials. All collected information sourced from ethnobotanical literature together with the results of existing pharmacological data have been approved by EMA. Following this extensive literature search, it can be concluded that many traditional uses of them are supported by modern *in vitro* or *in vivo* pharmacological studies. As discovery of pronounced natural antimicrobials, among aromatic plants, for future experimental studies, are still of high growing interest, well-designed studies are required to establish links between their traditional /ethnopharmacological uses and evaluated bioactivities. Moreover, their safe uses have to be fully ensured, before any potential exploitation in pharmaceutical and/or agricultural industries.

References

- Aksoy A, Duran N, Koksali F (2006) *In vitro* and *in vivo* antimicrobial effects of mastic chewing gum against *Streptococcus mutans* and *mutans streptococci*. Arch Oral Biol 51:476–481
- Al-Habbal MJ, Al-Habbal Z, Huwez FU (1986) A double-blind controlled clinical trial of mastic and placebo in the treatment of duodenal ulcer. Clin Exp Pharmacol Physiol 11:541–544
- Aneva I, Zhelev P, Kozuharova E, Danova K, Nabavi SF, Behzad S (2019) Genus *Sideritis*, section *Empedoclia* in southeastern Europe and Turkey – studies in ethnopharmacology and recent progress of biological activities. DARU J Pharm Sci 27:407–421
- Ait Lahcen S, EL HATTABÍ L, Benkaddour R, Chahboun N, Ghanmi M, Satrani B, Tabyaoui M, Zarrouk A (2020) Chemical composition, antioxidant, antimicrobial and antifungal activity of Moroccan *Cistus Creticus* leaves. Chemical Data Collections, 26, 100346

- Bankova V, Marcucci MC, Simova S, Nikolova N, Kujumgiev A, Popov S (1996) Antibacterial diterpenic acids from Brazilian propolis. *Zeitschrift für Naturforschung C* 51(5–6) 277–280. <https://doi.org/10.1515/znc-1996-5-602>
- Barrajón-Catalán E, Fernández-Arroyo S, Roldán C, Guillén E, Saura D, Segura-Carretero A (2011) A systematic study of the polyphenolic composition of aqueous extracts deriving from several *Cistus* genus species: evolutionary relationship. *Phytochem Anal* 22:303–312
- Baser KHC, Buchbauer G (eds) (2010) *Handbook of essential oils: science, technology, and applications*. CRC Press Taylor & Francis Group FL, Boca Raton
- Bojovic D, Jankovic S, Potrava Z, Tadic V (2011) Summary of the phytochemical research performed to date on *Sideritis* species. *Ser J Exp Clin Res* 12:109–122
- Bona SG, Bono L, Dagheta L, Marone P (2001) Bactericidal activity of *Pistacia lentiscus* gum mastic against *Helicobacter pylori*. *Am J Gastroenterol* 96:S49
- Bouamama H, Noel T, Villard J, Benharref A, Jana M (2006) Antimicrobial activities of the leaf extracts of two Moroccan *Cistus* L. species. *J Ethnopharmacol* 104:104–107
- Browicz K (1987) *Pistacia lentiscus* L. *Plant Syst Evol* 155:189–195
- Cacak-Pietrzak GZ, Rózyło R, Dziki D, Gawlik-Dziki U, Sulek A, Biernacka B (2019) *Cistus incanus* L. as an innovative functional additive to wheat bread. *Foods* 8(8):349
- Chatzopoulou A, Karioti A, Gousiadou C, Lax Vivancos V, Kyriazopoulos P, Golegou S, Skaltsa H (2010) Depsides and other polar constituents from *Origanum dictamnus* L. and their *in vitro* antimicrobial activity in clinical strains. *J Agric Food Chem* 58:6064–6068
- Chinou I, Demetzos C, Harvala C, Roussakis C, Verbist JF (1994) Cytotoxic and antibacterial labdane type diterpenes from the aerial parts of *Cistus incanus* subsp. *creticus*. *Planta Med* 60:34–36
- Daifas DP, Smit JP, Blanchfield B, Sanders G, Austin JW, Koukoutsis J (2004) Effects of mastic resin and its essential oil on the growth of proteolytic *Clostridium botulinum*. *Int J Food Microbiol* 94:313–322
- Danne A, Petereit F, Nahrstedt A (1993) Proanthocyanidins from *Cistus incanus*. *Phytochemistry* 34:1129–1133
- Demetzos C, Harvala C, Philianos SM, Skaltsounis AL (1990) A new labdane type diterpene and other compounds from the leaves of *Cistus creticus* subsp. *creticus* L. *J Nat Prod* 53:1365–1367
- Demetzos C, Katerinopoulos H, Kouvarakis AS, Stratigakis A, Loukis A, Ekonomakis C (1997) Composition and antimicrobial activity of the essential oil of *Cistus creticus* subsp. *eriocephalus*. *Planta Med* 63:477–479
- Droebner K, Ehrhardt C, Poetter A, Ludwig S, Planz O (2007) CYSTUS052, a polyphenol-rich plant extract, exerts anti-influenza virus activity in mice. *Antivir Res* 76:1–10
- Ekonomakis CD, Skaltsa H, Demetzos C, Sokovic M, Thanos CA (2002) Effect of phosphorus concentration of the nutrient solution on the volatile constituents of leaves and bracts of *Origanum dictamnus*. *J Agric Food Chem* 50:6276–6280
- Ehrhardt C, Hrinčius ER, Korte V, Mazur I, Droebner K, Poetter A (2007) A polyphenol rich plant extract, CYSTUS052, exerts anti influenza virus activity in cell culture without toxic side effects or the tendency to induce viral resistance. *Antivir Res* 76:38–47
- EMA European Union herbal Monograph of *Origanum dictamnus* L., *Origanum dictamnus* herba, Dittany of Crete herb, EMA/HMPC/200429/2012 Corr
- EMA European Union herbal Monograph on *Sideritis scardica* Griseb.; *Sideritis clandestina* (Bory & Chaub.) Hayek; *Sideritis raeseri* Boiss. & Heldr.; *Sideritis syriaca* L., herba EMA/HMPC/39455/2015a
- EMA European Union herbal Monograph on *Pistacia lentiscus* L. resina, mastic EMA/HMPC/46757/2015b
- Fraga BM (2012) Phytochemistry and chemotaxonomy of *Sideritis* species from the Mediterranean region. *Phytochemistry* 76:7–24
- González-Burgos E, Carretero ME, Gomez-Serranillos MP (2011) *Sideritis* spp.: uses, chemical composition and pharmacological activities – a review. *J Ethnopharmacol* 135:209–225
- Gruenwald J, Brendler T, Jaenicke C (2007) *PDR for herbal medicines*. Thomson, Reuters, Toronto

- Hanlidou E, Karousou R, Kleftoyanni V, Kokkini S (2004) The herbal market of Thessaloniki (N Greece) and its relation to the ethnobotanical tradition. *J Ethnopharmacol* 91:281–299
- Heinrich M (2000) Ethnobotany and its role in drug development. *Phytother Res* 14:479–488
- Huwez FU, Al-Habbal MJ (1986) Mastic in treatment of benign gastric ulcers. *Gastroenterol J* 21:273–274
- Huwez F, Thirwell D, Cockayne A, Ala'Aldeen D (1998) Mastiha gum kills *Helicobacter pylori*. *N Engl J Med* 339:1946
- Kalus U, Grigorov A, Kadecki O, Jansen J, Kesewetter H, Radke H (2009) *Cistus incanus* (CYSTUS052) for treating patients with infection of the upper respiratory tract A prospective, randomised, placebo- controlled clinical study. *Antivir Res* 84:267–271
- Kalus U, Kiesewetter H, Radke H (2010) Effect of CYSTUS052® and green tea on subjective symptoms in patients with infection of the upper respiratory tract. *Phytother Res* 24:96–100
- Karanika MS, Komaitis M, Aggelis G (2001) Effect of aqueous extracts of some plants of Lamiaceae family on the growth of *Yarrowia lipolytica*. *Int J Food Microbiol* 64:175–181
- Kontaxakis E, Filippidi E, Stavropoulou A, Daferera D, Tarantilis PA, Lydakis D (2020) Evaluation of eight essential oils for postharvest control of *Aspergillus carbonarius* in grapes. *J Food Prot* 83:1632–1640
- Kostadinova E, Alipieva K, Stefanova M, Antonova D, Evstatieva L, Stefkov G, Tsvetkova I, Naydenski H, Bankova V (2008) Influence of cultivation on the chemical composition and antimicrobial activity of *Sideritis* spp. *Pharmacogn Mag* 4:102–106
- Kougioumoutzis K, Kokkoris IP, Panitsa M, Kallimanis A, Strid A, Dimopoulos P (2021) Plant endemism centres and biodiversity hotspots in Greece. *Biology* 10:1–27
- Koutelidakis AE, Andritsos ND, Kabolis D, Kapsokefalou M, Drosinos EH, Komaitis M (2016) Antioxidant and antimicrobial properties of tea and aromatic plant extracts against bacterial foodborne pathogens: a comparative evaluation. *Curr Tops Nutr Res* 14:133–142
- Kuchta K, Tung NH, Ohta T, Uto T, Raekiansyah M, Grötzinger K, Rausch H, Shoyama Y, Rauwald HW, Morita K (2020) The old pharmaceutical oleoresin labdanum of *Cistus creticus* L. exerts pronounced *in vitro* anti-dengue virus activity. *J Ethnopharmacol* 257: Art No 112316
- Liolios CC, Gortzi O, Lalas S, Tsaknis J, Chinou I (2009) Liposomal incorporation of carvacrol and thymol isolated from the essential oil of *Origanum dictamnus* L. and *in vitro* antimicrobial activity. *Food Chem* 112:77–83
- Liolios CC, Graikou K, Skaltsa E, Chinou I (2010) Dittany of Crete: a botanical and ethnopharmacological review. *J Ethnopharmacol* 131:229–241
- Lisiecka K, Wójtowicz A, Dziki D, Gawlik-Dziki U (2019) The influence of *Cistus incanus* L. leaves on wheat pasta quality. *J Food Sci Technol* 56:4311–4322
- Magiatis P, Melliou E, Skaltsounis AL, Chinou IB, Mitaku S (1999) Chemical composition and antimicrobial activity of the essential oils of *Pistacia lentiscus* var. *Chia*. *Planta Med* 65:749–752
- Marone P, Bono L, Leoane E, Bona S, Carretto E, Perversi L (2001) Bactericidal activity of *Pistacia lentiscus* mastic gum against *Helicobacter pylori*. *J Chemother* 13:611–614
- Mastino PM, Mauro M, Jean C, Juliano C, Marianna U (2018) Analysis and potential antimicrobial activity of phenolic compounds in the extracts of *Cistus creticus* subspecies from Sardinia. *Nat Prod J* 8:166–174
- Mitropoulou G, Sidira M, Skitsa M, Tsochantaridis I, Pappa A, Proestos C, Kourkoutas Y (2020) Assessment of the antimicrobial, antioxidant, and antiproliferative potential of *Sideritis raeseri* subsps. *raeseri* essential oil. *Foods* 9: Article no. 860
- Miyamoto T, Okimoto T, Kuwano M (2014) Chemical composition of the essential oil of mastic gum and their antibacterial activity against drug-resistant *Helicobacter pylori*. *Nat Prod Bioprospect* 4:227–231
- Móricz M, Szeremeta D, Knaś M, Długosz E, Ott PG, Kowalska T, Sajewicz M (2018) Antibacterial potential of the *Cistus incanus* L. phenolics as studied with use of thin-layer chromatography combined with direct bioautography and in situ hydrolysis. *J Chromatogr A* 1534:170–178

- Pachi VK, Mikropoulou EV, Dimou S, Dionysopoulou M, Argyropoulou A, Diallinas G, Halabalaki M (2021) Chemical profiling of *Pistacia lentiscus* var. *Chia* Resin and essential oil: Ageing markers and antimicrobial activity. *PRO* 9:1–20
- Paloukopoulos C, Govari S, Soulioti A, Stefanis I, Matheussen A, Capasso C, Cos P, Supuran CT, Karioti A (2021) Phenols from *Origanum dictamnus* L. and *Thymus vulgaris* L. and their activity against *Malassezia globosa* carbonic anhydrase. *Nat Prod Res* 1–7. <https://doi.org/10.1080/14786419.2021.1880406>
- Paolini J, Falchi A, Quilichini Y, Desjobert JM, Cian MC, Varesi L (2009) Chemical and genetic differentiation of two subspecies of *Cistus creticus* L. (*C. creticus* subsp. *eriocephalus* and *C. creticus* subsp. *corsicus*). *Phytochemistry* 70:1146–1160
- Papaefstathiou G, Aligiannis N, Fokialakis N, Halabalaki M, Termentzi A, Skaltsounis AL (2014) Metabolic profiling and antioxidant activity of *Sideritis* species growing in Southeast Europe. *Planta Medica GA Congress: Coimbra Portugal*
- Papaefthimiou D, Papanikolaou A, Falara V, Givanoudi S, Kostas S, Kanellis A (2014) Genus *Cistus*: a model for exploring labdane-type diterpenes' biosynthesis and a natural source of high value products with biological, aromatic and pharmacological properties. *Front Chem* 2:33
- Paraschos S, Magiatis P, Mitakou S, Petraki K, Kalliaropoulos A, Maragkoudakis P, Mentis A, Sgouras D, Skaltsounis AL (2007) *In vitro* and *in vivo* activities of Chios mastic gum extracts and constituents against *Helicobacter pylori*. *Antimicrob Agents Chemother* 51:551–559
- Paraschos S, Mitakou S, Skaltsounis AL (2012) Chios gum mastic: a review of its biological activities. *Curr Med Chem* 19:2292–2302
- Petereit F, Kolodziej H, Nahrstedt A (1991) Flavan-3-ols and proanthocyanidins from *Cistus incanus*. *Phytochemistry* 30:981–985
- Petreska J, Stefova M, Ferreres F, Moreno DA, Tomas-Barberan FA, Stefkov G, Kulevanova S, Gil-Izquierdo A (2011) Potential bioactive phenolics of Macedonian *Sideritis* species used for medicinal “Mountain Tea”. *Food Chem* 125:13–20
- Pirintosos SA, Bariotakis M, Kampa M, Sourvinos G, Lionis C, Castanas E (2020) The therapeutic potential of the essential oil of *Thymbra capitata* (L.) Cav., *Origanum dictamnus* L. and *Salvia fruticosa* Mill. and a case of plant-based pharmaceutical development. *Front Pharmacol* 11, Article number 522213
- Proestos C, Sereli D, Komaitis M (2006) Determination of phenolic compounds in aromatic plants by RP-HPLC and GC-MS. *Food Chem* 95:44–52
- Rebensburg S, Helfer M, Schneider M, Koppensteiner H, Eberle J, Schindler M, Gürtler L, Brack-Werner R (2016) Potent *in vitro* antiviral activity of *Cistus incanus* extract against HIV and Filoviruses targets viral envelope proteins. *Sci Rep* 6: Art No 20394
- Seifert G, Jeitler M, Stange R, Michalsen A, Cramer H, Brinkhaus B, Esch T, Kerckhoff A, Paul A, Teut M, Ghadjar P, Langhorst J, Häupl T, Murthy V, Kessler CS (2020) The relevance of complementary and integrative medicine in the COVID-19 pandemic: a qualitative review of the literature. *Front Med* 7: Art No 587749
- Şener B (2020) Antiviral activity of natural products and herbal extracts. *Gazi Med J* 31:474–477
- Stamatis G, Kyriazopoulos P, Golegou S, Basayiannis A, Skaltsas S, Skaltsa H (2003) *In vitro* anti-*Helicobacter pylori* activity of Greek herbal medicines. *J Ethnopharmacol* 88:175–179
- Stange R, Uehleke B (2020) Covid-19: considerations on requirements to recommend herbal preparations. *Zeitschr Phytother* 41:160–164. (Letter to the Editor)
- Strid A (1986) Mountain Flora of Greece, vol 1. Cambridge University Press, Cambridge
- Strid A (2016) Atlas of the Aegean Flora. Part 1: text & plates. Part 2: maps. Botanic Garden and Botanical Museum Berlin, Berlin
- Strid A, Tan K (1991) Mountain flora of Greece, vol 2. Edinburgh University Press, Edinburgh
- Strid A, Tan K (1997) Flora Hellenica 1. Koeltz Scientific Books, Königstein
- Strid A, Tan K (2002) Flora Hellenica 2. Koeltz Scientific Books, Ruggell
- Szeremeta D, Knaś M, Długosz E, Krzykała K, Mrozek-Wilczkiewicz A, Musioł R, Kowalska T, Ott PG, Sajewicz M, Móricz ÁM (2018) Investigation of antibacterial and cytotoxic potential

- of phenolics derived from *Cistus incanus* L. by means of thin-layer chromatography-direct bioautography and cytotoxicity assay. *J Liq Chromatogr Relat Technol* 41:349–357
- Tadic VM, Jeremic I, Dobric S, Isakovic A, Markovic I, Trajkovic V, Bojovic D, Arsic (2012) Anti-inflammatory, gastroprotective, and cytotoxic effects of *Sideritis scardica* extracts. *Planta Med* 78:415–427
- Tisserand R, Young R (2014) Essential oil safety a guide for health care professional, 2nd edn. Churchill Livingstone, Edinburgh
- Todorova M, Trendafilova A (2014) *Sideritis scardica* Griseb., an endemic species of Balkan peninsula: traditional uses, cultivation, chemical composition, bioactivity. *J Ethnopharmacol* 152:256–265
- Tseliou M, Pirintsos SA, Lionis C, Castanas E, Sourvinos G (2019) Antiviral effect of an essential oil combination derived from three aromatic plants (*Coridothymus capitatus* (L.) Rchb. f., *Origanum dictamnus* L. and *Salvia fruticosa* Mill.) against viruses causing infections of the upper respiratory tract. *J Herb Med* 17-18:100288
- Varsani M, Graikou K, Velegraki A, Chinou I (2017) Phytochemical analysis and antimicrobial activity of *origanum dictamnus* traditional herbal tea (decoction). *Nat Prod Commun* 12:1801–1804
- Vassilopoulou CG, Kontogianni VG, Linardaki ZI, Iatrou G, Lamari FN, Nerantzaki AA, Gerothanassis IP, Tzakos AG, Margarity M (2013) Phytochemical composition of “mountain tea” from *Sideritis clandestina* subsp. *clandestina* and evaluation of its behavioural and antioxidant effects on adult mice. *Eur J Nutr* 52:107–116
- Viapiana A, Konopacka A, Waleron K, Wesolowski M (2017) *Cistus incanus* L. commercial products as a good source of polyphenols in human diet. *Ind Crops Prod* 107:297–304
- Vokou D, Varelzidou S, Katinakis P (1993) Effects of aromatic plants on potato storage - sprout suppression and antimicrobial activity. *Agric Ecosyst Environ* 47:223–235
- Wittpahl G, Kölling-Speer I, Basche S, Herrmann E, Hannig M, Speer K, Hannig C (2015) The Polyphenolic composition of *Cistus incanus* herbal tea and its antibacterial and anti-adherent activity against *Streptococcus mutans*. *Planta Med* 81:1727–1735
- World Health Organization [WHO] (2002) WHO traditional medicinal strategy 2002–2005. World Health Organization, Geneva
- Yaneva I, Balabanski V (2013) History of the uses of Pirin mountain tea (*Sideritis scardica* Griseb). *Bulg J Publ Health* 1:48–57. (in Bulgarian)

Chapter 7

The Spectrum of Berberine Antibacterial and Antifungal Activities



Ivan Kosalec, Maja Jazvinščak Jembrek, and Josipa Vlainić

Abstract Isoquinoline alkaloid berberine is a typical multitarget or multicomponent molecule of botanical origin. It is extensively used for hundreds of years in the treatment of various infectious diseases and in traditional medicine. Numerous studies from academic institutions and pharma industry reported several pharmacological effects and efficacy of berberine in the treatment of inflammation, cancer, and diabetes. The plethora of beneficial activities rely on the existence of multiple mechanisms of its action, mainly related to cell cycle arrest and apoptosis. Considering its antifungal and antibacterial activities, berberine targets bacterial cell walls and cell membranes, as well as DNA and RNA. It disturbs membrane potential and rigidity by forming reactive oxygen species (ROS). The influence on protein expression is evident in affecting efflux pumps. Berberine also potentiates activity of several antibiotics and antifungal drugs, demonstrating synergistic antibacterial activity, and has detrimental effect on biofilm matrix of several pathogens, including multidrug-resistant (MDR) bacteria and yeasts. In this chapter, we focus on the antimicrobial effects of berberine and plausible mechanisms of actions involved, and give future perspective on the potential of berberine-based therapy.

Keywords Antibacterial · Antifungal · Mechanism · Berberine · Multidrug resistance

Abbreviations

ALP	Alkaline phosphatase
ATCC	American Type Culture Collection

I. Kosalec (✉)
University of Zagreb, Faculty of Pharmacy and Biochemistry, Institute for Microbiology,
Zagreb, Croatia
e-mail: ikosalec@pharma.hr

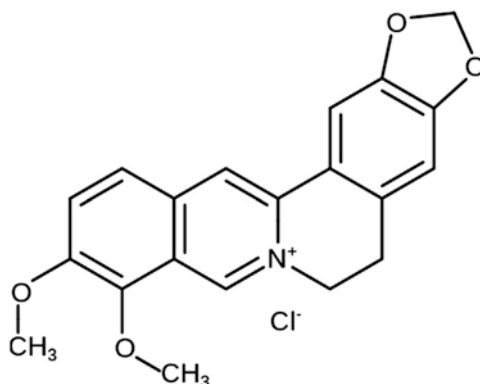
M. J. Jembrek · J. Vlainić (✉)
Ruđer Bošković Institute, Zagreb, Croatia
e-mail: josipa.vlainic@irb.hr

cAMP-PKA	Cyclic adenosine monophosphate–dependent protein kinase
CYP51	Fungal sterol 14 α -demethylase
DNA	Deoxyribonucleic acid
ERG	ETS (erythroblast transformation-specific)-related gene
ESKAPE pathogens	<i>Enterococcus faecium</i> , <i>Staphylococcus aureus</i> , <i>Klebsiella pneumoniae</i> , <i>Acinetobacter baumannii</i> , <i>Pseudomonas aeruginosa</i> , and <i>Enterobacter</i> species
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH/GSSH	Reduced glutathione/oxidized glutathione ratio
HSF1	Heat shock factor 1
LC-MS/MS	Liquid chromatography–tandem mass spectrometry
MAPK	Mitogen-activated protein kinase
MDR	Multidrug resistant
MIC	Minimal inhibitory concentration
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MSSA	Methicillin-sensitive <i>Staphylococcus aureus</i>
NAcDG	N-acetyl-D-glucosamine
ROS	Reactive oxygen species
SEM	Scanning electron microscopy
SOD	Superoxide dismutase
TEM	Transmission electron microscopy
YPD	Yeast extract–peptone–dextrose

1 Introduction

Centuries ago there was a search for plants that could solve medical problems. Similar problems we have nowadays, and still investigate many phytomolecules, rediscover their pharmacological and biological effectiveness, resolve mechanisms of their action, and finally introduce (or try to do so) them into drug list. One of them is berberine, a typical isoquinoline type of alkaloids, chemically entitled 5,6-dihydro-9,10-dimethoxybenzo(g)-1,3-benzodioxolo(5,6-a) quinolizinium. It belongs to the protoberberine group of alkaloids, forming salts with mineral acids (hydrogen chloride, sulfuric acid). It is isolated mainly from *Coptis* spp. (Ranunculaceae), *Berberis* spp. (Berberidaceae), *Argemone* spp. (Papaveraceae), *Zanthoxylum* L. (Rutaceae), and *Zanthoxylum* spp. (Rutaceae) using different isolation techniques, where the reaction of interconversion of protoberberine and base is of major importance (Singh and Katare 2020). The long ethnobotanical use of herbal species containing high concentrations of berberine engaged a huge number of scientific studies, published from the early 1960s till nowadays (Gaoi et al. 2020). Berberine-rich herbals are considered generally nontoxic and are used as alternative treatments for various antimicrobial and noninfectious diseases, and in Traditional Chinese Medicine (TCM) as official drug for treatment of diarrhea and

Fig. 7.1 Chemical structure of berberine



detoxification. The official Chinese Pharmacopoeia includes the *Cortex phellandria* and *Rhizoma coptidis*, which are rich in berberine for removing “heat” during fever (Huang and Williams 1999) (Fig. 7.1).

Berberine has many therapeutic niches and its potential against various diseases has been subject of many researches. Berberine has been widely studied for its anti-inflammatory potential (Yao et al. 2015). On the other hand, berberine may act as an antiproliferative agent against cells involved in the reinitiation and progression of rheumatoid arthritis. In particular, berberine effectively triggers apoptosis following the disruption of mitochondrial membrane potential, increase in the expression of proapoptotic proteins, and activation of caspase-3, caspase-9, and poly (ADP-ribose) polymerase (Wang et al. 2011). Berberine inhibits the proliferation of several cell lines by affecting cell cycle progression, induces apoptotic death by activating caspases, induces autophagy, inhibits expression of proteins involved in tumor metastasis and cell invasion, and regulates inflammatory response of an organism (for review, see Wang et al. 2020). As an inhibitor of inflammatory processes, berberine upregulates transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) and AMP-activated protein kinase/the mechanistic target of rapamycin (AMPK/mTOR) signaling pathway in macrophages, and downregulates production of inflammatory cytokines such as *tumor necrosis factor- α* (TNF- α), *interleukin-1 β* (IL-1 β), and interleukin-6 (IL-6) (Fan et al. 2015). All mentioned events make berberine a candidate for immunotherapy. Berberine has been also shown to have antioxidant activity, since it suppresses generation of ROS and upregulates superoxide dismutase (SOD) levels in neuronal tissue (Zhuang et al. 2018). Similar has been shown in mice intestinal tissue where berberine increased catalase and glutathione peroxidase (GPx) activity (Guna et al. 2018). Antiobesity and anti-dyslipidemia efficacy of berberine has been studied and proposed mechanism of action is related to the inhibition of mitochondrial function, stimulation of glycolysis, activation of AMPK pathway, suppression of adipogenesis, and induction of low-density lipoprotein (LDL) receptor expression (Lee et al. 2006).

One of the most studied effects of berberine is its antimicrobial potential. The extensive research was carried out on antimicrobial activity of berberine

(antibacterial, antifungal, antiviral, tuberculostatic) as preclinical step of drug-candida development, and these studies are good starting point for clinical type studies. The aim of this chapter is to summarize the recent progress in antifungal and antibacterial researches of berberine. Data presented will be mainly focused on the mechanisms of antifungal and antibacterial activities of berberine.

2 Antifungal Activity of Berberine

Opportunistic fungal infections, and among them invasive *Candida* infections, especially in immunocompromised hosts, have brought resistance to existing therapeutics, pointing to the need for the discovery and development of new antifungal drugs. Global rise in antifungal resistance makes fungal infections harder to treat, and the search for novel agents that are effective, both at planktonic phase and at biofilm layers, and safe for an organism, is an urgent demand. As mentioned previously, berberine, the isoquinoline alkaloid derived from many plants, has multiple biological effects (Imenshahidi and Hosseinzade 2019), including the antifungal activity. Berberine can act not only on the outer parts of a cell (e.g., cell wall and cell membrane), but also on all intracellular organelles. Almost linear increment of fluorescence intensity inside fungal cell was observed along with the gradual increase of the dose of berberine applied (accumulation of berberine is a dose-dependent in *Candida* cells) (Zorić et al. 2016). Although studies on the activity of berberine are not numerous, its antifungal activity has been demonstrated at relatively low doses, which are nontoxic to humans. Research was mainly conducted on *Candida* species, both regular and MDR species (Jantová et al. 2003; Kyoumi et al. 1990). The study of Xiao and coauthors (2014) also showed antifungal activity of berberine against *T. mentagrophytes* both *in vitro* and in clinical setting, and PAS experiments demonstrated that berberine chloride may cure fungal disease in rabbits as well (Xiao et al. 2014).

3 Mechanisms of Berberine Action

Once antifungal properties of berberine have been established, potential mechanisms of its actions need to be addressed in future studies. As with other natural products, there are probably multiple sites of action and several mechanisms involved in the antifungal effect of berberine.

It has been reported that fungal invasion is facilitated by the transition between yeast cells and filamentous growth, which occurs in response to external stimuli including temperature or pH change, nitrogen and/or carbon starvation, and the presence of the host macrophages (Midkiff et al. 2011). Our group showed that

berberine was less effective in yeast extract–peptone–dextrose (YPD) media supplemented with 10% fetal bovine serum (FBS), suggesting that serum constituents may affect tested compound or interfere with its action. The most prominent effect of berberine was observed at subminimal inhibitory concentration (MIC) during culturing in N-acetyl-D-glucosamine (NACDG) containing media in which mitogen-activated protein kinase (MAPK) pathways of morphogenesis were triggered. Berberine also inhibited germ-tube formation of *C. albicans* cells in sub-MIC in Spider's medium where the transition was mediated by cAMP-protein kinase A (PKA) pathway. Namely, inhibition of germ-tube formation of *C. albicans* by berberine was stronger at sub-MIC concentration in media where MAPK and cAMP-PKA pathways of budded-to-hypha transition were employed. On the other hand, in Lee's media (Cph2 to Tec1 regulation of hyphal transition) and in YPD media with addition of 10% of FBS, the effect of berberine was less pronounced. Taken together, the interference of berberine with the metabolic pathways of yeast is possible (Zorić et al. 2017).

Results show that berberine impairs mitochondrial function, enhances generation of ROS, targets cell wall and cell membrane, and is able to affect heat shock factor 1 (HSF1). Our group (Zorić et al. 2017) showed that following berberine treatment, membranes of *C. albicans* were damaged, leading to the leakage of cell content (DNA and proteins) in a time- and dose-dependent manner, whereas in a culture of fluconazole-resistant *Candida* and *Cryptococcus neoformans*, berberine caused alterations of the yeast mitochondrial transmembrane potential that further may lead to transient pore openings and the release of mitochondrial proapoptotic factors into the cytosol (da Silva et al. 2016). Li et al. (2017) demonstrated that berberine causes cell cycle arrest in *C. albicans* cells. Morphological nuclear changes, for example, DNA fragmentation and damage, were also associated with the action of berberine in *C. albicans* cells. Studies suggested that berberine can bind to DNA, cause DNA replication and transcription arrest, and affect the cell cycle (Bhadra and Kumar 2011). Although studies indicate that the main type of *C. albicans*-induced death following berberine treatment is apoptosis, reports also show that accumulation of berberine inside mitochondria and consequential arrest of proliferation, mitochondrial fragmentation, and oxidative stress induction may initiate other death pathways. Studies of its antitumor effect revealed significant inhibition of HT-29 cell growth due to cell growth arrest, with no apoptosis induction (Su et al. 2015) (Fig. 7.2).

Transcription regulator HSF1 protects cells from thermal assault by activating the expression of heat shock proteins (HSPs) that act as molecular chaperones involved in the adaptation of *Candida* cells not only during stress (e.g., thermal stress), but also under basal conditions (Nicholls et al. 2011). In yeasts, HSF1 binding was observed on several genes responsible for oxidative and osmotic stress response, cell wall integrity, iron homeostasis, and mitochondrial, hyphal, and multidrug transporters, some of which are essential for fungal viability and virulence. The exact mechanism by which berberine interacts with HSF1 is not clear yet, but certainly, several signaling pathways have been involved. Namely, studies have shown that fluctuations in ergosterol, which maintains membrane fluidity and is

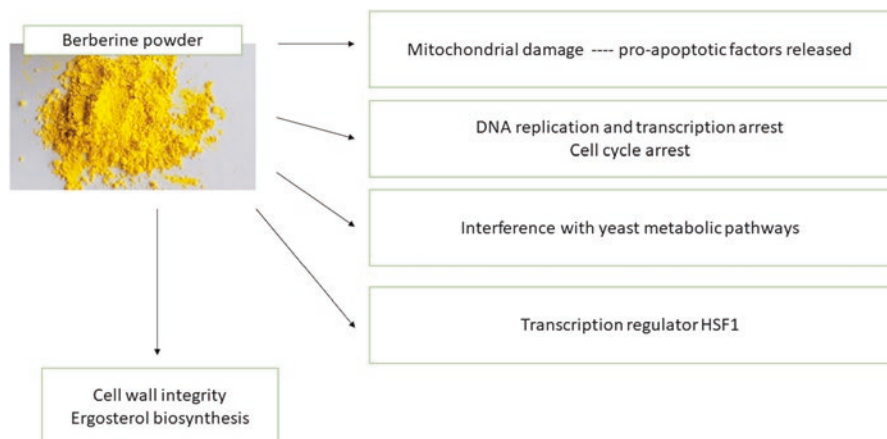


Fig. 7.2 Antifungal properties – berberine targets

involved in membrane lipid arrangement, are also associated with the reciprocal regulation of two major genes *ERG3* and *ERG11*, whereas the promoters of *ERG3* and *ERG11* possess multiple putative HSF1-binding sites (Nair et al. 2017). Iron (and other essential metals) limitations enhance the drug susceptibility of yeast cells, which may also be linked to defective mitochondria, since there is a reduction in iron-binding proteins involved in respiratory process of a yeast. In addition, HSF1 levels are associated with cell wall integrity, since HSF1 mutants are susceptible to cell wall inhibitors and this effect is independent of calcineurin stress pathway in *Candida* cells (Dhamgaye et al. 2014). Transcriptome analyses have shown that expression of genes involved in metabolic pathways, biosynthesis of secondary metabolites, and microbial metabolism are changed following berberine treatment in *T. mentagrophytes* (Xiao et al. 2019). The study revealed downregulation of an important ergosterol precursor 14 α -demethylase that belongs to the cytochrome P450 (*CYP51*) superfamily of fungal metabolic proteins, thus indicating potential blockage of ergosterol biosynthesis by berberine.

One of the proposed mechanisms of berberine's antifungal action is a dose-dependent generation of ROS by berberine itself (Zorić et al. 2017). Many living organisms can adapt antioxidant defense system following stress exposure by activation of antioxidant enzymes, whereas levels of antioxidant enzymes are usually decreased due to apoptotic process (Dhamgaye et al. 2014). Study proved that antioxidant mechanism may be activated in *Candida*. This results in upregulation of the production of antioxidant enzymes as a result of defensive system utilization due to noxious environment (Dhamgaye et al. 2014). This was also the case when cells were treated with berberine (overexpression of *SOD* mRNA and enhancement of *SOD* activity), probably as a result of cellular damage prevention induced by berberine-mediated ROS increase (Poopedi et al. 2020). In the same study, authors showed that this mechanism could be important in overcoming the drug resistance of *Candida*, as disruption of antioxidant defensive system in MDR strains could

support our fight against them. On the other hand, although GPx is the most prominent antioxidant enzyme in *C. albicans* cells responsible for reducing H₂O₂ and overcoming detrimental effects of oxidant events in a cell, increased GPx activity only partially represents the antioxidant defense response against berberine-induced stress. Whether catalase and its activity are increased or reduced following treatment of *C. albicans* cells with different oxidants and antifungal drugs must be further clarified, since current research gives two contradictory outcomes that may result from the differences caused by different exposure period, lower number of viable cells following treatment (sub-MIC and MIC doses), or the use of different oxidizing agents. In a study where berberine at sub-MIC concentrations was applied, the importance of increased glutathione reductase (GR) activity was emphasized in the maintenance of glutathione (GSH) level as well as GSH/GSSG ratio (Garcerá et al. 2010). Differential expression profiling of genes involved in oxidative stress in *Candida* species was confirmed by microarray analysis (Hua et al. 2017). Despite all defense mechanisms activated, *C. albicans* is not resistant to robust oxidative stress induced by berberine, even at sub-MIC level (Zorić et al. 2017).

Although the vast majority of studies on the antifungal action of berberine was focused on the effects on *Candida albicans*, similar effectiveness was observed for other *Candida* species e.g., *C. tropicalis* and *C. glabrata* (Nakamoto et al. 1990).

4 Antibacterial Activity of Berberine

The antibacterial activity of berberine was proved long ago, mainly using the *in vitro* approach. The results showed wide range of MICs, which were influenced by bacterial species and strain tested, and antimicrobial susceptibility method used as well. A recent survey of literature presented as a bibliometric review from 1985 to 2018 reveals a great interest in clinical usage of berberine in watery diarrhea, but not only due to its direct antibacterial activity against *Vibrio cholerae*. Potential clinical application of berberine is also based on the mechanisms of inhibition of intestinal smooth muscle movements, regulation of motility, inhibition of intestinal mucosa K⁺ influx, and restoration of intestinal barrier function (Gaoi et al. 2020). Recent data on *in vitro* selective inhibitory activities against intestinal bacteria showed that berberine is selectively active against *Clostridium perfringens* (MIC 256 µg/mL), *Salmonella typhimurium* (512 µg/mL), and *Vibrio parahaemolyticus* (512 µg/mL). However, the berberine also showed cytotoxic activity against cancer cell lines (HT29, Caco-2) with average IC₅₀ value 12.2 ± 7 µg/mL and normal intestinal cell line FHs 74 Int with IC₅₀ 1.0 ± 0.1 µg/mL (selective index -1.1) (Kudera et al. 2020). Berberine also has an influence on endospore formation and their germination. Based on the studies performed on spores of *Bacillus subtilis* and *Clostridioides* (ex *Clostridium*) *difficile*, berberine demonstrated interesting influence on bacterial spores of these two medicinally important Gram-positive pathogens. Berberine did not affect germination of *B. subtilis* and *C. difficile*, but however MICs were 256 µg/mL against *B. subtilis* and 640 µg/mL against *C. difficile*. Berberine acts as spores

inhibitor by accumulating in spores soon after germination is initiated (Wang et al. 2016). Wang and coauthors (2016) demonstrated that berberine not only has bactericidal activity toward vegetative cells of *B. subtilis* and *C. difficile*, but also exerts activity on spore outgrowth by inhibiting protein synthesis during outgrowth phase. Thus, positive effect of berberine on the inhibition of germinated spores could be considered as beneficial, because some toxins are synthesized during spore germination (Wang et al. 2016).

Although a large body of studies showed potent antimicrobial activity of berberine against both Gram-positive and Gram-negative bacterial species (Singh and Katare 2020), recently, the promising new area of research is to elucidate the underlying mechanisms of antimicrobial activity focusing on the inhibition of the following:

1. Bacterial adhesion to epithelial cells, as the first step in infection.
2. Biofilm formation.
3. Efflux pumps and downregulation of genes involved in efflux-pump expression.

Berberine also acts synergistically when used simultaneously with some antibiotics.

All these newly recognized activities suggest that berberine is a multitarget-active molecule of natural origin.

5 Mechanisms of Antibacterial Activity

Berberine (in the form of sulfate) inhibited (acted bacteriostatic) the *in vitro* growth of *Streptococcus pyogenes* at concentrations ≥ 30 $\mu\text{g/mL}$, and early works were performed to determine the inhibitory effects of berberine on adherence. Sun et al. (1988) showed that berberine blocks adhesion of *Streptococcus pyogenes* to the fibronectin and hexadecane on epithelial cells in sub-MIC concentrations. The authors also found that berberine increases release of lipoteichoic acid. On the same bacterial model, Du et al. (2020) found that berberine stimulates excessive production of ROS. Consequently, berberine suppresses DNA, protein, and fatty acid synthesis as determined by proteomic analysis or using liquid chromatography–mass spectrometry (LC-MS/MS) (Du et al. 2020). Berberine hydrochloride also inhibited DNA synthesis by affecting the activity of DNA topoisomerase on the model of *Streptococcus agalactiae* (Peng et al. 2015). On the other hand, He and Yin (2015) found that berberine caused cytoplasm pyknosis and bacterial death by using transmission electron microscopy (TEM) and SDS-PAGE/DAPI analysis on the model of *Actinobacillus pleuropneumoniae*, a Gram-negative bacterium from the pig's lungs. Peng et al. (2015) noticed that berberine causes damage of outer bacterial membrane, but could not suggest the specific target site(s). Berberine in MIC value (128 $\mu\text{g/mL}$) strongly interacts with bacterial membrane, damaging it with K^+ and alkaline phosphatase (ALP) leakage from intracellular space to outside on MRSA as a model strain (Zhang et al. 2020). Even at half of MIC value, morphological changes could be observed using scanning electron microscope (SEM) and TEM,

Table 7.1 Some of the approaches to reveal antibacterial activity of berberine

Model	Methodological approach	Reference
<i>S. aureus</i> CCTCC AB 9105	Metabolite profiles using HPLC/ESI-MS	Yi et al. (2007)
<i>S. aureus</i> ATCC 25923	Global transcriptional patterns	Wang et al. (2008)
MRSA ATCC 33591	Biofilm formation via extracellular amyloid fibril	Chu et al. (2016)
MRSA 252	K ⁺ and ALP leakage and morphological alternations in cell membrane	Zhang et al. (2020)

thus confirming the cause of K⁺ and ALP leakage to extracellular space (Zhang et al. 2020).

An important antivirulence target in Gram-positive bacteria are sortase enzymes, a family of transpeptidases important for anchoring the virulence-associated proteins to the cell wall. Berberine chloride (at 20 µg/mL) acts as an inhibitor of *S. aureus* adhesion to fibronectin by inhibiting sortase isoforms SrtA and SrtB (Oh et al. 2006).

By using kinetic study in time by time-kill curves, it is possible to determine bacteriostatic or bactericidal effects of berberine in bacterial models. Applied at 1× and 2× of MIC value on *Streptococcus agalactiae*, berberine hydrochloride directly caused decline phase, without the appearance of adjustment phase, log-phase, and stable phase (Peng et al. 2015). At the concentration 1× and 2× MIC, all bacterial cells of *Streptococcus agalactiae* were killed within 4 h. MIC of berberine was 0.78 µg/mL (Peng et al. 2015).

As a very important human pathogen, especially because of its resistant phenotype (MRSA), several studies used *Staphylococcus aureus* as a model to elucidate the mechanisms of antibacterial activity of berberine. Table 7.1 represents methodological approaches for the detection of the target sites of berberine action after treatment of *S. aureus* bacterial cells. After acquiring metabolites profile using high-performance liquid chromatography electrospray ionization mass spectrometry (HPLC/ESI-MS), Yi et al. (2007) elucidated the very close mechanism of berberine bactericidal activity to rifampicin and norfloxacin, whose targets are RNA polymerase, gyrase, and topoisomerase IV. The authors suggested that target site for antistaphylococcal activity is nucleic acid. Like the above-mentioned results on bacterial model, Yadav et al. (2005) found that berberine chloride strongly interacts with binding to the single-stranded poly(rA) structure in eukaryotic mRNA, causing inhibition of protein synthesis, as proved in S180 tumor cell line (Creasey 1979). As it is known, resistant phenotype of *S. aureus* MRSA causes high morbidity and mortality in infected patients, especially in those with comorbidities. The detected MIC value using berberine on the MRSA ATCC 33591 was 128 µg/mL, demonstrating strong bacteriostatic effects (Chu et al. 2016). Berberine also inhibited formation of MRSA biofilm in a dose-dependent manner and decreased MRSA density at concentrations between 16 and 64 µg/mL. Moreover, berberine below 32 µg/mL decreased number of amyloid-fibril-producing colonies, suggesting that it inhibits biofilm formation by disturbing aggregation of phenol-soluble modulins into the

biofilm matrix (Chu et al. 2016). Berberine also inhibited adhesion of MRSA cell to human gingival fibroblasts, and consequently inhibited the first step in biofilm formation (Yu et al. 2005). Exposure of *S. aureus* ATCC 25923 cells to sub-MIC concentrations of berberine chloride revealed interesting data regarding down- and upregulated genes after transcriptional profiles analysis (Wang et al. 2008). Following exposure to berberine, expression of a large number of putative transporter genes was upregulated in *S. aureus* cells. These include genes involved in multidrug resistance and toxin extrusion, and biocides and fluoroquinolone class of antibiotics transporters, among others (Wang et al. 2008).

6 Synergism with Antimicrobial Drugs

One of the promising strategies to combat MDR bacterial strains is combinational use of antibiotic with molecules, which possess synergistic activity with antibiotics and thus potentiate their activity on specific molecular targets. It is plausible that synergistic molecules possess activity different from classical antibiotic targets, or display activity toward virulence factors of pathogen (inhibition of toxin production, efflux-pumps, adhesion, capsule formation, etc.). The synergistic effect of berberine in combination with several antibiotics is shown in Table 7.2.

Berberine acts as a small molecule that can restore effectiveness of some β -lactam antibiotics, such as oxacillin and ampicillin, against MRSA, by inhibiting adhesion of MRSA cells. Furthermore, berberine inhibits intracellular invasion into human gingival fibroblasts (Yu et al. 2005). In the study of Yu et al. (2005), berberine lowered MICs of oxacillin and ampicillin against MRSA and MSSA with positive additive effects for both strains. The berberine chloride also acts synergistically with linezolid, ceftioxin, and erythromycin in $\frac{1}{4}$ MIC value against coagulase-negative *Staphylococci*, namely, *Staphylococcus epidermidis*, *S. haemolyticus*, *S. capitis* subs. *capitis*, *S. gallinarum*, *S. hominis* subsp. *hominis*, *S. intermedius*, and *S. lugdunensis* (Wojtyczka et al. 2014). The authors suggested

Table 7.2 Overview of synergistic effects of berberine with antibiotics

Antibiotic	Microbial species	Reference
Oxacillin	MRSA	Yu et al. (2005)
Ampicillin		Yu et al. (2005)
Fusidic acid	MRSA	Liang et al. (2014)
Linezolid	Coagulase-negative <i>Staphylococci</i>	Wojtyczka et al. (2014)
Ceftioxin		
Erythromycin		
Penicillin	<i>Staphylococcus epidermidis</i>	Zhou et al. (2015)
Lincomycin		
Amoxicillin		
Azithromycin	<i>Pseudomonas aeruginosa</i>	Li et al. (2017)

berberine is capable to augment the antibacterial activity of some antibiotics, thus providing more effective therapies (Wojtyczka et al. 2014). Among the coagulase-negative *Staphylococci*, *S. epidermidis* has a crucial place as it is a leading cause of hospital-acquired infections and mastitis in veterinary medicine (Zhou et al. 2015). Moreover, *S. epidermidis* has high-level intention to develop resistance to antibiotics and biocides (Becker et al. 2014; Oliviera et al. 2018). On the model of MDR strain *S. epidermidis*, Zhou et al. (2015) demonstrated the synergistic effects of berberine in combination with penicillin, lincomycin, or amoxicillin.

The effectiveness of berberine in combination with fusidic acid was also explored *in vitro* against MRSA (Liang et al. 2014). Liang et al. (2014) demonstrated synergistic antimicrobial interaction between fusidic acid and berberine in a fusidic acid-resistant MRSA bacterial strain. Authors also found that berberine in combination with fusidic acid destroyed mature biofilm in culture of fusidic acid-resistant MRSA strain.

The Gram-negative bacteria in the group of ESKAPE bacteria, especially *Pseudomonas aeruginosa*, are one of the most prominent MDR strains (De Oliveira et al. 2020a, b). The antimicrobial activity of berberine and synergism with antibiotics are less studied among Gram-negative bacteria. Using various *in vitro* antimicrobial approaches, Li et al. (2017) found that berberine potentiates activity of azithromycin against clinical isolates of azithromycin-resistant *P. aeruginosa* strain. Further studies revealed that berberine attenuated production of *P. aeruginosa* virulence factors, such as LasA/LasB proteases, pyoverdine, pyocyanin, chitinase, and alginate production (Li et al. 2017). Authors also demonstrated promising synergistic effect of berberine and azithromycin on biofilm-destruction activity in *P. aeruginosa*.

Furthermore, it has been reported that berberine acts in synergism with amphotericin B in the treatment of candidiasis in mice. When combined with fluconazole against *Candida albicans* resistant to fluconazole, berberine damaged membrane and fluconazole was able to enter the cell and exert its activity. Fluconazole-resistant clinical isolates of *C. albicans* exposed to berberine in combination with fluconazole collapsed and showed deep surface folds, even at concentrations of both drugs that *per se* showed no intrinsic activity (Iwazaki et al. 2010).

7 Conclusion

Berberine, an isoquinoline alkaloid, is a very promising compound of natural origin for future clinical studies. In order to introduce berberine to broader use, as it has many beneficial effects, additional efforts are needed, of both, scientific community and legislative. Namely, a long ethnobotanical history of its medicinal use and the research conducted give strong evidence on its effectiveness, in many medicinal applications. Studies also showed antimicrobial potential (here reviewed antifungal and antibacterial) of berberine, with molecular targets revealed (cell wall and membrane, cell cycle, mitochondria, etc.). Molecular events induced by berberine in bacteria and fungi lead to apoptosis, without affecting healthy human cells (use as

wound dressing). Also, a large body of preclinical data on antifungal and antibacterial activities, especially those that are focused on mechanisms at cellular and molecular level, could be an incentive for further *in vivo* studies and clinical trials that will reveal the full potential of berberine as an antimicrobial agent, or as a synergistic activator of other medicinal products.

References

- Becker K, Heilmann C, Peters G (2014) Coagulase-negative staphylococci. *Clin Microbiol Rev* 27:870–926
- Bhadra K, Kumar GS (2011) Therapeutic potential of nucleic acid binding isoquinoline alkaloids: binding aspects and implications for drug design. *Med Res Rev* 31:821–862
- Chu M, Zhang MB, Liu YC, Kang JR, Chu ZY, Yin KL, Ding LY, Ding R, Xiao RX, Yin YN, Liu XY, Wang YD (2016) Role of berberine in the treatment of methicillin-resistant *Staphylococcus aureus* infections. *Sci Rep* 22:24748
- Creasey WA (1979) Biochemical effects of berberine. *Biochem Pharmacol* 28:1081–1084
- da Silva AR, de Andrade Neto JB, da Silva CR, Campos RDS, Costa Silva RA, Freitas DD, do Nascimento FBSA, de Andrade LND, Sampaio LS, Grangeiro TB, Magalhães HIF, Cavalcanti BC, de Moraes MO, Nobre Júnior HV (2016) Berberine antifungal activity in fluconazole-resistant pathogenic yeasts: action mechanism evaluated by flow cytometry and biofilm growth inhibition in *Candida* spp. *Antimicrob Agents Chemother* 60:3551–3557
- De Oliveira DMP, Forde BM, Kidd TJ, Harris PNA, Schembri MA, Beatson SA, Paterson DL, Walker MJ (2020a) Antimicrobial resistance in ESKAPE pathogens. *Clin Microbiol Rev* 13:e00181–e00119
- De Oliveira DMP, Forde BM, Kidd TJ, Harris PNA, Schembri MA, Beatson SA, Paterson DL, Walker MJ (2020b) Antimicrobial resistance in ESKAPE pathogens. *Clin Microbiol Rev* 33:e00181–e00119
- Dhamgaye S, Devaux F, Vandeputte P, Khandelwal NK, Sanglard D, Mukhopadhyay G et al (2014) Molecular mechanisms of action of herbal antifungal alkaloid berberine, in *Candida albicans*. *PLoS One* 9:e104554. <https://doi.org/10.1371/journal.pone.0104554>
- Du G-F, Le Y-J, Sun X, yang X-Y, He Q-Y (2020) Proteomic investigation into the cation mechanism of berberine against *Streptococcus pyogenes*. *J Proteome* 215:103666
- Fan X, Wang J, Hou J et al (2015) Berberine alleviates ox-LDL induced inflammatory factors by up-regulation of autophagy via AMPK/mTOR signaling pathway. *J Transl Med* 13:92. <https://doi.org/10.1186/s12967-015-0450-z>
- Gaoi Y, Wang F, Song Y, Liu H (2020) The status of and trends in the pharmacology of berberine. A bibliometric review [1985-2018]. *Chin Med* 15:7
- Garcerá A, Casas C, Herrero E (2010) Expression of *Candida albicans* glutathione transferases is induced inside phagocytes and upon diverse environmental stresses. *FEMS Yeast Res* 10:422–431
- Guna V, Saha L, Bhatia A et al (2018) Anti-oxidant and anti-apoptotic effects of berberine in pentylenetetrazole-induced kindling model in rat. *J Epilepsy Res* 8(2):66–73. <https://doi.org/10.14581/jer.18011>
- He C, Yin L (2015) The antibacterial mechanism of berberine against *Actinobacillus pleuropneumoniae*. *Nat Prod Res* 29:2203–2206
- Hua X, Chi W, Su L, Li J, Zhang Z, Yuan X (2017) ROS-induced oxidative injury involved in pathogenesis of fungal keratitis via p38 MAPK activation. *Sci Rep* 7(1):10421. <https://doi.org/10.1038/s41598-017-09636-w>
- Huang KC, Williams WM (1999). Antibacterial, antiviral, and antifungal herbs) The pharmacology of Chinese herbs. CRC Press, New York, pp 381–383

- Imenshahidi M, Hosseinzade H (2019) Berberine and barberry (*Berberis vulgaris*): a clinical review. *Phytother Res* 33:504–523. <https://doi.org/10.1002/ptr.6252>
- Iwazaki RS, Endo EH, Ueda-Nakamura T, Nakamura CV, Garcia LB, Filho BP (2010) In vitro antifungal activity of the berberine and its synergism with fluconazole. *Antonie Van Leeuwenhoek* 97:201–205
- Jantová S, Cipák L, Cernáková M, Kost'álová D (2003) Effect of berberine on proliferation, cell cycle and apoptosis in HeLa and L1210 cells. *J Pharm Pharmacol* 55:1143–1149
- Kudera T, Doskocil I, Salmonova H, Petryl M, Skrivanova E, Kokoska L (2020) In vitro selective growth-inhibitory activities of phytochemicals, synthetic phytochemical analogs, and antibiotics against diarrheagenic/probiotic bacteria and cancer/normal intestinal cells. *Pharmaceuticals (Basel)* 13:233. <https://doi.org/10.3390/ph13090233>
- Kyouri N, Shinsuke S, Taizo H (1990) Effects of crude drugs and berberine hydrochloride on the activities of fungi. *J Prosthet Dent* 64:691–694
- Lee YS, Kim WS, Kim KH, Yoon MJ, Cho HJ, Shen Y, Ye JM, Lee CH, Oh WK, Kim CT, Hohnen-Behrens C, Gosby A, Kraegen EW, James DE, Kim JB (2006) Berberine, a natural plant product, activates AMP-activated protein kinase with beneficial metabolic effects in diabetic and insulin-resistant states. *Diabetes* 55(8):2256–2264. <https://doi.org/10.2337/db06-0006>
- Li Y, Huang J, Li L, Liu L (2017) Synergistic activity of berberine with azithromycin against *Pseudomonas aeruginosa* isolated from patients with cystic fibrosis of lung *in vitro* and *in vivo*. *Cell Physiol Biochem* 2017:1657–1669
- Liang R, Yong X, Duan Y, Tan Y-h, Zeng P, Zhou Z-y, Jiang Y, Wang S-h, Jiang Y-p, Huang X-c, Dong Z-h, Hu T-t, Shi H-q, Li N (2014) Potent in vitro synergism of fusidic acid (FA) and berberine chloride (BBR) against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA). *World J Microbiol Biotechnol* 30:2861–2869
- Midkiff J, Borochoff-Porte N, White D, Johnson DI (2011) Small molecule inhibitors of the *Candida albicans* bud-to-hyphal transition act through multiple signalling pathways. *PLoS One*. <https://doi.org/10.1371/journal.pone.0025395>
- Nair R, Shariq M, Dhamgaye S, Mukhopadhyay CK, Shaikh S, Rajendra P (2017) Non-heat shock responsive roles of HSF1 in *Candida albicans* are essential under iron deprivation and drug defense. *Biochim Biophys Acta Mol Cell Res* 1864:345–354
- Nakamoto K, Sadamori S, Hamada T (1990) Effects of crude drugs and berberine hydrochloride on the activities of fungi. *J Prosthet Dent* 64:691–694
- Nicholls S, MacCallum DM, Kaffarnik FAR, Selway L, Peck SC, Brown AJP (2011) Activation of the heat shock transcription factor Hsf1 is essential for the full virulence of the fungal pathogen *Candida albicans*. *Fungal Genet Biol* 48:297–305
- Oh K-B, Oh M-N, Kim J-G, Shin D-S, Shin J (2006) Inhibition of sortase-mediated *Staphylococcus aureus* adhesion to fibronectin via fibronectin-binding protein by sortase inhibitors. *Appl Microbiol Biotechnol* 70:102–106
- Oliveira WF, Silva PMS, Silva RCS et al (2018) *Staphylococcus aureus* and *Staphylococcus epidermidis* infections on implants. *J Hosp Infect* 98:111–117
- Peng L, Kang S, Yin Z, Jia R, Song X, Li L, Li Z, Zou Y, Liang X, Li L, He C, Ye G, Yin L, Shi F, Lv C, Jing B (2015) Antibacterial activity and mechanism of berberine against *Streptococcus agalactiae*. *Int J Clin Exp Pathol* 1:5217–5223
- Poopedi E, Marimani M, AlOmar SY, Aldahmash B, Ahmad A (2020) Modulation of antioxidant defence system in response to berberine in *Candida albicans*. *Yeast*:1–13. <https://doi.org/10.1002/yea.3531>
- Singh B, Katara AK (2020) Botanical sources, chemistry aspects and biological functions of berberine: an updated critical review. In: Singh B (ed) *Botanical leads for drug discovery*. Springer Nature Ltd, Singapore
- Su Y-H, Tang W-C, Cheng Y-W, Sia P, Huang C-C, Lee Y-C, Jiang H-Y, Wu M-H, Lai I-L, Lee J-W et al (2015) Targeting of multiple oncogenic signaling pathways by Hsp90 inhibitor alone or in combination with berberine for treatment of colorectal cancer. *Biochim Biophys Acta Mol Cell Res* 1853:2261–2272

- Sun D, Courtney HS, Beachey EH (1988) Berberine sulfate blocks adherence of streptococcus pyogenes to epithelial cells, fibronectin, and hexadecane. *Antimicrob Agents Chemother* 32:1370–1374
- Wang D, Yu L, Xiang H, Fan J, He L, Guo N, Feng H, Deng X (2008) Global transcriptional profiles of *Staphylococcus aureus* treated with berberine chloride. *FEMS Microbiol Lett* 279:217–225
- Wang X, Jiang S, Sun Q (2011) Effects of berberine on human rheumatoid arthritis fibroblast-like synoviocytes. *Exp Biol Med* 236(7):859–866. <https://doi.org/10.1258/ebm.2011.010366>
- Wang S, Setlow B, Setlow P, Yong-qing L (2016) Uptake and levels of the antibiotic berberine in individual dormant and germinating *Clostridium difficile* and *Bacillus cereus* spores as measured by laser tweezers Raman spectroscopy. *J Antimicrob Chemother* 71:1540–1546
- Wang Y, Liu Y, Du X, Ma H, Yao J (2020) The anti-cancer mechanisms of berberine: a review. *Cancer Manag Res* 12:695–702. <https://doi.org/10.2147/CMAR.S242329>
- Wojtyczka RD, Orlewska K, Kępa M, Idzik D, Dziedzic A, Mularz T, Krawczyk M, Mikłasińska M, Wąsik TJ (2014) Biofilm formation and antimicrobial susceptibility of *Staphylococcus epidermidis* strains from a hospital environment. *Int J Environ Res Public Health* 11:4619–4633
- Xiao CW, Ji QA, Rajput ZI, Wei Q, Liu Y, Bao GL (2014) Antifungal efficacy of *Phellodendron amurense* ethanol extract against *Trichophyton mentagrophytes* in rabbits. *Pak Vet J* 34:219–223
- Xiao CW, Liu Y, Wei Q et al (2019) Inhibitory effects of berberine hydrochloride on *Trichophyton mentagrophytes* and the underlying mechanisms. *Molecules* 24(4):742. <https://doi.org/10.3390/molecules24040742>
- Yadav RC, Kumar GS, Bhandra K, Giri P, Sinha R, Pal S, Maiti M (2005) Berberine, a strong polyriboadenylic acid binding plant alkaloid: spectroscopic, viscometric, and thermodynamic study. *Bioorg Med Chem* 13:165–174
- Yao J, Kong W, Jiang J (2015) Learning from berberine: treating chronic diseases through multiple targets. *Sci China Life Sci* 58(9):854–859. <https://doi.org/10.1007/s11427-013-4568-z>. PMID: 24174332
- Yi Z-B, Yu Y, Liang Y-Z, Zeng B (2007) Evaluation of the antimicrobial mode of berberine by LC/ESI-MS combined with principal component analysis. *J Pharm Biomed Anal* 44:301–304
- Yu HH, Kim KJ, Cha JD, Kim HK, Lee YE, Choi NY, You YO (2005) Antimicrobial activity of berberine alone and in combination with ampicillin or oxacillin against methicillin-resistant *Staphylococcus aureus*. *J Med Food* 8:454–461
- Zhang X, Sun X, Wu J, Wu Y, Wang Y, Hu X, Wang X (2020) Berberine damages the cell surface of methicillin-resistant *Staphylococcus aureus*. *Front Microbiol* 22:621
- Zhou X, Yang C, Li Y, Liu X, Wang Y (2015) Potential of berberine to enhance antimicrobial activity of commonly used antibiotics for dairy cow mastitis caused by multiple drug-resistant *Staphylococcus epidermidis* infection. *Genet Mol Res* 14:9683–9692
- Zhuang W, Li T, Wang C, Shi X, Li Y, Zhang S, Zhao Z, Dong H, Qiao Y (2018) Berberine exerts antioxidant effects via protection of spiral ganglion cells against cytomegalovirus-induced apoptosis. *Free Radic Biol Med* 121:127–135. <https://doi.org/10.1016/j.freeradbiomed.2018.04.575>. PMID: 29715550
- Zorić N, Kopjar N, Bobnjarić I, Horvat I, Tomić S, Kosalec I (2016) Antifungal activity of Oleuropein against *Candida albicans*-the in vitro study. *Molecules* 21:1631. <https://doi.org/10.3390/molecules21121631>
- Zorić N, Kosalec I, Tomić S, Bobnjarić I, Jug M, Vlainić T, Vlainić J (2017) Membrane of *Candida albicans* as a target of berberine. *BMC Complement Altern Med* 17:268. <https://doi.org/10.1186/s12906-017-1773-5>

Part II
Antimicrobial Agents/Secondary
Metabolites

Chapter 8

Plants to Drugs: A Case Study of Human Papilloma Virus and Traditional Chinese Medicine



Mohammed Rahmatullah, Taufiq Rahman, Anamul Hasan, Rownak Jahan, Md Shahadat Hossan, Khoshnur Jannat, and Tohmina Afroze Bondhon

Abstract Various types of viral diseases are emerging as the largest menace human beings have faced in the last few decades. Since the arrival of human immunodeficiency virus, the world has seen the emergence of deadly viruses like bird flu, Ebola, Nypah, Hanta, SARS, MERS, and currently the SARS-CoV-2. Other viral diseases like herpes, human papilloma virus, and hepatitis have become so common that despite their widespread infection rates, causes of liver and cervical cancer and consequent mortalities, they have not caught the attention of the general people in a way SARS-CoV-2 has done. Unlike small pox, polio, several types of hepatitis, and, to a certain extent, HPV, most other viral diseases have proved difficult to cure with vaccines or drugs. As with many other diseases, plants can form a possible source of therapeutics for HPV. There are around 250,000 species of flowering plants in the world; each species contain a range of phytochemicals with diverse pharmacological activities. For instance, over four dozen plants have been identified with antiviral activity against herpes virus, while a number of other plants and phytochemicals have shown promise against various viruses. Promising antiviral phytochemicals include coumarins, terpenoids, flavonoids, polyphenols, and alkaloids. This chapter will attempt to summarize the present state of knowledge regarding plants, formulations, and phytochemicals (against HPV) and discuss the potential of drug discovery from the promising phytochemicals.

Keywords Human papilloma virus · Traditional chinese medicine · Phytochemicals · Cervical cancer · Anti-HPV patents

M. Rahmatullah (✉) · A. Hasan · R. Jahan · K. Jannat · T. A. Bondhon
Department of Biotechnology & Genetic Engineering, University of Development
Alternative, Dhaka, Bangladesh

T. Rahman
Department of Pharmacology, University of Cambridge, Cambridge, UK

M. S. Hossan
School of Pharmacy, University of Nottingham, Nottingham, UK

Abbreviations

CIN	Cervical intraepithelial neoplasias/dysplasias
CIS	Carcinoma <i>in situ</i>
COVID-19	Corona virus–induced disease 2019
COX-2	Cyclooxygenase-2
DLA	Dalton’s lymphoma ascites
DNA	Deoxyribonucleic acid
E6AP	E6-associated protein
HPV	Human papilloma virus
HR-HPV	High-risk HPV
HSV	Herpes simplex virus
hTERT	Human telomerase reverse transcriptase
IFN- γ	Interferon- γ
LCR	Long control region
LR-HPV	Low-risk HPV
MAP kinase	Mitogen-activated protein kinase
ORF	Open reading frame
Rb	Retinoblastoma protein
RNA	Ribonucleic acid
SARS-CoV-2	Severe acute respiratory syndrome-corona virus-2
SJAMP	Marine japonicus polysaccharide
Sp1	Specificity protein 1
Tan IIA	Tanshinone IIT
T-bet	T-box transcription factor
Th1	T helper type 1 cells
Th2	T helper type 2 cells
TMP	Tetra- <i>O</i> -methyl nordihydroguaiaretic acid
VEGF	Vascular endothelial growth factor

1 Introduction

It has been said that interaction between viruses and humans has shaped human evolution (Leal and Zanotto 2000). Viruses like human papilloma virus (HPV) and herpes simplex virus (HSV) may have coevolved with humans since ancient times (Ong et al. 1993; McGeogh et al. 1995). In recent years, it has been reported that 219 virus species can infect humans (Woolhouse et al. 2012). In fact, viruses constitute more than two-thirds of all new pathogens that infect humans (Woolhouse and Gaunt 2007). Among the emerging viruses, zoonotic viruses play a major role in causing both human fatalities and worldwide economic disruptions. The latest virus causing the present pandemic (COVID-19) is a zoonotic virus (SARS-CoV-2), which was initially transmitted to humans from bats through a still undetermined animal species. A partial list of the more known viruses currently affecting humans is shown in Table 8.1.

Table 8.1 Selected viruses (with family) and diseases caused by them in humans

Virus family (characteristic)	Virus	Disease caused and symptoms
DNA viruses		
Adenoviridae (NE, DS linear)	Human adenoviruses A-F	Respiratory and/or ocular disease. According to American Thoracic Society, the viruses can cause a variety of illnesses such as upper and lower respiratory infections, gastrointestinal infection, neurological infection, and eye infection (Dela Cruz et al. 2019)
Hepadnaviridae (E, partial DS circular)	Hepatitis B virus (Z, possibly bat origin)	Chronic infection of liver leading to liver damage, cirrhosis, and liver cancer. Nonspecific symptoms in acute infections can be fatigue, poor appetite, nausea, vomiting, abdominal pain, low-grade fever, jaundice, and dark urine (Wilkins et al. 2010)
Herpesviridae (E, DS linear)	Human Herpes simplex virus 1	Herpetic gingivostomatitis, herpes labialis (sores around the mouth and lips, also called cold sores) (Mustafa et al. 2016)
	Human Herpes simplex virus 2	Genital herpes (clusters of inflamed papules and vesicles on the outer surface of the genitals resembling cold sores) (Mustafa et al. 2016)
	Human Herpes virus 3 or varicella-zoster virus	Chicken pox, shingles (Gebreyohannes 2014)
	Human Herpes virus 4 or Epstein Barr virus	Infectious mononucleosis, Burkitt's lymphoma, Hodgkin's lymphoma, stomach cancer, nasopharyngeal carcinoma, multiple sclerosis, and lymphomatoid granulomatosis, chronic fatigue syndrome and disorders of the immune system (Gebreyohannes 2014)
	Human Herpes virus 5 or human cytomegalovirus (CMV)	Significant morbidity, including low birth weight, hearing loss, visual impairment, microcephaly, hepatosplenomegaly, and varying degrees of mental retardation. CMV infection is strongly correlated with asymptomatic vascular diseases such as heart, coronary artery, and atherosclerosis (Gebreyohannes 2014)
	Human Herpes viruses 6, 7 and 8	Roseola infantum, pityriasis rosea, lichen planus, hypersensitivity reactions, graft-vs-host disease, and multiple other cutaneous manifestations (viruses 6 and 7); Kaposi sarcoma (virus 8) (Gebreyohannes 2014)
Papovaviridae (NE, DS circular)	Human papilloma virus (HPV)	Genital warts and warts in the throat (known as recurrent respiratory papillomatosis). Persistent infections can develop into anogenital warts, precancers, and cervical, anogenital, or oropharyngeal cancers in women and men. <i>The virus</i> can also cause cancers of the head and neck (Brianti et al. 2017)
Parvoviridae (NE, SS linear)	Adeno-associated viruses 1–6	Activate proto-oncogenes in human hepatocellular carcinoma (Berns et al. 2015)
	B-19 virus	The virus causes “fifth disease,” a mild rash illness that affects children and occasionally adults. It can also cause severe anemia and painful or swollen joints (Heegaard and Brown 2002)

Table 8.1 (continued)

Virus family (characteristic)	Virus	Disease caused and symptoms
Poxviridae (E, DS linear)	Bovine papular stomatitis virus (Z)	Localized skin lesions. Mainly affects cattle from which it is transmitted to human beings (Temizel 2015)
	Contagious ecthyma or contagious pustular dermatitis or orf virus (Z)	Cattle disease but can cause orf in humans following transmission from cattle. Orf is characterized by small, red, itchy, or painful lump (lesion) that usually appears on the fingers, hands, forearms, or face (Taghipour et al. 2015)
	Cowpox virus (Z)	Viral skin infection in cattle, from which it can be transmitted to humans via contact with infected teats of milking cows
	Monkeypox virus (Z)	A milder form of small pox, possibly transmitted to humans by rodents and squirrels in the rain forests of Africa
	Pseudo cowpox virus (milker's nodules) (Z)	Mild infection of udders and teats in cows, from which it is commonly transmitted to ranchers, milkers, and veterinarians
	Small pox virus (variola)	Cause of small pox; a contagious, disfiguring, and often deadly disease, which has been eradicated
	Vaccinia virus (Z)	Transmitted from cattle to humans, the viral disease is characterized by focal red skin areas, fever, and general symptoms similar to those of a cold. It mostly occurs in Brazil (Silva et al. 2010)
RNA viruses		
Arenaviridae (E, SS linear segments)	Lassa virus (Z)	Causes Lassa fever, an acute viral hemorrhagic illness, transmitted through infected African mouse, <i>Mastomys natalensis</i> . Prevalent in West African countries. The virus causes multisystemic dysfunction through infecting every tissue of the body (Azeez-Akande 2016)
	Lymphocytic choriomeningitis virus (Z)	Rodent-borne viral infectious disease that appears as aseptic meningitis, encephalitis, or meningoencephalitis. Initial phase symptoms are fever, malaise, lack of appetite, muscle aches, headache, nausea, and vomiting, followed by second phase symptoms, which may consist of meningitis (fever, headache, stiff neck, etc.), encephalitis (drowsiness, confusion, sensory disturbances, and/or motor abnormalities, such as paralysis), or meningoencephalitis (inflammation of both the brain and meninges) (Bonthius 2012)
	Machupo virus (Bolivian hemorrhagic fever) (Z)	Preliminary symptoms of Bolivian hemorrhagic fever are fever, headache, fatigue, myalgia, and arthralgia. In some patients, hemorrhagic signs develop, including bleeding from nasal and oral mucosa, as well as the bronchopulmonary, gastrointestinal, and genitourinary tracts. <i>Calomys callosus</i> , a forest rodent, is the primary host of the virus (Kilgore et al. 1997)
Astroviridae (NE, SS linear)	Human astroviruses 1–8	Major cause of diarrhea in the young and the elderly. Recently, the virus has been linked to encephalitis and meningitis (Johnson et al. 2017)

Table 8.1 (continued)

Virus family (characteristic)	Virus	Disease caused and symptoms
Bunyaviridae (E, SS linear segments)	Cache valley virus	Can cause an illness with fever and in more severe cases encephalitis or meningitis. Transmitted by mosquitoes to human from infected sheep (Waddell et al. 2019)
	California encephalitis virus (Z)	Belongs to the California encephalitis virus group, which includes California encephalitis virus, La Crosse virus, and Jamestown Canyon virus in USA and Tahyna virus in Russia. California encephalitis and La Crosse virus causes encephalitis in children; Jamestown Canyon virus affects elderly individuals. Symptoms of encephalitis in children include fever, headache, vomiting, seizures, and altered mental status. These viruses infect rabbits, squirrels, and chipmunks and are transmitted by the mosquito <i>Aedes triseriatus</i> (Newhouse et al. 1963)
	Crimean-Congo hemorrhagic fever virus (Z)	Causes severe viral hemorrhagic fever with case fatality rates up to 40%. Transmitted by ticks from livestock animals like cattle, sheep, and goats. Endemic in Africa, the Balkans, the Middle East, and Asian countries south of the 50th parallel north (Flick and Whitehouse 2006)
	Hantaviruses (several serotypes) (Z)	Viral infection can cause hemorrhagic fever with renal syndrome or pulmonary syndrome. Original host found to be infected field rodent <i>Apodemus agrarius</i> near Hantan river in South Korea. Later on, it was discovered that the common deer mouse (<i>Peromyscus maniculatus</i>) was also an agent in USA (Máttar et al. 2015)
	Jamestown Canyon virus (z)	See California encephalitis virus (above)
	La Crosse virus (Z)	See California encephalitis virus (above)
	Nairobi sheep disease virus (Z)	Tick-borne virus in sheep and goats. Can cause a mild influenza-like disease in humans
	Rift Valley fever virus (Z)	Occurs in livestock. Transmitted to humans through mosquitoes causing mild flu-like illness to severe hemorrhagic fever (Bird et al. 2009)
Calciviridae (NE, SS linear)	Hepatitis E virus (Z) genotypes 1–7	Causes inflammation of liver. Depending on genotype, it can also cause acute pancreatitis, glomerulonephritis, and severe thrombocytopenia. Zoonotic Hepatitis E virus (HEV) genotype HEV-3 has been found among pigs and wild boars in Europe, whereas zoonotic genotype HEV-4 is more common in pigs in some Asian countries (Kantala and Maunula 2018)
	Noroviruses (Norwalk and Norwalk-like viruses)	Causes vomiting, diarrhea, nausea, and stomach pain (Hardy 1999)

Table 8.1 (continued)

Virus family (characteristic)	Virus	Disease caused and symptoms
	Vesicular exanthema of swine virus (Z)	Vesicular exanthema of swine virus is clinically indistinguishable from vesicular disease caused by foot-and-mouth disease virus, and vesicular stomatitis virus. Occurs in pigs and marine animals. Occasionally isolated from humans with blisters
Coronaviridae (E, SS linear)	Human coronaviruses (colds)	Four coronaviruses, namely, HCoV-OC43, H-CoV-229E, H-CoV-NL63, and HCoV-HKU1, cause common cold in children and elderly people (Tyrrell and Bynoe 1965; Hamre and Procknow 1966; van der Hoek et al. 2004; Woo et al. 2005)
	Severe Acute Respiratory Syndrome (SARS) coronavirus (Z)	Emerged in Guangdong Province in China in November 2002. Possible mode of transmission from bats to humans was through masked palm civets (<i>Paguma larvata</i>) and the raccoon dog (<i>Nyctereutes procyonoides</i>). The major clinical features include persistent fever, chills/rigor, myalgia, malaise, dry cough, headache, dyspnea, and diarrhea (Hui 2005)
	Middle East Respiratory Syndrome (MERS) coronavirus (Z)	Emerged in Saudi Arabia and other Middle Eastern countries in 2012. Possible mode of transmission from bats to humans was through camels or goats. Causes mild to severe pneumonia often accompanied by acute respiratory distress syndrome (ARDS), renal failure, pericarditis, and disseminated intravascular coagulation (DIC) (Mortazavi et al. 2014)
	Severe Acute Respiratory Syndrome (SARS) coronavirus (SARS-CoV-2)	Emerged in Wuhan, China in late December of 2019. Possible mode of transmission is from bats to humans through a yet-to-be-identified host, pangolins being the most suspected. The initial clinical features include fever, cough, shortness of breath, and muscle ache. These can progress to ARDS and multiple organ dysfunction leading to death (Tu et al. 2020)
Filoviridae (E, SS linear)	Ebola virus (Z)	First reported in the Democratic Republic of Congo in 1976. Mode of transmission to humans seems to be from fruit bats to simians to humans. Initial features of the disease include high fever, headache, vomiting, anorexia, diarrhea, and aching muscles. Advanced stages of the virus-induced disease include unexplained bleeding in the eyes, nose, gums, and gut (Kimura et al. 2015)
	Marburg virus (Z)	The first case was reported in 1967. Initial mode of transmission was possibly from the Egyptian fruit bat (<i>Rousettus aegyptiacus</i>) to African green monkeys to humans. The disease can initially start with abrupt onset of high fever, severe headache, and severe malaise followed by severe watery diarrhea, abdominal pain with cramping, nausea, and vomiting. Many patients develop severe hemorrhagic manifestations later as the disease progresses (Asad et al. 2020)

Table 8.1 (continued)

Virus family (characteristic)	Virus	Disease caused and symptoms
Flaviviridae (E, SS linear)	Dengue virus (Z)	The virus can circulate in both human and nonhuman primates and is mosquito-transmitted, particularly the <i>Aedes aegypti</i> species. Other <i>Aedes</i> species, which can transmit, include <i>Aedes albopictus</i> , <i>Aedes polynesiensis</i> , and <i>Aedes scutellaris</i> . Symptoms of dengue include sudden onset of fever, headache (typically located behind the eyes), muscle and joint pain, and rashes. Nausea, vomiting, and hemorrhage occur in some patients (Singh et al. 2015)
	Hepatitis C virus (HCV)	25–30% of those infected may develop symptoms like fever, jaundice, and abdominal pain. 15–20% of people with chronic hepatitis may develop cirrhosis leading to hepatocellular carcinoma. HCV can lead to extrahepatic diseases like diabetes mellitus, cryoglobulinemia, non-Hodgkin's B cell lymphoma, membranoproliferative glomerulonephritis, lichen planus, and porphyria cutanea tarda (Millman et al. 2017)
	Japanese encephalitis virus (Z)	Japanese encephalitis virus (JEV) is related to dengue virus (DENV), West Nile virus (WNV), Zika virus (ZIKV), and tick-borne encephalitis virus (TBEV). Transmitted by mosquitoes from migrating birds like herons, egrets, and ducks, which may be asymptomatic carriers. Wild and domesticated pigs also play a role in the infection chain (Filgueira and Lannes 2019)
	Louping ill virus (Z)	Tick-borne pathogen causing illness in sheep (<i>Ovis aries</i>) and red grouse (<i>Lagopus lagopus scoticus</i>) and occasionally humans (Gilbert 2016)
	Murray valley encephalitis virus (Z)	Related to the Kunjin virus, it is found in northeastern Australia and Papua New Guinea. The major vertebrate hosts are herons and egrets, particularly the Nankeen night heron (<i>Nycticorax caledonicus</i>). Transmitted through <i>Aedes aegypti</i> mosquitoes. Clinical symptoms include febrile illness with headache, myalgia, and occasional rash. Clinical encephalitis occurs in a few cases with disease varying from fever, headache, and altered mental state to coma and severe flaccid paralysis (Mackenzie et al. 2017)
	Omsk hemorrhagic fever virus (Z)	Omsk hemorrhagic fever virus (OHFV) was first reported from Omsk oblast (province) in Russia. The virus is transmitted to humans from infected muskrats (<i>Ondatra zibethicus</i>) via ticks belonging to the species, <i>Dermacentor reticulatus</i> . The initial symptoms are chills, fever, headache, and severe muscle pain with vomiting, gastrointestinal symptoms, and bleeding problems, which may be followed by inflammation of the brain (encephalitis) (Gould and Solomon 2008)

Table 8.1 (continued)

Virus family (characteristic)	Virus	Disease caused and symptoms
	St. Louis encephalitis virus (Z)	The virus was isolated for the first time from St. Louis, Missouri, USA. <i>Culex</i> mosquitoes are vectors and birds serve as hosts. Clinical manifestations include nonspecific febrile syndrome to febrile headache, aseptic meningitis, and encephalitis (Ortiz-Martínez et al. 2017)
	Tick-borne encephalitis viruses (several subtypes) (Z)	Diseases caused by these virus subtypes range throughout Europe and the northern parts of Asiatic Russia. The principal vector is the tick, <i>Ixodes ricinus</i> , the intermediary hosts being various types of rodents and carnivores. The different subtypes differ in the development of various disease forms (febrile, meningeal, meningoencephalitic, polyencephalitic, poliomyelitic, poliomyelitic, poliomyelitic) and the extent of severity of these disease forms (Donoso-Mantke et al. 2011)
	Yellow fever virus (Z)	The disease is spread by <i>Aedes</i> mosquitoes with primates as hosts and is prevalent in Africa, Central and South American countries. The viral disease symptoms include asymptomatic to severe clinical signs such as muscle pain with a prominent backache, headache, loss of appetite and nausea or vomiting, jaundice dark urine and abdominal pain, and kidney and liver failure (Mulatu and Feyisa 2018)
	Wesselsbron virus (Z)	Wesselsbron virus (WSLV) was first detected in South Africa. The virus is mosquito-transmitted and its natural hosts include camels, cattle, pigs, donkeys, and horses besides wild animals like South African zebras and wild ruminants in Chad and possibly black rats (<i>Rattus rattus</i>). The disease is essentially confined to southern African countries. In humans, the infection causes arthralgia, myalgia, and fever during a short and mild acute phase (Diagne et al. 2017)
	West Nile virus (Z)	The virus is transmitted from passerine birds (like American robin or <i>Turdus migratorius</i>) by <i>Culex</i> species of mosquitoes (<i>Culex pipiens</i> , <i>Culex quinquefasciatus</i> , and <i>Culex tarsalis</i>). Infected humans can develop fever and neuroinvasive diseases (Petersen et al. 2013)
Orthomyxoviridae (E, SS linear segments)	Influenza viruses A (Z), B (Z) and C	Aquatic birds are the main carriers of the virus A and B. Host ranges of influenza virus A are humans, swine, equine, avian, canine, and marine mammals; that of B is humans only; for C the host range includes humans, swine, and canine. 500 million people die from influenza (flu) every year. Flu symptoms include fever, dry cough, sore throat, headache, fatigue, and body ache; similar symptoms are also seen in SARS-CoV-2-induced viral disease, COVID-19. Majority of human infections are caused by Type A and B viruses (Regea 2017)

Table 8.1 (continued)

Virus family (characteristic)	Virus	Disease caused and symptoms
	Avian influenza (Z)	All avian influenza viruses are Type A influenza viruses. Their hosts range from various avian species to mammalian species like swine, ferrets, mink, felids, horses, seals, whales, civets, dogs, and humans. The highly pathogenic avian influenza (HPAI) H5N1 strain is of concern to humans worldwide for its ability to create an epidemic or a pandemic (Kelly et al. 2008)
	Swine influenza (Z)	The classical swine influenza virus is a Type A (H1N1) virus, first discovered in 1930 in swine. Swine influenza can be caused by other subtypes like H1N1, H1N2, H2N3, H3N1, and H3N2. The virus can infect humans, the clinical symptoms being aches and fever, upper respiratory symptoms, weakness, and gastrointestinal disorders (Rajesh et al. 2011)
Paramyxoviridae (E, SS linear)	Hendra virus (Z)	All four species of Australian flying foxes (<i>Pteropus</i> spp.) also known as fruit bats are the natural host. Their transmission of the virus to horses is not clear. Humans can get infected through coming in contact with body fluid of horses (Hess et al. 2011)
	Human parainfluenza viruses (HPIV) 1–4	Can infect other animals apart from humans. Causes both lower respiratory infection (LRI) and upper respiratory infection (URI) (Henrickson 2003)
	Nipah virus (Z)	<i>Pteropus</i> spp. bats are the natural hosts. Can be transmitted to humans through pigs and horses. Direct transmission has also been reported from bats to humans when humans drank raw date palm sap contaminated by bats. Initial symptoms are fever, headache, and myalgia. Features of encephalitis can develop after that, the most common symptoms being altered mental status, limb weakness, hypotonia, and segmental myoclonus (Aditi and Shariff 2019)
	Respiratory syncytial virus (RSV)	The virus is the leading cause of lower respiratory tract infections in infants (Meng et al. 2014)
Togaviridae (E, SS linear)	Chikungunya virus (CHIKV) (Z)	First reported from Tanzania in 1953. Primates can be hosts of the virus. Transmitted by <i>Aedes aegypti</i> and <i>Aedes albopictus</i> mosquitoes. Symptoms begin with high fever. Arthralgia and rash, which can progress to rheumatic symptoms and gastrointestinal disorders (Runowska et al. 2018)

E enveloped, NE nonenveloped, DS double stranded, SS single stranded, Z zoonotic

A total of 16 virus families (6 DNA virus families and 10 RNA virus families) comprising of 17 DNA virus species and 39 RNA virus species out of 219 species are shown in Table 8.1. The surprising thing about viral diseases is that most of them lack therapeutics in the form of vaccines or drugs. Because of space constraints of the chapter, only one virus will be discussed. We have chosen to review one DNA virus

(human papilloma virus or HPV): the basis for our selection is that unlike the widely known current COVID-19 pandemic, cervical cancer due to HPV infection is a “silent” pandemic in the sense that it is the fourth most common malignant tumor affecting women in the world with around 265,672 deaths annually according to one report (Bruni et al. 2019). Another report attributes 4.5% of new cancer cases (640,000 cases) diagnosed in the world to persistent HPV infections (de Sanjosé and Tsu 2020).

Human papilloma virus has caused 79 million infections in USA alone and 14 million more are infected every year. HPV mostly affects women of underdeveloped countries. There are more than 40 sexually transmitted types of HPV affecting the epithelial lining of the anogenital tract and other mucosal areas of the human body (Findik et al. 2019). HPV causes anogenital cancers, cervical cancers, precancerous anogenital lesions, genital warts, and even can cause head and neck cancers (de Sanjosé and Tsu 2020). However, HPV infections are curable, even cervical cancer, if detected in the early stages although allopathic drugs or other measures like surgery for treatment of HPV infections may have adverse effects.

The outline followed in the present review for HPV is that of (i) describing any traditional medicinal treatments for HPV and/or its symptoms, (ii) identifying any medicinal plants and their phytochemicals responsible for their possible antiviral activities, and (iii) discussing the drug potential of phytochemicals or crude extracts of the plant(s) as shown by actual clinical trials. From scientific literature searches, it was concluded that the focus should be on traditional chinese medicines (TCMs) for TCM only showed a high diversity of plant-based formulations and potentially more promising sources of novel anti-HPV agents.

We have based our searches in the scientific literature on PubMed, SCOPUS, and Google Scholar using the terms [ethnomedicine, viral disease, or its symptoms], [phytochemicals, botanical name of the plant], [botanical name of the plant, review], [botanical name of the plant, antiviral activity], [ethnomedicine, antiviral plants], and other combinations of the above terms.

2 Human Papilloma Virus (HPV)

Human papilloma virus (HPV) is a double-stranded closed circular DNA genome virus. The virus is enveloped by a capsid containing two proteins L1 and L2. Each capsid is composed of 72 capsomeres, and each capsomere in turn is composed of five monomeric proteins of 55Kda each, forming a pentamer (Sapp et al. 1995). The HPV genome is divided into three distinct regions: the first comprises of a long control region (LCR) responsible for the regulation of transcription of E6 and E7 viral genes; the second region is an early region (E), consisting of six ORFs: E1, E2, E4, E5, E6, and E7; and the third region is a late (L) region that encodes the L1 and L2 structural proteins (Fehrmann and Laimins 2003; Jo and Kim 2005). Besides L1 and L2, the virus contains several other proteins E1–E7 of varying functions, one of the important E proteins being E6 against which most in silico inhibitor studies have been carried out. The E6 protein is responsible for binding and degrading the tumor-suppressor protein p53, inhibiting

apoptosis, interacting with proteins responsible for innate immune response (initial response to pathogens), contributing to immune evasion and persistence of virus, and activating the expression of telomerase (and so contributing to cancer).

HPV infection is common and has been reported to be detectable at least once in the lifetime of sexually active people (Park et al. 2015). There are two types of HPVs, the high-risk HPV (HR- HPV) and low-risk HPV (LR-HPV). In most cases HPV infections disappear without any interventions; in some cases of HPV 6, 11, 12, 13, 15, 32, 34, 40, 42, 43, 44, 53, 54 (LR-HPVs), infections may cause genital warts (condylomata acuminata with itching), benign papilloma, precancerous lesions, and if allowed to progress may lead to cancer. Types 6 and 11 HPVs cause genital warts in more than 90% of the cases (Gómez et al. 2012; Menéndez et al. 2004). Genital warts are one of the most common sexually transmitted diseases in the world with an estimated 600 million individuals believed to be affected. Lesions can form on the penis, vulva, scrotum, perineum, the perianal area, cervix, urethra, anus, mouth, and also in the conjunctiva, nose, and larynx (Centers for Disease Control and Prevention 2002). HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 70 (HR-HPVs) can cause cancer of cervix, vulva, vagina, penis, anus, and oropharynx, head and neck carcinomas, and contribute to over 40% of oral cancers (Forman et al. 2012; Stanley 2010), but according to Timmons et al. (2010), they cause cancer most frequently of the uterine cervix. HPV 16, 31, 32, 34, 35, 37, 42 cause Bowenoid papillosis (Zanotti and Belinson 2002). Pathogenesis of HPV and related symptoms has been reviewed by Wang et al. (2014b).

All cervical intraepithelial dysplasias (also neoplasias or CIN) are linked with certain types of cervical HPV infection. Cervical dysplasia is when there are abnormal or precancerous cells in and around a woman's cervix, cervix being the lower part of the uterus. It is well established that invasive carcinoma of cervix is preceded by a precursive lesion that morphologically resembles adjacent invasive squamous carcinomas (when the lesion has grown and progressed to the point where it has breached, penetrated, and infiltrated adjacent structures), and which lesion is termed "carcinoma *in situ*" (CIS). CIS is preceded by a spectrum of lesions that vary in degrees of abnormality with CIN 1 (cervical intraepithelial neoplasia 1) to CIN 3 ranging from small to highest levels of abnormality.

Various allopathic procedures exist to combat the HPV infections, like antiviral drugs (cidofovir), immunoenhancers (imiquimod, interferon), cytotoxic agents (5-fluorouracil), photodynamic therapy, vaccines, and surgery. All of these treatments have adverse effects or only result in partially successful treatment. Success of surgical excision of HPV-induced lower genital tract neoplasia largely depends on secondary prevention programs; chemoradiation therapy does not result in improved prognosis in about a third of cervical cancer patients; antiviral drugs, topical agents, or photodynamic therapy is somewhat successful (50–60%) in treatment of high-grade vulvar intraepithelial neoplasia (Stem et al. 2012). But the expenses associated with these allopathic methods make traditional medicines a possibly better approach, with traditional chinese medicine (TCM) playing a major role in HPV treatment. On the other hand, TCM has the disadvantage of not being known widely throughout the world and a sort of "look-down" approach from a section of scientists and allopathic doctors as to their real or perceived toxicity.

3 Traditional Chinese Medicine (TCM) Against HPV

The findings of medical research on some TCM formulations and isolated compounds have been listed (Lin et al. 2017). Essentially, traditional Chinese formulations against HPV infections can be divided into three types: internal applications taken in oral form, external applications applied on the body surface, and a combination of internal and external applications. Not all the formulations act directly on the virus; some are immunoenhancers, some strengthen the spleen or other body organs, and some are heat-clearing and detoxifying. In other words, various TCM formulations act against both HPV (anticancer, antiviral) and its symptoms, and can be both preventive and therapeutic. A number of TCM polyherbal and monoherbal formulations with their constituents and effects are shown in Table 8.2. For the sake of clarity, Table 8.2 will also give the botanical name(s) of the herbs used in the formulations. Promising phytochemicals and formulations in the light of modern scientific studies will be discussed.

4 Plant-Based Anti-HPV Formulations

HPV can cause cancer; however, before it reaches the precancerous stage and gradually progresses to cancer, HPV can cause cervical dysplasia, which, if left untreated, progresses to precancer and then cancer. TCM has formulations for various stages of HPV-induced cervical dysplasia as well as cervical cancer. In this chapter, the TCM medications for cervical dysplasia and cancer will not be differentiated; not only they will be grouped together but TCM medications discussed or given in the chapter will cover other symptoms associated with both cervical dysplasia and cervical cancer. Despite the quite large number of TCM plant-based formulations given in Table 8.2, the list is exclusive; there are other formulations, some of which will be discussed here along with an integrated medicine approach to treat HPV (combination of TCM with modern drugs). A number of them have undergone scientific testing with encouraging results. For instance, *Pinellia* extract fraction (extract of rhizomes of *Pinellia ternata* (Thunb.) Ten. ex Breitenb. (Crow dipper in English, Ban Xia in Chinese) has been shown to downregulate HPV E6 and upregulate p53 in CaSki (epidermoid cervical cancer cell line) and HeLa (human cervical cancer) cells (Li et al. 2012). In randomized control trial, Youdujing cream and external lotion were found to be effective in clearing HR-HPV infection and reversing the cervical precancerous changes, which was attributed to downregulating the mRNA expression of hTERT (human telomerase reverse transcriptase) (Xiao et al. 2012). Tanshinone IIA (TanIIA, 1,6,6-trimethyl-8,9-dihydro-7H-naphtho[1,2-g]benzofuran-10,11-dione), found in the root of *Salvia miltiorrhiza* Bunge, has been found to inhibit oncogene expression, arrest cell cycle, induce p53, and cause apoptosis in CaSki, SiHa (human cervical tumor cell), HeLa, and C33a (HPV-negative cervical cancer cell line) cells (Munagala et al. 2015).

Table 8.2 Traditional Chinese medicine formulations for treatment of HPV infections and its various symptoms with scientific justifications of their uses (if any)^a

Formulations for internal applications		Relevant phytochemicals and pharmacological actions with reference(s)
Name of formulation	Botanical with (English name) and parts used	
Rhizoma Atractylodis	<i>Atractylodes macrocephala</i> Koidz. Baizhu in Chinese, (Atractylodes), Rhizome	Beneficial effect on spleen function and fluid metabolism. (Lin et al. 2017). Atractylone is the main component in the plant. Other uses include benefiting vital energy, eliminating dampness, hydroschesis, and soothing fetuses Gu et al. (2019)
Cortex Phellodendri	<i>Phellodendron amurense</i> Rupr. (Amur cork tree) or <i>Phellodendron chinense</i> Schneid. (Chinese cork tree), Bark	Beneficial effect on spleen function and fluid metabolism (Lin et al. 2017). Known as Huang Bai in Chinese. Crude bark contains alkaloids, isoquinoline alkaloid, limonoids, phenolic acid, quinic acid, lignans, and flavonoid. Major alkaloids include berberine, palmatine, and jatrorrhizine. Limonoids include limonin and obakumone. Pharmacological actions include antiinflammatory, antibacterial, antiviral, antitumor, antigout, antiulcer, neuroprotective, and antiatopic dermatitis effects (Sun et al. 2019). The effect of berberine was tested on HPV16-positive cervical cancer cell line, SiHa and HPV18-positive cervical cancer cell line, HeLa. Berberine modulated the activity of the transcriptional factor activator protein-1 (AP-1), which plays a central role in HPV-mediated cervical carcinogenesis (Mahata et al. 2011) Cervical erosion (CE), or cervical ectropion, is a common condition among women who have married, and is considered to be a risk factor for cervical carcinoma. An empirical formulation consisting of six Chinese herbs viz. Cortex Phellodendri, Rhizoma Coptidis, Olibanum, Myrrha, borneol and catechu has been reported to be very effective (Zhou et al. 2012)
Semen Coicis	<i>Coix lacryma-jobi</i> L. (Job's tears), Mature kernel Known as Yi Yi Ren in Chinese	Beneficial effect on spleen function and fluid metabolism (Lin et al. 2017). Recent studies showed that active ingredients in coicis semen could be used to treat flat wart, verruca vulgaris, and infectious condyloma (Lee et al. 2011a, b). Coicis semen is also applied as an adjuvant to treat stomach, colon, and cervical cancers (Hu et al. 2009). <i>Coix lacryma-jobi</i> L. var. ma-yuen (Rom.Caill.) Stapf ex Hook. f. sprout extract has been shown to significantly inhibit cell proliferation in human cervical cancer HeLa cells by inducing cell cycle arrest and apoptotic cell death through inactivation of the PI3K/AKT pathway (Son et al. 2019). Triolein from the plant has been shown to induce cell cycle arrest in MCF-7 breast cancer cells (Hien et al. 2016)
Poria	<i>Wolfiporia extensa</i> (Peck) Ginns (China root), Whole mushroom (fungus) Synonym: <i>Poria cocos</i>	Known in Chinese as Fuling. Beneficial effect on spleen function and fluid metabolism (Lin et al. 2017). It is also used to promote urination and to calm the mind. One of the active ingredient is known as <i>Poria cocos</i> polysaccharide (PCP). Its activities include antitumor, immunomodulation, anti-inflammation, antioxidant, antiangiogenic, antidiabetic, and antihemorrhagic fever effects (Li et al. 2019)

Radix Astragali	Dried root of perennial herbs, <i>Astragalus membranaceus</i> (Fisch.) Bunge (Mongolian milkvetch) and <i>Astragalus mongholicus</i> Bunge. Dried roots	Known as Huangqi in Chinese. To enhance immunity (Lin et al. 2017). According to the Chinese Pharmacopoeia (CP Volume 1 of 2015 Edition), it possesses tonic, hepatoprotective, diuretic, and expectorant properties (Ma et al. 2002). More than 200 compounds have been isolated and identified from <i>Astragalus</i> root, including saponins, flavonoids, and polysaccharides (Liu et al. 2017). Isoflavonoids, triterpene saponins, and polysaccharides are the main bioactive compounds (Song et al. 2007). One of the constituents, formononetin, could significantly inhibit the PI3K/AKT signaling pathway to induce the apoptosis of cervical cancer HeLa cells and is considered as a potential drug (Jin et al. 2014). Besides cervical cancer, formononetin is also active against ovarian, breast, prostate, non-small cell lung, and bladder cancer (reviewed in Guo et al. 2019)
Radix Angelicae Sinensis	<i>Angelica sinensis</i> (Oliv.) Diels (Winter cherry root), Dried root	Known as Danggui in China. Used in TCM to enrich blood, promote blood circulation, treat blood deficiency pattern and menstrual disorders such as dysmenorrhea and irregular menstrual cycle, and modulate the immune system. Contains phthalides (both monomeric and dimeric) (Lin et al. 2017; Wu and Hsieh 2011). The herb has been in use in China for thousands of years for treatment of women's reproductive disorders. Ethanol extract of roots, when tested on human breast (MCF-7 and 7368) and cervical (CaSki and SiHa) cancer cells, killed over 90% of cancer cells at a dose of 0.32 µg/ml (Zhu et al. 2012)
Flos Lonicerae	<i>Lonicera japonica</i> Thunb. (Japanese honeysuckle), Flower More known as Lonicerae japonicae flos	Known as Shan Yin Hua in Chinese. Chinese Pharmacopoeia (2005) distinguishes between Lonicerae flos (all other species of <i>Lonicera</i>) and Lonicerae japonica flos (<i>Lonicera japonica</i>), although they have similar efficacies. Heat-clearing and detoxifying herb, which is capable of relieving genital itching and pain. Extracts and its active components including chlorogenic acid, flavonoid, caffeoylquinic acid, and iridoid glycoside can inhibit herpes simplex keratitis, influenza virus pneumonia, influenza A virus, porcine reproductive and respiratory syndrome virus, Newcastle disease virus, respiratory syncytial virus, influenza virus, human cytomegalovirus, Coxsackie β virus, and enteric cytopathic human orphan 19 virus (Lin et al. 2017; Li et al. 2015)
Herba Hedyotis Diffusae	<i>Hedyotis diffusa</i> Willd. (Snake needle grass), Whole plant Known as Bai Hua She She Cao in Chinese	Heat-clearing and detoxifying herb, which is capable of relieving genital itching and pain. For thousands of years, has been used in TCM for inflammation-linked diseases such as urethritis, appendicitis, and hepatitis. Recent scientific studies have shown the herb's efficacy against colorectal cancer, leukemia, liver cancer, lung cancer, breast cancer, cervical tumor, prostate cancer, and multiple myeloma (reviewed by Chen and others) (Lin et al. 2017; Chen et al. 2016). Contains the iridoid compounds asperuloside, geniposidic acid, diffusoside A and B; triterpenes – arborinol, isoarborinol, oleanolic and ursolic acids; flavonoids – amentoflavone, quercetin, rutin, kaempferol; various anthraquinones; and phenolic acids including ferulic and caffeic acids (Chen et al. 2016). Amentoflavone has been shown to induce apoptosis in human cervical cancer cells via suppressing HPV E7 expression (Lee et al. 2011a, b). Also see below

Herba Scutellariae Barbatae	<i>Scutellaria barbata</i> D. Don (Chinese skullcap), Whole plant	Known as Ban Zhi Lian in Chinese. Heat-clearing and detoxifying herb, which is capable of relieving genital itching and pain. Over hundreds of years, the whole herb has been in use in TCM for treating symptoms associated with carbuncle, scrofula, hematemesis, epistaxis, ascites, traumatic injuries, and especially tumors (mainly lung, breast, and digestive system cancers). The plant contains scutellarin, which is active against breast cancer cells, hepatoma, and colon cancer cells. Diterpenoid alkaloids of the plant, Scutebarbatines A-L, X, are active against nasopharyngeal cancer, oral epidermoid carcinoma, lung cancer, colorectal carcinoma, colon cancer, prostate cancer, melanoma, breast cancer, gastric cancer, leukemia, and hepatoma cancer (Lin et al. 2017 ; Gao et al. 2019)
Herba Lobeliae Chinensis	<i>Lobelia chinensis</i> Lour. (Chinese Lobelia herb), Whole plant Known in Chinese as Ban Bian Lian	Heat-clearing and detoxifying herb, which is capable of relieving genital itching and pain. The main constituents of the plant include piperidine alkaloids and flavonoids. Anticancer effects are shown by the piperidine alkaloids, namely, norlobelamine, lobeline, lobelanidine, lobelamine, radicansine A, and radicansine B. A decoction of the herb can restrain growth of liver cancer and the alkaloids can inhibit growth of BC-38 gastric cancer cells. Constituent flavonoids like apigenin and luteolin can promote apoptosis in colorectal cancer cell line, COLO205, as well as HCT 116 and HeLa cells of cervical cancer. A review of the anticancer effects of luteolin reported the compound to be effective against cancer cells of breast, colon, pancreatic, prostate, oral, lung, kidney, cervical, placental, ovarian, skin, liver, gastric, esophageal, and bladder cancers as well as glioblastoma. Apigenin reportedly inhibited proliferation in human head and neck and oral squamous cancer cells, induced apoptosis in anaplastic thyroid carcinoma cells, blocked progression of progesterin-dependent BT-474 breast cancer cell, suppressed proliferation of several colorectal adenocarcinoma cell lines, and was effective against skin, liver, and pancreatic cancer cells (Lin et al. 2017 ; Chen et al. 2014 ; Inrnan et al. 2019 ; Madumitc et al. 2018)

<p>Modified Simiao Decoction*, main components containing Radix Astragali 20 g, Rhizoma Atractylodis 15 g, Cortex Phellodendri 15 g, Semen Coicis 30 g, Radix Angelicae Sinensis 15 g, Poria 15 g, Faeces Trogopteris 10 g, Flos Lonicerae 15 g, Herba Hedyotidis Diffusae 30 g, Herba Scutellariae Barbatae 10 g, Herba Lobeliae Chinensis 10 g, Radix Glycyrrhizae 10 g *Simiao means temple in Chinese. However, in this case the name comes from Sun Simiao, author of the earliest Chinese Encyclopedia for Clinical Practice (circa 652 AD) [http://www.itmonline.org/arts/sunsimiao.htm]</p>	<p>Radix Astragali: see above. Rhizoma Atractylodis: see above Cortex Phellodendri: see above Semen Coicis: see above Radix Angelicae Sinensis: see above Poria: see above Flos Lonicerae: see above Herba Hedyotidis Diffusae: see above Herba Scutellariae Barbatae: see above Herba Lobeliae Chinensis: see above Radix Glycyrrhizae: Root of <i>Glycyrrhiza glabra</i> L. (licorice) Faeces Trogopteris: Faeces of flying squirrel (<i>Trogopterus xanthipes</i>)</p>	<p>The decoction reportedly exhibited better improvement of clinical symptoms compared with the classic Chinese medicine formulation Baofukang in patients with cervical HPV infection. Clearance of virus was greater and there was higher level of interferon-α (IFN-α) and tumor necrosis factor-α (TNF-α), those being positive signs of antiviral and immune-regulatory effects (Dou et al. 2013; Cheng et al. 2015)</p> <p><i>Glycyrrhiza glabra</i> roots have been reported to contain the triterpenoid saponins glycyrrhizin, 18β-glycyrrhizic acid and other triterpenes including liquiritic acid, glycyrrhetol, glabrolide, isoglabrolide, and liquorice acid. Flavonoids and chalcones isolated from <i>Glycyrrhiza glabra</i> included flavonoids like liquiritin, liquiritigenin, hammoliquiritin, and neoliquiritin; chalcones included isoliquiritin, isoliquiritigenin, neoisoliquiritin, licuraside, glabrolide, icoflavonol, 5,8-dihydroxy-flavone-7-O-β-D-glucuronide, glychionide A, and 5-hydroxy-8-methoxyl-flavone-7-O-β-D-glucuronide and glychionide B. Reported isoflavones included glabridin, galbrene, glabrone, shinteroicarpin, licoisoflavones A and B, formononetin, glyzarin, kumatakenin, hispaglabridin A, hispaglabridin B, 4'-O-methylglabridin, 3'-hydroxy-4'-O-methylglabridin, glabroisoflavanone A and B. <i>Glycyrrhiza glabra</i> extracts and glycyrrhizic acid have been reported to inhibit the replication of several viruses included Epstein-Barr virus, Herpes simplex virus, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Human cytomegalovirus, Human immunodeficiency virus, Influenza virus, SARS coronavirus, and Varicella zoster virus. Root methanolic extract had a growth inhibitory action against intestinal carcinoma cell line Caco-2 and prostate carcinoma cell line PC-3 with IC₅₀ values of 40 and 40.6 μg/ml, respectively. Isoliquiritigenin, present in the root, prevented the incidence of 1,2-dimethylhydrazine-induced colon and lung tumors in mice (reviewed by Al-Snafi 2018a). Glycyrrhizic acid along with a food supplement has been found to be effective for treatment of HPV-induced anogenital warts (Gómez et al. 2012). The roots of <i>Glycyrrhiza uralensis</i> are also used in TCM against recurrent respiratory papillomatosis (Yarnell 2015)</p> <p>A total of 54 fecal medicines are used in traditional Chinese formulations; the common ones are Wu-Ling-Zhi, Jiu-Fen and Hei-Bing-Pian. Most fecal medicines are used to treat gastrointestinal, nervous system, skin, and gynecological diseases (Du et al. 2019).</p>
---	---	--

<p>Yiqi Huashi Jiedu Decoction, composed of Radix Astragalii 15 g, Poria 20 g, Rhizoma Atractylodis Macrocephalae 15 g, Cortex Phellodendri 10 g, Fructus Amomi 10 g, Radix Angelicae Sinensis 10 g, Rhizoma Chuanxiong 10 g, Radix Gentianae 6 g, Radix Glycyrrhizae 6 g</p> <p>Added Fructus Toosendan and Rhizoma Corydalis for patients with abdominal pain; Cortex Magnoliae Officialis for poor appetite; and Semen Euryales for leukorrhagia</p>	<p>Radix Astragalii: see above Poria: see above Rhizoma Atractylodis Macrocephalae: see above Cortex Phellodendri: see above Rhizoma Cyrtomii: Rhizome with leafstalk of <i>Dryopteris crassirhizoma</i> Nakai (thick stemmed wood fern) Fructus Amomi: Mature fruit of <i>Amomum villosum</i> Lour. (Cocklebur-like Amomum) Radix Angelicae Sinensis: see above Rhizoma Chuanxiong: <i>Rhizome of Ligusticum chuansong</i> Hort. (Sichuan lovage) Radix Gentianae: Root of <i>Gentiana lutea</i> L. (Gentian root) Radix Glycyrrhizae: see above Fructus Toosendan: Fruit of <i>Melia toosendan</i> Siebold & Zucc. (Toosendan fruit) Rhizoma Corydalis: Rhizome of <i>Corydalis tuberosa</i> DC. (Hollowroot) Cortex Magnoliae Officialis: The dried bark or bark of branch or root of <i>Magnolia officinalis</i> Rehd. (Magnolia) Semen Euryales: Seed of <i>Euryale ferox</i> Salisb. (Gordon Euryale)</p>	<p>This decoction is another classic formulation that is believed to have the power of activating blood circulation, dissipating blood stasis, eliminating necrotic tissues, promoting granulation, dissipate heat, and enhancing diuresis (Lin et al. 2017). According to He et al. (2015), Yiqi Huashi Jiedu Decoction showed higher clinical healing rate, better virus clearance, and less recurrence than routine western medical treatment in patients with HPV infection and cervicitis (Lin et al. 2017; He et al. 2015)</p> <p>Bornyl acetate of <i>Dryopteris crassirhizoma</i> reportedly inhibited all four serotypes of dengue virus (Maryam et al. 2020). Bornyl acetate is the main constituent in <i>Amomum villosum</i> fruit; it reportedly showed analgesic and anti-inflammatory activity in rodents (Wu et al. 2005)</p> <p>Essential oil in rhizomes of <i>Ligusticum chuansong</i> contains <i>ligustilide</i>, <i>enidilid</i>, <i>neocnidilide</i>, <i>senkyunolide</i>, <i>barylidenephthalide</i>, <i>sabinene</i>, α-<i>fiprene</i>, and <i>myrcene</i>; rhizomes contain the alkaloids <i>chuanxiongine</i> and <i>pelotylfene</i> as well as <i>phenolic compounds like ferulic and sedaronic acids</i>, and other compounds like <i>spathulenol</i> and <i>apigenin</i>, <i>quercetin</i> and <i>cosmosin</i> (Ran et al. 2011). A Chinese herbal medicine containing <i>Ligusticum chuansong</i> has been shown to block cancer transformation of experimental oral precancerous lesion (Chen et al. 2004)</p> <p><i>Gentiana lutea</i> is also an Ayurvedic plant, used for its immunostimulatory properties and contains bitter phytoconstituents like amarogentin, gentiopicrinor, gentiopicroside, gentiolutein and its dimethyl acetal, gentioluteol, gentamine, amaroswerin, gentioside, and gentiolutein (Prakash et al. 2017). The plant can be used against chemotherapy-induced mucositis (Meyer-Hamme et al. 2013)</p> <p>Fruits of <i>Melia toosendan</i> contain limonoids including 12-O-methyl-1-O-deacetylrimbolinin B, 12-O-methyl-1-O-tylloyl-1-O-deacetylrimbolinin B, 12-O-ethylrimbolinin B, and 1-O-cinnamoyl-1-O-debenzoylocholinal and tirucallane-type triterpenoids, named meliasenins S and T (Hu et al. 2011). Significant cytotoxic activity against KB* cells has been reported for limonoids (meliatoxin B1 and toosendanin) from the plant (Tada et al. 1999). [*KB is now known to be a subline of the ubiquitous KERATIN-forming tumor cell line HeLa. KB cells have been reported to contain human papillomavirus18 (HPV-18) sequences.]</p> <p><i>Corydalis tuberosa</i> is known to contain the isoquinoline type alkaloid thalictricavine (Manske 1953). The quinoline/isoquinoline nucleus can play a major role in the development of anticancer drugs (Diaz et al. 2015)</p> <p><i>Magnolia officinalis</i> bark contains two major polyphenolic neolignans, magnolol and honokiol (Amblard et al. 2007; Kong et al. 2005). The two compounds are active against multiple human cancer cell lines including HeLa (human cervix adenocarcinoma cell line) and RKO (human rectal carcinoma cell line); for a complete review, see Poivre and Duez (2017). Other compounds found in the bark essential oil include bornyl acetate, camphene, caryophyllene epoxide, α-, β- and γ-eudesmol, cryptomeridol, α- and β-pinene; bark constituents further include bornyl-magnoliol, caddenic acid, quercetin, and kaempferol; alkaloids present in the bark include anonaïne, liriodenine, and magnocurarine</p> <p><i>Euryale ferox</i> seeds are also used in Ayurveda for uterine weakness; the main bioactive component has been reported to be valerianic acid (Sidh and Sharma 2019). Other constituents isolated from ethanol extract of seeds include protocatechuic acid, gallic acid, gallic acid ethyl ester, 5,7-dihydroxychromone, β-sitosterol, daucosterol, and 5,7-dihydroxy-6,4'-dimethoxyflavone (Sun et al. 2014). Seeds also contain 2β-hydroxybutelnic acid 3β-caprylate, which reportedly has antidiabetic and hepatoprotective potential (Ahmed et al. 2015)</p>
---	---	---

(continued)

Formulations for external applications		Action(s) with Reference(s)
<p>Name of formulation</p> <p>Baofukang Suppository. Main contents are Rhizoma Curcumae and Borneolum</p>	<p>Botanical with (English name) and parts used</p> <p>Rhizoma Curcumae is rhizome of <i>Curcuma longa</i> L. (turmeric) Borneolum is the processed item from resin of <i>Dryobalanops aromatica</i> Gaertn. f. (Borneo camphor in English)</p>	<p><i>One study (Shen et al. 2013) found that a 3-month medication of Baofukang reduced human papilloma virus (HPV) by 38%. Comparison of Baofukang with INF-α2b (interferon-α2b) showed that Baofukang had a better HPV negative rate and higher cervical intraepithelial neoplasia I (CIN I) reversal rate. Curcumin, present in rhizomes of Curcuma longa, has been shown in several studies to have anti-HPV effects, which has been reviewed by Mishra and Das (2015). A patent (CN103041283A) has been granted for a Chinese medicine composition to treat cervical HPV infection, which contains borneolum. Patent No. CN103041283A, granted 2014-10-22</i></p> <p>Essential oil of exudates from <i>Dryobalanops aromatica</i> reportedly contained terpenoid compounds like borneol, α- and β-caryophyllene, α-pinene, α-terpineol, and terpinen-4-ol (Le et al. 2016)</p>
<p>Radix Sophorae Flavescentsis</p>	<p>Root of <i>Sophora flavescens</i> Ait. (Ku Shen in Chinese, yellow sophora in English)</p>	<p>A polyherbal formulation named YIKEER containing root of <i>Sophora flavescens</i> is used to treat verruca (warts). Bioactive components include matrine, an alkaloid present in <i>Sophora flavescens</i>, which reportedly demonstrated antitumor effects against liver, breast, pancreatic, myeloma, and gastric cancer cell lines. (Jiang et al. 2019). Other flavonoids such as kuramione and sopheraflavone are also thought to be biologically active and may have antiviral effects. [https://www.msckc.org/cancer-care/integrative-medicine/herbs/sophora-flavescens]</p>
<p>Zhimiling suppository (ZMLS) intravaginal suppository, ingredients including Cortex Phellodendri, Radix Sophorae Flavescentsis, Catechu, and Borneolum</p>	<p>Catechu (betel nut)</p>	<p>An empirical formulation consisting of six Chinese herbs viz. Cortex Phellodendri, Rhizoma Coptidis, Olibanum, Myrrha, borneol, and catechu has also been reported to be very effective against cervical erosion associated with chronic cervicitis. Note that this formulation contains three ingredients of ZMLS</p> <p>It is also interesting that both formulations contain catechu. Catechu is regarded as one of the most potent substances associated with oral carcinoma by HPV-16 and HPV-18: both are also causative agents for cervical carcinoma (Zhou et al. 2012; Chakraborty et al. 2014)</p>

<p>Paiteling, composed of Herba Hedyotis Diffusae, Folium Isatidis, Fructus Chidii, and Fructus Bruceae</p>	<p>Herba Hedyotis Diffusae is the whole plant of <i>Hedyotis diffusa</i> Willd. (see above) Folium isatidis is the leaf of <i>Isatis indigotica</i> Fort. (known as Da Qing Ye in Chinese and Dyers Woad Leaf in English) Fructus Chidii is the fruit of <i>Cnidium monnieri</i> (L.) Cusson (known in English as Cnidium and in Chinese as She chuang zi) Fructus Bruceae is the fruit of <i>Brucea javanica</i> (L.) Merr. (known in Chinese as Ya Dan Zi and in English as Java Brucea)</p>	<p><i>Hedyotis diffusa</i> (Bai Hua She Cao or BHSSC) has been shown to induce murine and human antigen-presenting cell (APC) activation via the MAPK signaling pathway and enhance antigen presentation in bone marrow-derived dendritic cells (BMDCs) <i>in vitro</i>. Variant peptide-based vaccines combined with BHSSC were shown to improve antitumor activity in preventive, therapeutic, and recurrent HPV-related tumor models. Rutin was found to be the active constituent, which induced a strong specific immune response against HPV-related tumors <i>in vivo</i> (Song et al. 2020) Extract of Folium Isatidis has been found to be active against influenza A virus (IAV), coxsackie virus B3 (CVB3), respiratory syncytial virus (RSV), and adenovirus type 7 (Ad-7) (Deng et al. 2013). Application of Paiteling to vaginal stump accelerated the positive to negative conversion of high-risk HPV (HR-HPV) after hysterectomy for cervical intraepithelial neoplasia (CIN) (Zhao et al. 2018) Courmarins may play a role in the activity of Cnidium fruits in alleviating HPV symptoms or inhibiting the virus itself. Some of the coumarins present are xanthotoxin, isopimpinellin, bergapten, imperatorin, and osthole, which are present in crude extract used for treatment of pain in female genitalia. Bergapten possesses anticancer, analgesic, and anti-inflammatory activities (Qian et al. 2007). Imperatorin showed strong cytotoxic activity on human leukemia, and chemopreventive effects on hepatitis and skin tumor (Li and Chen 2004) Various quassinoid group of compounds (some with anticancer activity) are the main constituents of <i>Brucea javanica</i> (Zhao et al. 2014)</p>
<p>Zibai gel, the active ingredients being Radix Arnebiae, Rhizoma Curcumae, Cortex Phellodendri, Flos Lonicerae, and Radix Sophorae Flavescens</p>	<p>Radix Arnebiae (Arnebia Root or Gromwell Root in English, Zi Cao in Chinese) is the dried root of <i>Arnebia euchroma</i> (Royle) Johnston. or Lithospermum erythrorhizon Sieb. et Zucc. or <i>Arnebia gutifata</i> Bunge (Family Boraginaceae)</p>	<p>The gel has been found to reduce HPV load, effectively relieve symptoms, and improve cytological and pathological results for cervical infected patients (Ma et al. 2012). Any scientific reports on Radix Arnebiae and HPV have not been reported thus far. Certain polyphenols inhibit the proliferation of HPV cells; it has been reported that in a clinical trial, a daily oral dose of 0.5–12 g curcumin (present in <i>Rhizoma Curcumae</i>) resulted in histologic improvement of precancerous lesions in one out of four patients with uterine cervical intraepithelial neoplasms (Moga et al. 2016) According to TCM, <i>Arnebia</i> root is useful in cardiovascular and skin diseases (Ma et al. 2014). <i>Arnebia</i> species are rich in naphthoquinones such as alkannins, shikonins, and their derivatives, which may have antitumor activity (Hosseini et al. 2018)</p>
<p>Youdijing cream containing Fructus Curcumae, Rhizoma Curcumae, and Radix Arnebiae</p>	<p>For more about the ingredients, see above</p>	<p>The cream has been reported to be clinically effective against cervical infected patients and also used against condyloma acuminatum (anogenital warts caused by HPV). <i>In vitro</i> experiments with the cream showed inhibition of HPV-DNA amplification (Xiao et al. 2011; Hou et al. 1998; Feng et al. 2004)</p>
<p>Formulations for both internal and external applications</p>		
<p>Name of formulation</p>	<p>Botanical with (English name) and parts used</p>	<p>Action(s) with Reference(s)</p>

(continued)

<p>Combination of Folium Isatidis, Radix Isatidis, and Herba Portulacae</p>	<p>Radix isatidis is the dried roots of the plant <i>Isatis indigoica</i> Fort. or <i>Isatis tinctoria</i> L. (Fam. Brassicaceae). Known in Chinese as Ban Lan Gen and in English as woad dyer's woad or glastum. Folium isatidis is the leaf blade of the same plant. Herba Portulacae is <i>Portulaca oleracea</i> L. (known in Chinese as Ma Chi Xian and in English as purslane)</p>	<p>In traditional Chinese medicine, pathogenic heat and toxins (inflammatory factors) are causes of cancer and can promote its virulence (Zhang et al. 2017). The combination of Folium Isatidis, Radix Isatidis, and Herba Portulacae is thought to be good for cancer cure through eliminating pathogenic heat and toxins. Herba Portulacae and Radix Isatidis are among the two most used herbs to treat HPV. Clemastatin B, 7S,8R,8'R-(−)-harciresinol-4,4'-bis-<i>O</i>-β-D-glucopyranoside, a lignan isolated from roots of <i>Isatis indigoica</i>, has been found to be active against different subtypes of human (H1N1, including swine-origin H1N1; H3N2 and influenza B) and avian influenza viruses (H6N2, H7N3, H9N2) (Yang et al. 2013). Bioactive flavonoids of <i>Portulaca oleracea</i> include kaempferol, myricetin, luteolin, apigenin, quercetin, and genistin (Zhu et al. 2010)</p>
---	--	--

<p>Decoction containing Poria 30 g, Rhizoma Dioscoreae Hypoglaucis 15 g, Radix Achyranthis Bidentatae 12 g, Semen Coicis 30 g, Radix Stephaniae Tetrandrae 10 g, Fructus Forsythiae 12 g, Radix Angelicae Dahuricae 10 g, Rhizoma Arctylodis Macrocephalae 10 g, Herba Viola 15 g, Cortex Phellodendri 12 g, Radix Glycyrrhizae 6 g, with Herba Hedyotidis Diffusae, Herba Parimiae, and Herba Portulacae for patients with excessive heat and toxin; Flos Carthami and Semen Persicae for genital drying; Fructus Kochiae and Cortex Dictamnii for genital itching.</p>	<p>Rhizoma Dioscoreae Hypoglaucis is the <i>rhizome</i> of perennial herbaceous plant <i>Dioscorea septemloba</i> Thunb. It is known in Chinese as Bei Xie and in English as fish poison yam</p> <p>Radix Achyranthis Bidentatae is the dried roots of <i>Achyranthes bidentata</i> Bl. It is known in Chinese as Niu Xi and ox knee in English</p> <p>Radix Stephaniae Tetrandrae is the root of <i>Stephania tetrandra</i> S. Moore. Known in Chinese as Han Fang Ji and Fourstamen Stephania in English</p> <p>Fructus Forsythiae is the dried fruit of <i>Forsythia suspensa</i> (Thunb.) Vahl. It is known in English as Weeping forsythia and in Chinese as Lian Qiao</p> <p>Radix Angelicae Dahuricae is the root of <i>Angelica dahurica</i> (Fischer ex Hoffmann) Bentham & J. D. Hooker ex Franchet & Savatier, Enum. Known in Chinese as Bai Zhi and in English as Chinese Angelica</p> <p>Herba Viola (<i>Viola tricolor</i> L.), Chinese name Zi Hua Di Ding, English name Wild Pansy</p> <p>Herba Parimiae is the entire plant of <i>Parimia scabiosaeifolia</i> Fisch. ex Trev. Known in English as Patrimia herb and in Chinese as Bai Jiang Cao</p>	<p>The decoction exhibited significant improvement of cervicitis combined with intravaginal Baofukang suppository (Qin et al. 2016)</p> <p>Cholestane glycosides, diosepemlosides A and B, together with six spirostane glycosides, diosepemlosides C-H, were isolated from the rhizomes of <i>D. septemloba</i>. Other compounds isolated from the rhizomes include diaryl-heptanoids, dioscorol A, dioscorosides E1, E2; two new stilbenes, dioscorosides F1 and F2; 1,7-bis(4-hydroxyphenyl)-hepta-4E,6E-dien-3-one, 1,7-bis(4-hydroxy-phenyl)-1,4,6-heptatrien-3-one, 3,5-dithy-droxy-1,7-bis(4-hydroxyphenyl)heptane, (3R,5R)-3,5-dihydroxy-1,7-bis(4-hydroxy-phenyl)heptane 3-O-β-D-glucopyranoside, (3R,5R)-3,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-heptane 3-O-β-D-glucopyranoside, and 3-O-[α-L-arabinopyrano-syl(1→6)-β-D-glucopyranosyl]oct-1-ene-3-ol (Salehi et al. 2019)</p> <p>Stigmasterol, stigmasteryl glucoside, β-sitosterol, chrysofamine hydrate, betaine hydrochloride, succinic acid, oxalic acid, γ-aminobutyric acid, α-spinalsterol, β-sitosterol, glyrsophanol, dibutyl phthalate, palmitic acid, and daucosterol have been reported from <i>A. bidentata</i>. Two new isoflavonoid glucosides, achyranthosides A and B, were separated from the roots of <i>A. bidentata</i> (reviewed by Yang et al. 2019). It is to be noted that another plant belonging to the same genus, <i>Achyranthes aspera</i>, is used in Ayurveda for removing warts; intralésional infiltration of Apamarga Ksharodaka (AK), i.e., aqueous solution of Apamarga (<i>Achyranthes aspera</i>) Kshara (alkaline ash of the herb) took 2–6 days for the warts to shed off (Gundeti et al. 2014)</p> <p><i>Stephania tetrandra</i> roots contain monobenzyltetrahydroisoquinoline alkaloids like N-methylcoclaurine, juziphine, coclaurine, protisinomenine, reticuline, and oblongine among others; BISBENZYL-TETRAHYDROISOQUINOLINE alkaloids – tetrandrine, fangchinoline, oxo-fangchinoline, cycleanorine, cycleanine, homoaromaline, stephibaberine, cepharanthine, and obaberine among others; aporphine alkaloids like dicentrine, tazopsine, isoboldine, and corytuberine among others; and protoberberine and tetrahydroprotoberberine alkaloids (reviewed in Jiang et al. 2020). A number of the first three group of alkaloids have reported antitumor and anticancer activities (Jiang et al. 2020)</p> <p>A total of three hundred and twenty-one compounds were identified from <i>Forsythiae Fructus</i>, including fifty-one phenylethanoid glycosides, fifty lignans, nineteen aliphatic alcohols with the C6-C2 skeleton, two iridoids, nineteen diterpenoids, twenty-seven triterpenoids, six sterols, nineteen flavonoids, fifty-two volatiles, seven alkaloids, twenty-eight organic acids, six amino acids, nine sugar derivatives, two allyl/benzene glycosides, and twenty-four others. Some flavonoids are rutin, quercetin, isorhamnetin, kaempferol, hyperin, baicalin, and hesperidin (Dong et al. 2017)</p> <p>Angelicae dahuricae radix is a traditional herbal medicine used to treat various diseases in China and Korea, such as colds, headaches, rhinitis, and psoriasis (Jeong et al. 2015). Imperatorin, a furocoumarin isolated from the roots, was found to inhibit the human liver cancer cell line HepG2 through induction of apoptosis by both death receptor- and mitochondria-mediated pathways (Luo et al. 2011). Other coumarins in the root INCLUDE isomeripatorin, bergapten, oxypeucedanin, byakangelicin, oxypeucedanin hydrate, and cnidilin (Yang et al. 2020). Roots of a related species <i>Angelica sinensis</i> (Oliv.) Diels, Dong Quai in Chinese, are used in TCM for treatment of recurrent respiratory papillomatosis (Yarnell 2015)</p> <p><i>Viola tricolor</i> contains 0.3% of salicylic acid and its derivatives such as the methyl ester and violotuside (the glucosidic acetyl derivative of salicylic acid methyl ester), phenol carboxylic acids such as <i>trans</i>-caffeic acid, protocatechuic acid, <i>p</i>-coumaric acid, flavonoids (rutin, violaquercitrin, violanthin, scoparin, saponaritin, orientin, vicenin, and anthocyanidin glycosides); carotenoids (violaxanthin, zeaxanthin); and coumarins: umbelliferone (Rimkienė et al. 2003). The herb has been reported to be used after surgery to prevent reoccurring tumors (McGuffin et al. 1997). Various solvent fractions, especially ethyl acetate fraction, showed activity against MCF-7 human breast cancer cells and inhibited angiogenesis in chicken chorioallantoic membrane (Sadeghnia et al. 2014)</p>
--	--	---

(continued)

<p>Flos Carthami is dried floret of <i>Carthamus tinctorius</i> L. (safflower in English and Da Hong Hua in Chinese)</p> <p>Semen Persicae is the dry ripe seed of deciduous tree <i>Prunus persica</i> (L.) Batsch or <i>Prunus davidiana</i> (Carr.) Franch. (known as peach seed in English and Tao Ren in Chinese)</p> <p>Fructus Kochiae is the desiccative ripe fruit from <i>Kochia scoparia</i> (L.) Schrad. It is known as Di Fu Zi in Chinese</p> <p>Cortex Dictamnii is the dried root bark of <i>Dictamnus dasycarpus</i> Turcz</p> <p>Chinese name is Bai Xian Pi, English name is dittany</p>	<p>Two species, <i>Patrinia scabiosaeifolia</i> Fisch. ex Trev. and <i>Patrinia villosa</i> Juss., are considered as Herba Patrimiae in TCM. Various classes of compounds are present in Herba Patrimiae, including triterpenoid aglycones and triterpenoid saponins (scabiosides, patrinivilosides), flavonoids (acetetin, luteolin, apigenin, scutellarin, quercetin, kaempferol, cathartcin), organic acids, iridoids, and volatiles. Anticancer effects of Herba Patrimiae have been shown in CRC cell line SW480, SMMC-7721, A375-S2, A549, HeLa; HepG2, HT1080, K562, HL-60 and U937 cells, AGS, SGC-7901, BV-2, 5-FU/HCT-8, HepG2, HT-29, HeLa and MDA-MB-231 cells, A498, A549, BEL-7402, HT-29, MCF-7, K562, SGC-7901, and various other cell lines (reviewed in Gong et al. 2020)</p> <p>Flos Carthami, the dried floret of <i>Carthamus tinctorius</i> L. (safflower in English and Da Hong Hua in Chinese), is used in TCM to treat coronary heart disease, angina pectoris, gynecologic disease, stroke, and hypertension (Tu et al. 2015). According to Zhang et al. (2016), flavonoids and alkaloids, especially the quinochalcone c-glycoside hydroxysafflor yellow A, <i>N</i>-(<i>p</i>-Coumaroyl)serotonin, and <i>N</i>-feruloylserotonin, are responsible for most of the pharmacological activities of the plant. Flower extract has been found to relieve inflammation and retard progression of skin tumors (Hiramatsu et al. 2009)</p> <p>Semen Persicae can promote blood circulation and dissipate stasis (Lin et al. 2017). Phytochemicals present include neochlorogenic acid, chlorogenic acid, rutin, and cyaniding-3-rutinoside (Lara et al. 2020). It has been shown that curcumin and rutin can downregulate cyclooxygenase-2 (COX-2) and reduce tumor-associated inflammation in HPV-16 transgenic mice (Moutinho et al. 2018)</p> <p>Various formulations containing Cortex Dictamnii have been found useful for atopic dermatitis and other skin diseases (Yan et al. 2020). Anti-inflammatory limonoid compounds have been isolated from Cortex dictamnii, namely, dictamilimonol A, dictamilimonoside B, and dictamilimonols C-F, as well as limonin, limonin diosphenol, obacunon, 7α-obacunyl acetate, fraxinellone, 9β-hydroxyfraxinellone, and dasylactone A; fraxinellone is the main anti-inflammatory compound in the root bark (Chen et al. 2020)</p> <p>Fructus Kochiae total flavonoids have been shown to give an anti-inflammatory effect through activation of the pERK1/2/TLR4/NF-κB pathway (Xiao et al. 2018). [ERK = Extracellular signal-regulated kinase; TLR4 = toll-like receptor 4, its activation leads to activation of NF-κB or nuclear factor-kappa B, which in turn is responsible for activating the innate immune system]. While the Chinese use it for the treatment of diseases of the skin, urinary tract, and eyes, in Korean traditional medicine, it is used as tonic, diuretic, analgesic, and antidote and for the treatment of cutaneous pruritus and thermal skin diseases, and in China, Japan, and Korea, Fructus Kochiae is used to treat dysuria, skin diseases, and cancers (Al-Snafi 2018b). Triterpenoid glycosides isolated from the fruits of <i>Kochia scoparia</i> include momordin Ic, the 6'-methyl ester of momordin Ic, its 2'-<i>O</i>-β-D-glucopyranoside, momordin IIc, scoparinosides A, B, and C, 2'-<i>O</i>-β-D-glucopyranosyl momordin Ic, 2'-<i>O</i>-β-D-glucopyranosyl momordin IIc, momordin Ib, its 6'-<i>O</i>-methyl ester, and oleanolic acid; flavone glycosides isolated from Fructus Kochiae include quercetin-3-<i>O</i>-β-D-apiofuranosyl-[1 \rightarrow 2]-β-D-galactopyranosyl-7-<i>O</i>-β-D-glucopyranoside, quercetin 3-<i>O</i>-α-L-rhamnopyranosyl-[1 \rightarrow 6]-β-D-galactopyranosyl-7-<i>O</i>-β-D-sophoroside, quercetin 7-<i>O</i>-β-D-glucopyranoside, quercetin 3-<i>O</i>-β-D-apiofuranosyl-[1 \rightarrow 2]-β-D-galactopyranoside, quercetin 3-<i>O</i>-β-D-galactopyranosyl-7-<i>O</i>-β-D-glucopyranoside, and quercetin 7-<i>O</i>-β-D-sophoroside (reviewed by Al-Snafi 2018b). The anti-inflammatory and analgesic effects of methanol, ethanol, and aqueous extracts of dried fruits have been shown experimentally (Kim et al. 2016; Shin et al. 2004; Choi et al. 2014). Methanol extract of Fructus Kochiae inhibited human breast cancer cell MDA-MB-231 and oral squamous cell carcinoma (OSCC). Momordin Ic induced HepG2 cell apoptosis (Han et al. 2014, 2016); Wang et al. 2014a)</p>
---	--

1. ^aThe main point to be noted is that not all TCM formulations for treatment of HPV and its symptoms have been given in Table 8.2. Traditional Chinese medicine has more formulations for HPV and associated symptoms; to discuss them is beyond the scope of this chapter. Another main point to be noted is that in Column 2 of Table 8.2, English and botanical names of plants and plant parts are provided to facilitate understanding. Also to be noted is that the English names of a number of TCM formulations are not available, so the Chinese names have been given in English, as they are quoted in the English scientific literature

Dehydrocostus lactone, a natural sesquiterpene lactone, can be obtained from *Saussurea lappa* Clarke (Lin et al. 2015). The compound can inhibit cell proliferation, inhibit invasion, and induce apoptosis in HeLa and C33a (human cervical cancer cell line) cells (Jiang et al. 2015). Butein is a chalcone (2',3,4,4'-tetrahydrochalcone), which can be found in *Toxicodendron vernicifluum* (Stokes) F A Barkley and *Butea monosperma* (Lam.) Taubert. Reported activities of this compound include reduced cell viability, increased apoptosis, and DNA damage in MCF-7 (Michigan Cancer Foundation-7, a breast cancer cell line), HeLa, and ME180 (human cervical carcinoma cell line) cells (Tong et al. 2016). Erhuang powder is composed of Daihuang and Huangqi (Guo et al. 2016). The root or rhizome of perennial herbaceous plant *Rheum palmatum* L., or *Rheum tanguticum* Maxim. ex. Balf. or *Rheum officinale* Baill is called Da Huang or Daihuang; Radix Astragali is also known as Huangqi or Huang Qi. In vaginal lavage and cervical tissue of HPV-infected CIN I patients, Erhuang powder regulates Th1/Th2 [helper T cells expressing cytokines, Th1 expresses the proinflammatory cytokine interferon- γ (IFN- γ), while Th2 expresses interleukins 4, 5, and 13 and interleukin 10, an anti-inflammatory cytokine] balance and increases IFN- γ and T-bet (T-box transcription factor, also called TBX21, facilitates IFN- γ expression) (Xu and Yuan 2016).

Yi Gan Kang, a TCM formulation of which three of the major constituents are whole plant of *Angelica sinensis* (Oliv.) Diels (Apiaceae/Umbelliferae family), *Radix Astragali* (roots of *Astragalus mongholicus* Bge. (Leguminosae family), and whole plant of *Salvia miltiorrhiza* Bge. (Labiatae family), has been shown to benefit the liver and used for treatment of liver fibrosis (Yao et al. 2005). It was discovered that the medication downregulated E6 and E7 oncogenes while upregulating p53 and p21 expression in the HeLa cervical cancer line, thus proving its efficacy in HPV treatment (Deng et al. 2006).

5 Marine-Based Anti-HPV Formulations

Nonplant items used for liver ailments have been found to remove lumps and warts (Lin et al. 2017); an example is Concha Margaritifera (Zhen Zhu Mu or mother-of-pearl shell), which in TCM is used to pacify liver [<https://tcmwiki.com/wiki/zhen-zhu-mu>]. Concha Ostreae (Mu-li in Pinyin Chinese) can be the shell of any or all three species of oyster [*Crassostrea gigas* Thunberg (*Ostrea gigas* Thunberg), *Crassostrea talienwhanensis* Crosse, and *Crassostrea rivularis* Gould]; among other uses, its TCM uses include tinnitus, scrofula, subcutaneous nodules (Yang et al. 2012). Concha Ostreae has also been reported to remove lumps and warts (Lin et al. 2017).

Wang et al. (2014b) have listed several anti-HPV compounds from marine organisms. From red algae, they have listed γ -, κ -, and τ -carragenan and agar; from brown algae, alginic acid, and fucoidan; and from marine fungus, gliotoxin, neoehinulin A, physcion, and (+)-epoxydon. Marine japonicus polysaccharide (SJAMP) isolated from *Stichopus japonicus* Selenca could significantly inhibit the proliferation of human cervical carcinoma HeLa cell *in vitro* (Niu and Song 2010).

6 Integrative Treatment for HPV-Induced Conditions

Integrated treatment can consist of one TCM herbal formulation with another TCM herbal formulation, combination of two different methods of treatment (both within TCM like acupuncture/moxibustion/Tai Chi with herbal formulation), or a TCM formulation/method with a Western drug or surgery. Acupuncture has been used in combination with TCM herbal treatment for HPV infections. A 32-year-old woman was treated for myoma for 2 years prior to treatment for HPV. Myoma treatment consisted of 10 acupuncture treatments, *Cordyceps sinensis* (Berk.) Sacc. (a mushroom type known as caterpillar fungus in English and Dong Chong Xia Cao in Chinese) tea capsules 1.5 g per day and Yunnan Baiyao tea capsules 1 g per day in a period of 4 months. HPV treatment was done with 10 acupuncture treatments and 1.5 g *Cordyceps sinensis* tea capsules per day in a period of 3 months. Following treatment, clinical examinations did not show any signs of myoma or HPV (Jihe et al. 2020). The mushroom contains cordycepin (Liu et al. 2015), a compound, which has reportedly shown anticancer activities against B16 mouse melanoma and Lewis lung carcinoma (LLC) cells (Nakamura et al. 2015). The exact composition of Yunnan Baiyao is a closely guarded secret; however, some details are provided in Table 8.3.

A combination of Ezhuyou- N-CWS [*Nocardia rubra* cell wall skeleton (N-CWS) having antitumor and adjuvant activities; *Nocardia* is a genus of weakly staining Gram-positive, catalase-positive, rod-shaped bacteria] and Chinese medicine Ezhuyou (having antimicrobial activities) inhibited *in vitro* proliferation of HeLa cells and gave beneficial results in patients with cervical HPV infection (Chi and Li 2009). Ezhuyou or Ezhu You contains oil obtained from rhizomes of *Curcuma zedoaria* Rosc.; the main ingredients of the herb include curzerene, eucalyptol, curcumol, pyridine, germacrone, β -elemene, τ -elemene, and 28 other ingredients, including curdione; the oil was found to inhibit proliferation of AGS (human gastric adenocarcinoma hyperdiploid cell line) cells (Shi et al. 2013); curdione, which is also present in *Curcuma aromatica* Salisb., besides *Curcuma zedoaria*, plays an

Table 8.3 Composition of Yunnan Baiyao TCM formulation

Proprietary Blend	总成分	500 mg
Radix Notoginseng (root of <i>Panax notoginseng</i> (Burk.) ^a)	田七	200 mg
<i>Ajuga Forrestii</i> Diels (<i>Ajuga forrestii</i> Diels plant)	散瘀草	85 mg
Rhizoma Dioscoreae (dried rhizome of <i>Dioscorea opposita</i> Thunb.)	淮山药	66.5 mg
Rhizoma Dioscoreae Nipponicae (dried rhizome of <i>Dioscorea nipponica</i> Makino)	穿山龙	57.5 mg
Herba Geranii & Herba Erodii ^b	老鹤草	36 mg
Dioscoreae Parviflora Ting (<i>Dioscorea parviflora</i> C T Ting)	苦良姜	30 mg
Herba Inulae Cappae (<i>Inula cappa</i> (Buch.-Ham. ex D.Don) DC)	白牛胆	25 mg

Source: <https://www.activeherb.com/baiyao/>

^aA bioactive constituent of steamed ginseng showed cytotoxicity in HeLa and MS751 human cervical cancer cell lines (Liang et al. 2015)

^bHerba Geranii and Herba Erodii are *Geranium thunbergii* Siebold et Zuccarini and *Erodium stephanianum* Willd., respectively

important role in the inhibitory effect of the plant on cytochrome P450 3A4 (CYP3A4) in Caco-2 (human epithelial colorectal adenocarcinoma) cells (Hou et al. 2011). Isocurcumenol, another compound present in rhizomes of *Curcuma zedoaria*, showed concentration- and time-dependent increase in the percentage of cytotoxicity in Dalton's Lymphoma Ascites (DLA), adenocarcinomic human alveolar basal epithelial cells (A549), myelogenous leukemia cell line (K-562), and KB cells (subline of HeLa cells) (Lakshmi et al. 2011). Thus, although the anti-HPV component in essential oil remains to be identified, it is possible that the compound may have the potential of becoming a therapeutic against HPV.

Surprisingly, despite the widespread prevalence of HPV, allopathic anti-HPV drugs have not been discovered so far. The Food and Drug Administration of USA approved the use of Antiviral 2 (AV2[®], Cesa Alliance, Luxembourg) containing the phytochemicals carvone, eugenol, geraniol, and nerolidol; these compounds together have a broad spectrum of antiviral activities. Preliminary clinical studies have shown that the drug is quite efficacious against cervical lesions but did not show 100% efficacy (reviewed by Mutombo et al. 2017). It would be interesting to see clinical trials of AV2 along with a TCM anti-HPV formulation to determine whether full efficacy for HPV treatment can be achieved.

7 Phytochemicals as Anti-HPV Drugs

Unlike viruses of other diseases (sexually transmitted or not), human papillomaviruses lack specific targets for the drug to act, the result being that HPV therapy consists of antimetotics or immunomodulators (Mlynarczyk-Bonikowska et al. 2013). In the absence of synthetic antiviral drugs, initial attention has focused on plant secondary metabolites like podophyllotoxin, and catechins like epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate, the action of the catechins is to inhibit HPV E6 and E7 protein expressions (Mlynarczyk-Bonikowska et al. 2013). On the other hand, as shown in Table 8.2, a large number of phytochemicals can possibly be new sources of anti-HPV therapeutics. It has to be clarified at this point that while TCM considers even crude powdered plant(s) as “drugs,” in Western terminology, the paradigm of “one drug one therapy” means that a single compound should be considered as a drug even though many “drugs” may be used to combat the main disease and its associated symptoms. However, from TCM anti-HPV medications, a number of bioactive compounds appear to be promising anti-HPV candidates.

Berberine, formononetin, chlorogenic acid, amentoflavone, quercetin, rutin, kaempferol, scutellarin, apigenin, luteolin, glycyrrhizic acid, spathulenol, honokiol, genistein, baicalin, hesperidin, bergapten, and imperatorin can be possible candidates for HPV therapy, to name only a few. At the very least, some of the phytochemicals can act as therapeutics for HPV symptoms or boost the immune system, if not against the virus itself. The flavonoid quercetin has been reported to induce G2 phase arrest and apoptosis with the activation of p53 in HPV-positive human

cervical cancer-derived cells (Clemente-Soto et al. 2019). Another flavonoid compound, hesperidin (present in citrus species), reportedly induced apoptosis in MSTO-211H (established in 1985 from the pleural effusion of a patient with biphasic mesothelioma of the lung) cells by inhibiting specificity protein 1 (Sp1) transcription factor (Lee et al. 2012). Though hesperidin is yet to be reported for any activity against HPV, another flavonoid found in citrus species plants, naringin, is known to inhibit growth and induce apoptosis in HeLa cervical cancer cells (Zeng et al. 2014). Luteolin has been shown to disrupt binding between HPV16 E6 and E6AP (E6-associated protein) and so decrease viability and proliferation of HPV-positive cells (Cherry et al. 2013).

Besides flavonoids, α -linoleic acid, which is found in a number of TCM formulations presented in Table 8.2, can regulate Cox2/VEGF/MAP kinase pathway and decrease the expression of HPV oncoproteins E6/E7 through restoration of p53 and Rb expression in human cervical cancer cell lines (Deshpande et al. 2016). Limonoid group of compounds obtained from *Azadirachta indica* A. Juss. has been reported to be effective against gynecological cancers, including cervical cancers (Moga et al. 2018). Lignans have also been found to be effective against HPV. TMP or tetra-*O*-methyl nordihydroguaiaretic acid, originally found in the resin of the creosote bush, was made into a vaginal ointment and, when applied to women with HPV-linked cervical intraepithelial neoplasia, showed an excellent safety profile in Phase I/II trials (Khanna et al. 2007). The existing scientific reports suggest that quite a number of phytochemicals may prove to be of value in the treatment of HPV and/or its symptoms but at the moment lack adequate experiments done on them followed by proceeding for clinical trials (see Table 8.2 for more phytochemicals relevant for HPV treatment or prevention). The structures of some phytochemicals relevant to HPV treatment are given in Fig. 8.1.

8 Patents for Anti-HPV Formulations or Drugs

A number of patents have been granted in China for treatment of HPV and its symptoms, some of which are presented in this chapter. A patent has been granted in China in 2014 for 75% ethanol extract of *Euphorbia ebracteolata* Hayata, which purportedly contains “*Euphorbia ebracteolata* Hayata first element, *Euphorbia ebracteolata* Hayata second element, the plain A of *Euphorbia ebracteolata* Hayata, *Euphorbia ebracteolata* Hayata element B, 24-methylene cycloartanol, *Euphorbia ebracteolata* Hayata element C, β -Amyrin acetate, triterpenic acid, euphorbin A, 3-acetyl- α -Amyrin, rock Radix *Euphorbiae Pekinensis* lactone B, ebractelatinoside B, ebractelatinoside C, isoquercitrin, Radix *Euphorbiae Fischerianae* (Radix *Euphorbiae Ebracteolatae*) first element, Radix *Euphorbiae Fischerianae* (Radix *Euphorbiae Ebracteolatae*) second element, and 2-hydroxy-1-6-methoxy-3-methyl acetophenone 2-4- β -glucosidase” for treating human papillomavirus infection symptom. The patent CN102335223B was filed on 2010-07-29 and granted on 2014-01-01 [<https://patents.google.com/patent/CN102335223B/en>].

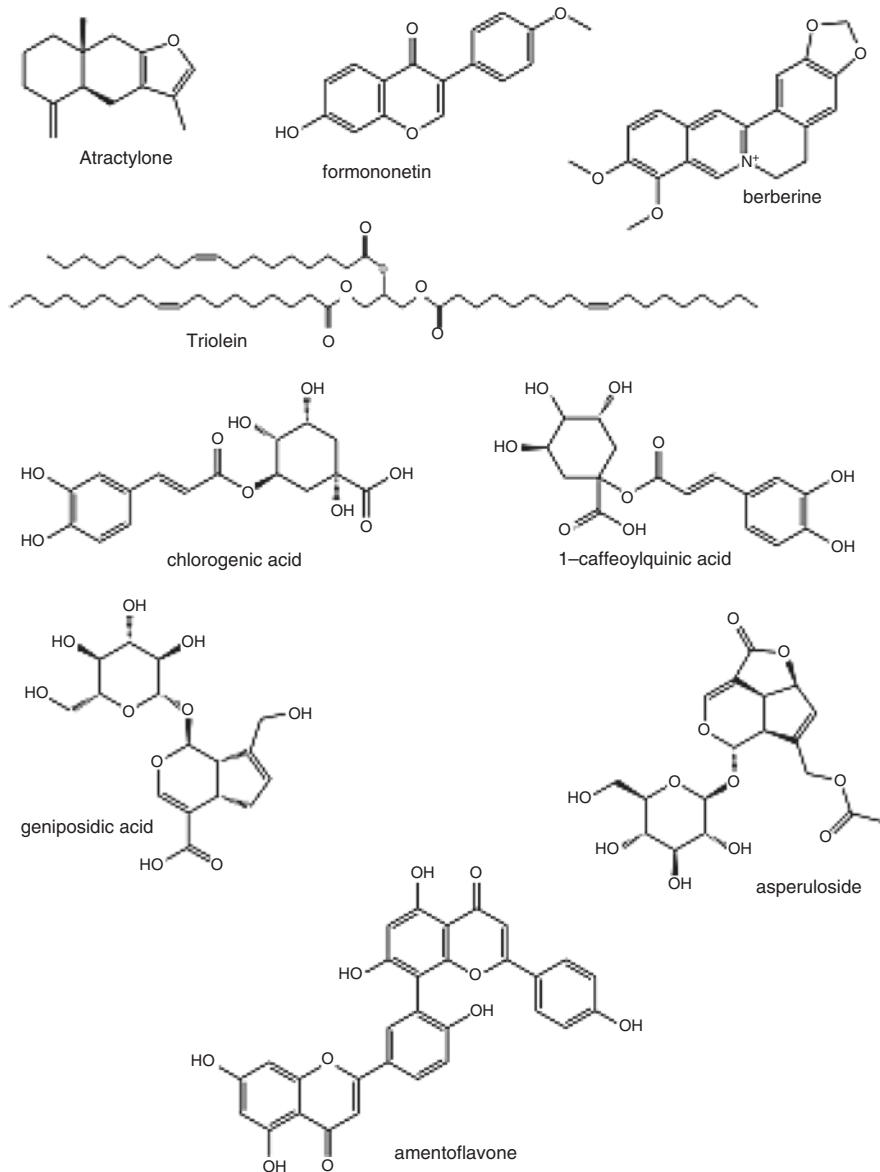


Fig. 8.1 Structure of selective phytochemicals with potential therapeutic significance against HPV and its symptoms

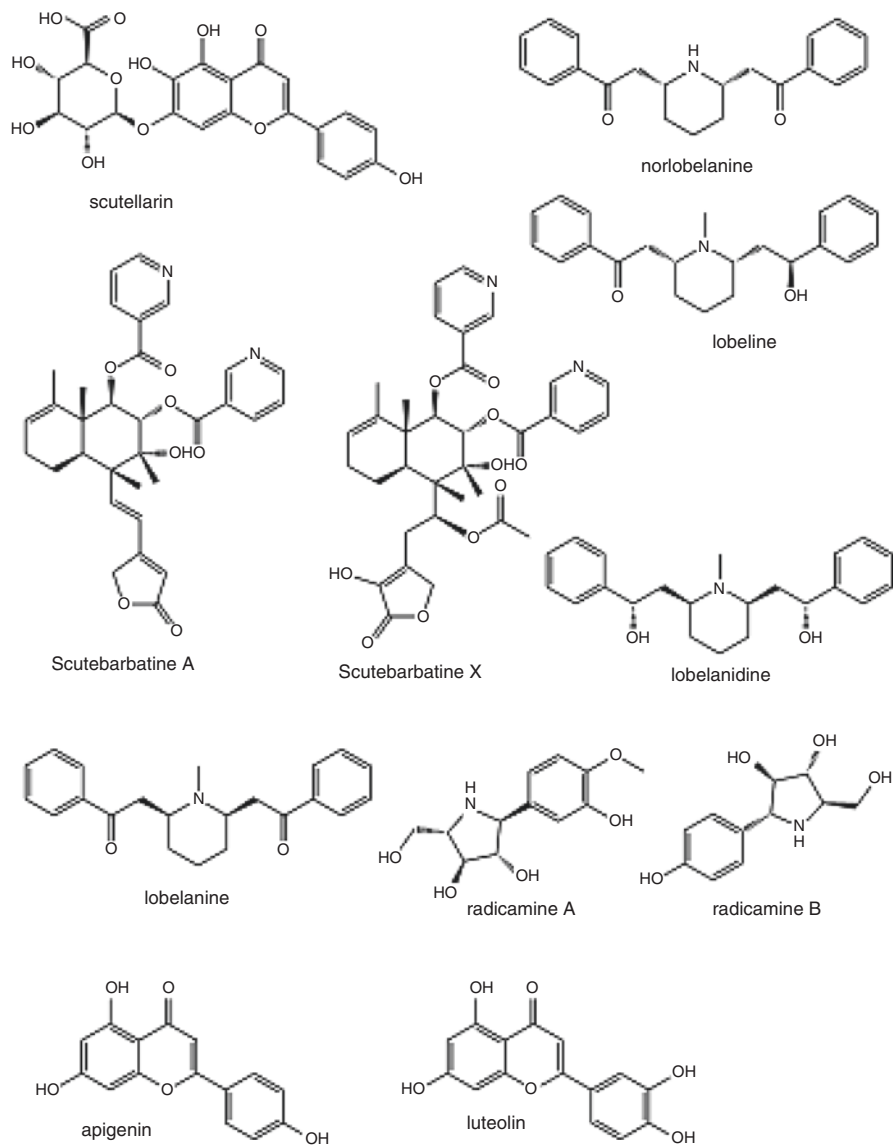


Fig. 8.1 (continued)

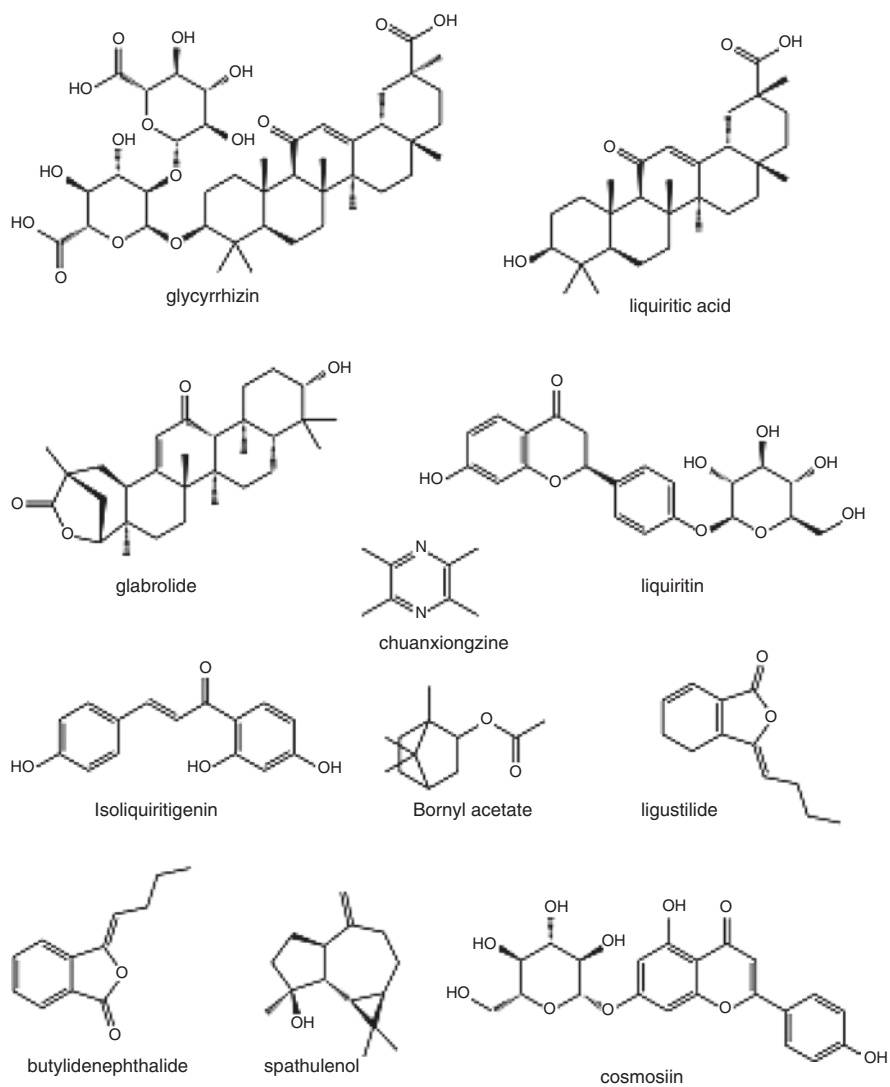


Fig. 8.1 (continued)

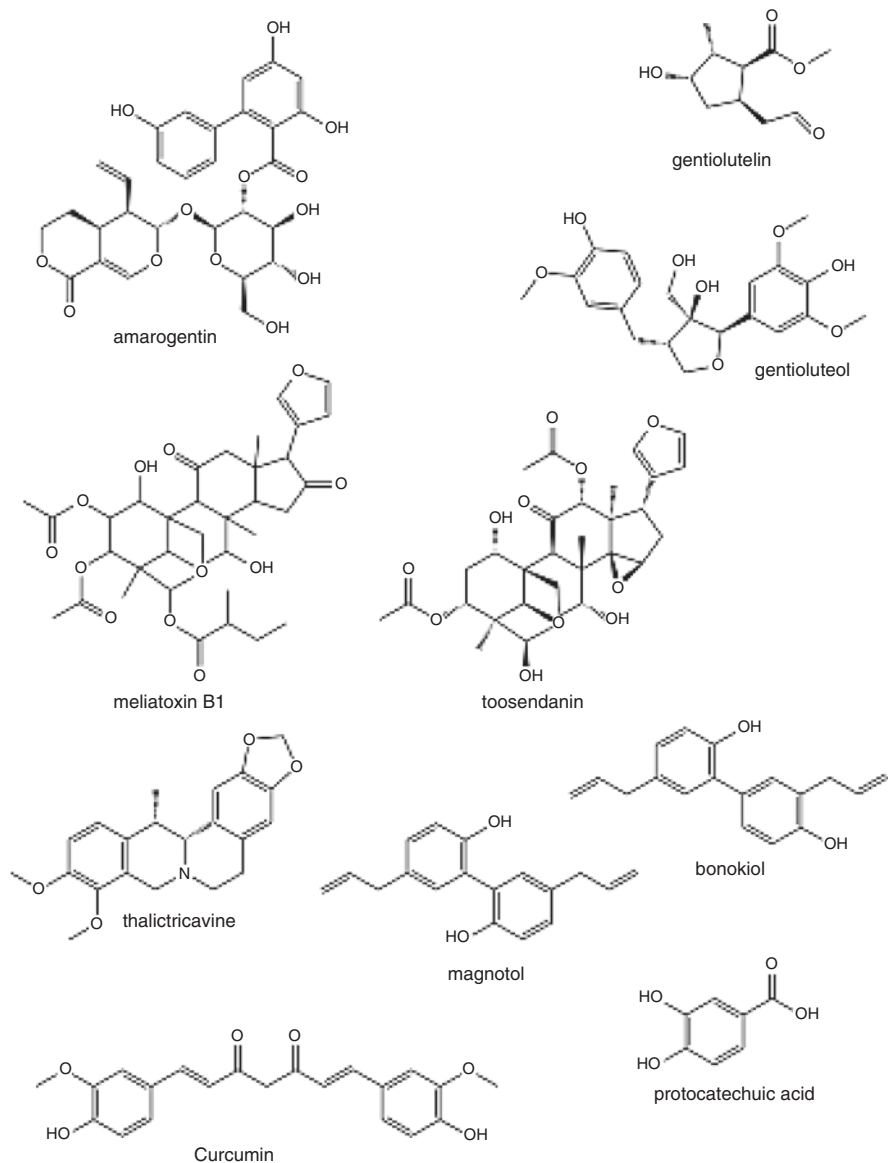


Fig. 8.1 (continued)

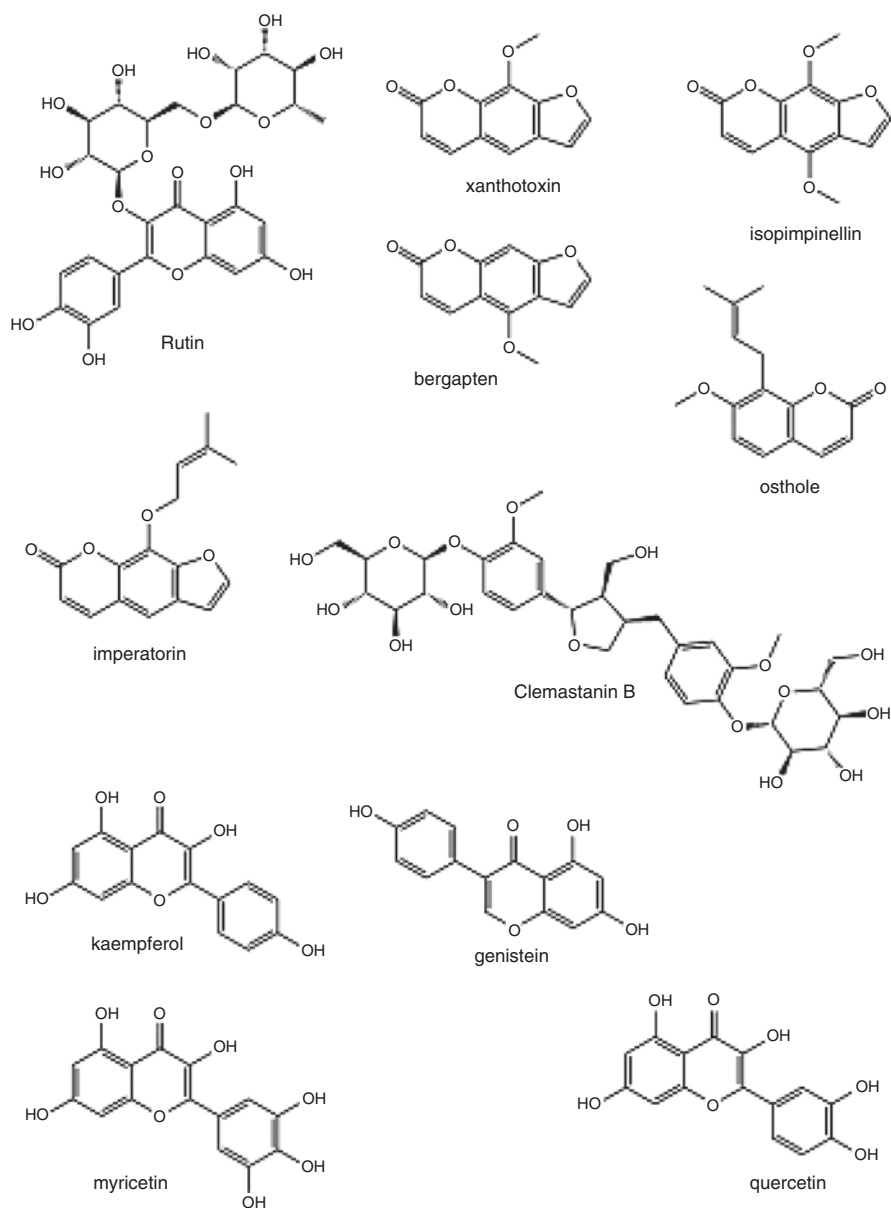


Fig. 8.1 (continued)

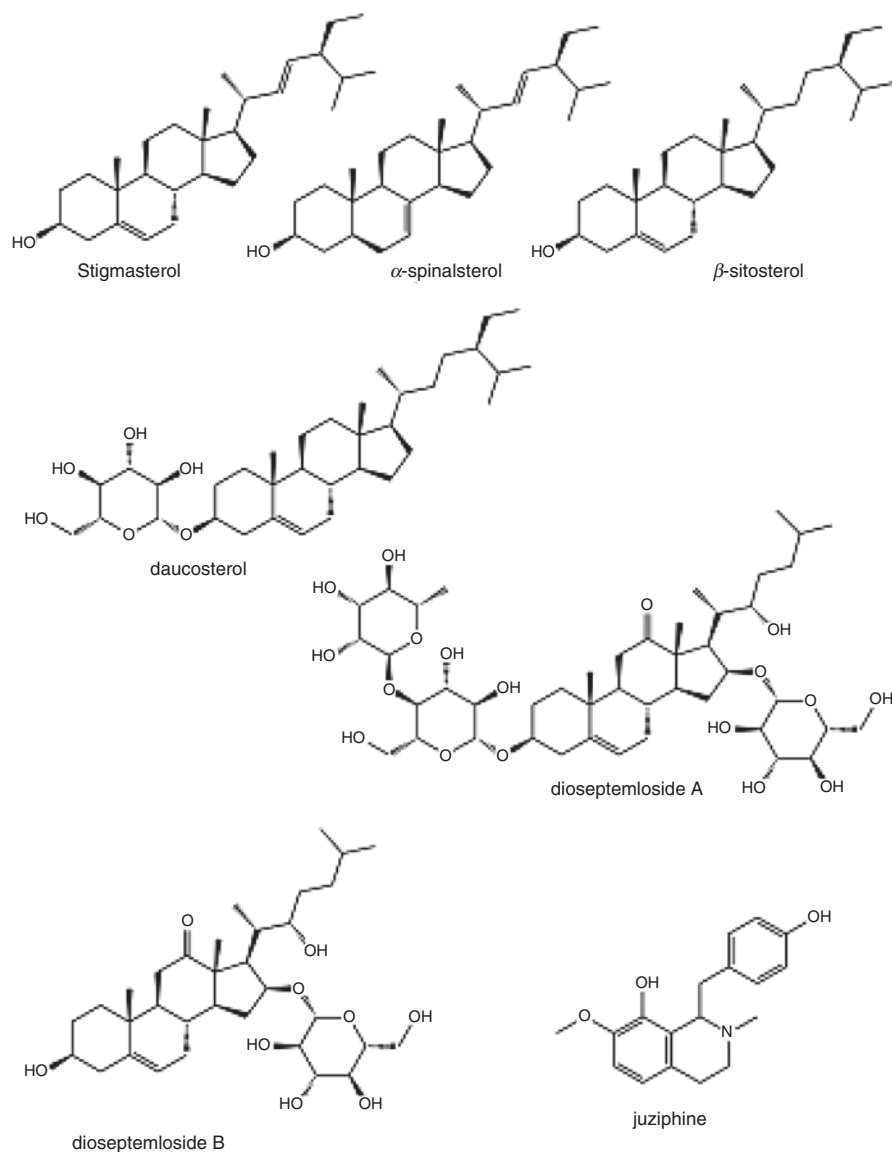


Fig. 8.1 (continued)

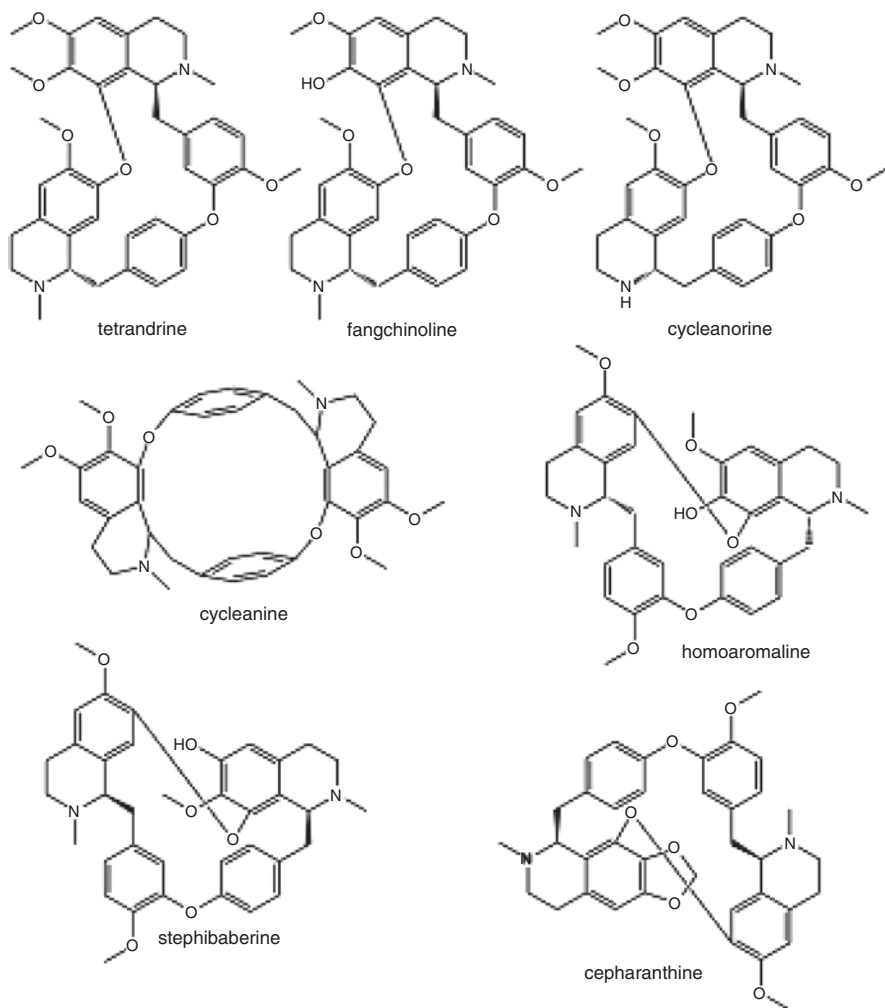


Fig. 8.1 (continued)

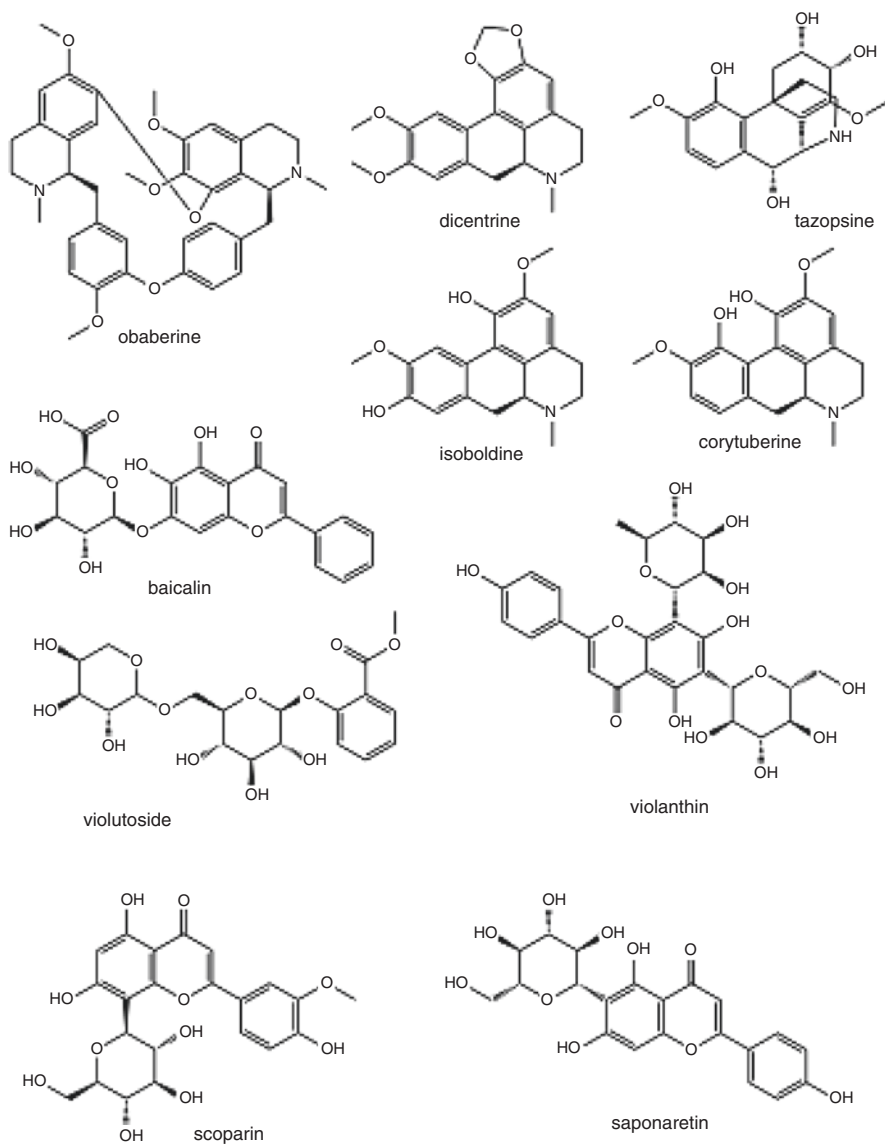


Fig. 8.1 (continued)

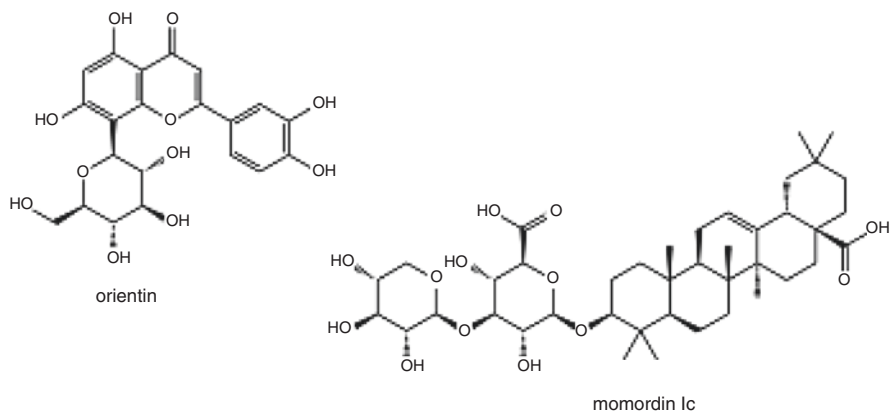


Fig. 8.1 (continued)

Patent number CN101366717A was filed on 2007-08-17 and granted on 2011-01-12. The invention relates to two successively applied medicinal preparations for treating condyloma acuminatum. The technical problem to be solved by the invention is to provide the two successively applied medicinal preparations for treating the condyloma acuminatum which can not damage normal tissues, are convenient to use and are effective. The compositions of a transdermal absorption type medicinal preparation are 30 to 80 weight percent of trichloroacetic acid homolog, 2 weight percent of water-soluble azone compound, 1 weight percent of menthol and the balance being water, wherein the azone compound comprises azone, Laurocapram, novel azone and derivatives of the azone, the Laurocapram and the novel azone and the trichloroacetic acid homolog comprises a trichloroacetic acid, a glacial acetic acid and derivatives of the trichloroacetic acid and the glacial acetic acid. The compositions of a coating medicinal preparation are 0.2 weight percent of glycyrrhetic acid, 2 weight percent of water-soluble azone compound, 10 weight percent of urea, 2 weight percent of nano-silver water solution and the balance being water. By utilization of the two medicinal preparations to coat affected parts, viruses can be eliminated, immunity can be strengthened and recrudescence can be prevented, thus the expected aims are achieved in practice.” [<https://patents.google.com/patent/CN101366717A/en>].

Patent CN102716112B claims to resist HPV infection. The patent was filed on 2012-07-13 and granted on 2014-05-14. The medication is said to contain germacrone, furanodiene, curdione, β -elemene, curcuminol, curzerene, and borneol. [<https://patents.google.com/patent/CN102716112B/en>].

Further information on Chinese patents for anti-HPV formulations can be found in “A61P 15 - Drugs for genital or sexual disorders; Contraceptives,” which gives the entire list and description of patents in this area though the patents (55,635 patents) are not necessarily solely on anti-HPV or its symptoms but covers the whole gamut of the title described. [https://www.google.com/patents/sitemap/en/Sitemap/A61/A61P/A61P_15_22.html].

9 Toxicity of TCM Formulations and Drugs

TCM formulations have the reputation, whether actual or not, of being toxic, the toxicity ranging from zero to highly toxic. As with any traditional medicinal systems and natural product, particularly plant-based formulations, the toxicity allegations have some merit based on just common grounds. A given drug in low amounts can be beneficial for health or to cure disease(s); however, once a threshold limit has been crossed, the drug can be even fatal when taken above that threshold dose. This paradigm applies to even modern over-the-counter (OTC) drugs like paracetamol or aspirin. Both relieve pain, but the former can cause hepatotoxicity and the latter gastric ulceration. Plant secondary metabolites are the phytochemicals used as drugs because of their pharmacological effects. However, the concentration of such secondary metabolites can vary widely depending on the region of cultivation, climate, and stress factors like drought, excess water, or pest attack. As a result, if a drug consists of crude plant powder (as in many TCM formulations), the concentration of the principal bioactive secondary metabolite can differ to an extent where instead of curative, it produces a toxic effect.

Ueng et al. (1997) have pointed out several factors behind toxic effects in TCM medications; toxicity can be due to contamination with heavy metals, pesticides, or even addition of a Western medicine to a TCM drug. Adverse effects can occur from improper dispensing and individual idiosyncrasy. They also pointed out that many TCM products show mutagenicity in Ames test and also that various TCM medications can adversely affect organs like liver, kidney, gastrointestinal tract, and nervous and cardiovascular systems.

10 Conclusion

Human papilloma virus (HPV) infections can range from being totally harmless and going away in a few days by itself to causing a number of cancers, the most important being cervical cancer in women. HPV is more prevalent in the low-income countries because of lack of adequate diagnostic and treatment facilities, and it is possible that because of this and other undefined factors, HPV has not attained the attention it deserves. It is possible that traditional medicinal systems in a number of countries may be able to provide effective treatment for HPV infections, but traditional Chinese medicine (TCM) appears to be the most advanced and quite thorough in this area, taking into account HPV infection and its various ramifications, and the number of TCM formulations in existence to deal with the complications associated with HPV infections. However, a major problem with TCM is the lack of adequate

studies regarding toxicity and pharmacokinetics of a given formulation or a drug. Once these two problems are adequately addressed, TCM can play a major role in the treatment and even possible eradication of this viral disease in its various manifestations.

Acknowledgments The authors are grateful to Professor Dr. ABM Anwarul Bashar for his helpful comments.

References

- Aditi, Shariff M (2019) Nipah virus infection: a review. *Epidemiol Infect* 147:e95. <https://doi.org/10.1017/S0950268819000086>
- Ahmed D, Sharma M, Kumar V, Bajaj HK, Verma A (2015) 2 β -hydroxybetulinic acid 3 β -caprylate: an active principle from *Euryale Ferox* Salisb. Seeds with antidiabetic, antioxidant, pancreas & hepatoprotective potential in streptozotocin induced diabetic rats. *J Food Sci Technol* 52:5427–5441. <https://doi.org/10.1007/s13197-014-1676-0>
- Al-Snafi AE (2018a) *Glycyrrhiza glabra*: a phytochemical and pharmacological review. *IOSR J Pharm* 8:1–17
- Al-Snafi AE (2018b) A review on pharmacological activities of *Kochia Scoparia*. *Indo Am J Pharm Sci* 5:2213–2221. <http://www.iajps.com>
- Amblard F, Govindarajan B, Lefkove B, Rapp KL, Detorio M, Arbiser JL, Schinazi RF (2007) Synthesis, cytotoxicity, and antiviral activities of new neolignans related to honokiol and magnanolol. *Bioorg Med Chem Lett* 17:4428–4431. <https://doi.org/10.1016/j.bmcl.2007.06.024>
- Asad A, Aamir A, Qureshi NE, Bhimani S, Jatoi NN, Batra S, Ochani RK, Abbasi MK, Tariq MA, Diwan MN (2020) Past and current advances in Marburg virus disease: a review. *Infez Med* 3:332–345
- Azeez-Akande O (2016) A review of Lassa fever, an emerging old world haemorrhagic viral disease in sub-Saharan Africa. *Afr J Clin Exper Microbiol* 17:282–289. <https://doi.org/10.4314/ajcem.v17i4.9>
- Berns KI, Byrne BJ, Flotte TR, Gao G, Hauswirth WW, Herzog RW, Muzyczka N, VandenDriessche T, Xiao X, Zolotukhin S, Srivastava A (2015) Adeno-associated virus type 2 and hepatocellular carcinoma? *Hum Gene Ther* 26:779–781. <https://doi.org/10.1089/hum.2015.29014.kib>
- Bird BH, Ksiazek TG, Nichol ST, Maclachlan NG (2009) Rift Valley Fever virus. *J Am Vet Med Assoc* 234:883–893. <https://doi.org/10.2460/javma.234.7.883>
- Bonthius DJ (2012) Lymphocytic choriomeningitis virus: an under-recognized cause of neurologic disease in the fetus, child, and adult. *Semin Pediatr Neurol* 19:89–95. <https://doi.org/10.1016/j.spn.2012.02.002>
- Brianti P, Flammoneis ED, Mercuri SR (2017) Review of HPV-related diseases and cancers. *New Microbiol* 40:80–85
- Bruni L, Albero G, Serrano B, Mena M, Gómez D, Muñoz J, Bosch FX, de Sanjosé S (2019) ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre). Human Papillomavirus and Related Diseases in the World. Summary Report 17 June 2019. Date Accessed 3 Nov 2020
- Centers for Disease Control and Prevention (2002) Guidelines for treatment of sexually transmitted diseases. *Morb Mortal Wkly Rep* 51(RR06):1–80
- Chakraborty B, Roy JG, Majumdar S, Uppala D (2014) Relationship among tobacco habits, human papilloma virus (HPV) infection, p53 polymorphism/mutation and the risk of oral squamous cell carcinoma. *J Oral Maxillofac Pathol* 18:211–216. <https://doi.org/10.4103/0973-029X.140752>

- Chen ZL, Guan YQ, Chen X, Chen XL, Chen JC (2004) Effect of Chinese herbal medicine 1023 recipe in blocking cancer transformation of experimental precancerous lesion and its mechanism. *Chin J Integr Med* 2:281–284. <https://doi.org/10.3736/jcim20040415>
- Chen M-W, Chen W-R, Zhang J-M, Long X-Y, Wang Y-T (2014) *Lobelia chinensis*: chemical constituents and anticancer activity perspective. *Chin J Nat Med* 12:0103–0107. [https://doi.org/10.1016/S1875-5364\(14\)60016-9](https://doi.org/10.1016/S1875-5364(14)60016-9)
- Chen R, He J, Tong X, Tang L, Liu M (2016) The *Hedyotis diffusa* Willd. (Rubiaceae): a review on phytochemistry, pharmacology, quality control and pharmacokinetics. *Molecules* 21:710. <https://doi.org/10.3390/molecules21060710>
- Chen Y, Ruan J, Sun F, Wang H, Yang S, Zhang Y, Yan J, Yu H, Guo Y, Zhang Y, Wang T (2020) Anti-inflammatory limonoids from Cortex Dictamni. *Front Chem* 8: Article 73. <https://doi.org/10.3389/fchem.2020.00073>
- Cheng JM, Tong Y, Chen SC, Zhang L (2015) Clinical effect of modified Simiao Decoction in treating cervical HPV infection. *Pharm Clin Chin Mater Med* 31:149–150. (in Chinese)
- Cherry JJ, Rietz A, Malinkevich A, Liu Y, Xie M, Bartolowits M, Davisson VJ, Baleja JD, Androphy EJ (2013) Structure based identification and characterization of flavonoids that disrupt human papillomavirus-16 E6 function. *PLoS One* 8:e84506. <https://doi.org/10.1371/journal.pone.0084506>
- Chi Y, Li J (2009) Ezhuyou-N-CWS carrier suppository for antagonizing HPV infection and preventing cervical cancer. *J Beijing Univ Tradit Chin Med* 32:419–421. (in Chinese)
- Choi YY, Kim MH, Lee JY, Hong J, Kim S, Yang WM (2014) Topical application of *Kochia scoparia* inhibits the development of contact dermatitis in mice. *J Ethnopharmacol* 154:380–385. <https://doi.org/10.1016/j.jep.2014.04.009>
- Clemente-Soto AF, Salas-Vidal E, Milan-Pacheco C, Sánchez-Carranza JN, Peralta-Zaragoza O, González-Maya L (2019) Quercetin induces G2 phase arrest and apoptosis with the activation of p53 in an E6 expression-independent manner in HPV-positive human cervical cancer-derived cells. *Mol Med Rep* 19:2097–2106. <https://doi.org/10.3892/mmr.2019.9850>
- de Sanjosé S, Tsu VD (2020) Spectrum of HPV related diseases. HPVW: The Newsletter on HPV. <https://www.hpvworld.com/articles/spectrum-of-hpv-related-diseases/>
- Dela Cruz CS, Pasnick S, Gross JE, Keller J, Carlos WG, Cao B, Jamil S (2019) Adenovirus infections and outbreaks: what you need to know. *Am J Respir Crit Care Med* 199:13–14. <https://doi.org/10.1164/rccm.1997P13>
- Deng WP, Chao MW, Lai WF, Sun C, Chung CY, Wu CC, Lin IH, Hwang JJ, Wu CH, Chiu WT, Chen CY, Redpath JL (2006) Correction of malignant behavior of tumor cells by traditional Chinese herb medicine through a restoration of p53. *Cancer Lett* 233:315–327. <https://doi.org/10.1016/j.canlet.2005.03.031>
- Deng Y-P, Liu Y-Y, Liu Z, Li J, Zhao L-M, Xiao H, Ding X-H, Yang Z (2013) Antiviral activity of Folium Isatidis derived extracts *In Vitro* and *In Vivo*. *Am J Chin Med* 41:957–969. <https://doi.org/10.1142/S0192415X1350064X>
- Deshpande R, Mansara P, Kaul-Ghanekar R (2016) Alpha-linolenic acid regulates COX2/VEGF/MAP kinase pathway and decreases the expression of HPV oncoproteins E6/E7 through restoration of p53 and Rb expression in human cervical cancer cell lines. *Tumour Biol* 37:3295–3305. <https://doi.org/10.1007/s13277-015-4170-z>
- Diagne MM, Faye M, Faye O, Sow A, Balique F, Sembène M, Granjon L, Handschumacher P, Faye O, Diallo M, Sall AA (2017) Emergence of *Wesselsbron* virus among black rat and humans in Eastern Senegal in 2013. *One Health* 3:23–28. <https://doi.org/10.1016/j.onehlt.2017.02.001>
- Diaz G, Miranda IL, Diaz MAN (2015) Quinolines, isoquinolines, angustureine, and congeneric alkaloids – occurrence, chemistry, and biological activity. In: Rao AV, Rao LG (eds) *Phytochemicals - isolation, characterisation and role in human health*. <https://doi.org/10.5772/59819>
- Dong Z, Lu X, Tong X, Dong Y, Tang L, Liu M (2017) *Forsythiae Fructus*: a review on its phytochemistry, quality control, pharmacology and pharmacokinetics. *Molecules* 22:1466. <https://doi.org/10.3390/molecules22091466>

- Donoso-Mantke O, Karan LS, Růžek D (2011) Tick-borne encephalitis virus: a general overview. In: *Flavivirus encephalitis*, chapter 7. InTechOpen. <https://doi.org/10.5772/21912>
- Dou BF, Zhang JG, Liu B (2013) Analysis on effect of modified Simiao decoction in treatment of cervical HPV infection. *Liaoning J Tradit Chin Med* 40:1141–1142. (in Chinese)
- Du H, Kuang T-T, Qiu S, Xu T, Huan C-LG, Fan G, Zhang Y (2019) Fecal medicines used in traditional medical system of China: a systematic review of their names, original species, traditional uses, and modern investigations. *Chin Med* 14:31. <https://doi.org/10.1186/s13020-019-0253-x>
- Fehrmann F, Laimins LA (2003) Human papillomaviruses: targeting differentiating epithelial cells for malignant transformation. *Oncogene* 22:5201–5207. <https://doi.org/10.1038/sj.onc.1206554>
- Feng Y, Deng YH, Xuan GW, Zhou D, Sun J, Yuan XH, Zhao RZ (2004) Preparation and *in vitro* pharmacodynamics effect of the Youdujing cream. *Chin Hosp Pharm J* 24:7–8. (in Chinese)
- Figueira L, Lannes N (2019) Review of Japanese encephalitis virus: new aspects and concepts about entry into the brain and inter-cellular spreading. *Pathogens* 8:111. <https://doi.org/10.3390/pathogens8030111>
- Findik S, Findik S, Abuoğlu S, Cihan FG, Ilter H, Iyisoy MS (2019) Human papillomavirus (HPV) subtypes and their relationships with cervical smear results in cervical cancer screening: a community-based study from the central Anatolia region of Turkey. *Int J Clin Exp Pathol* 12:1391–1398
- Flick R, Whitehouse CA (2006) Crimean-Congo hemorrhagic fever virus. *Curr Mol Med* 5:753–760. <https://doi.org/10.2174/156652405774962335>
- Forman D, Martel CD, Lacey CJ, Soerjomataram I, Tieulent JL, Bruni L, Vignat J, Ferlay J, Bray F, Plummer M, Franceschi S (2012) Global burden of human papillomavirus and related diseases. *Vaccine* 30:12–23. <https://doi.org/10.1016/j.vaccine.2012.07.055>
- Gao J, Yin W, Corcoran O (2019) From *Scutellaria barbata* to BZL 101 in cancer patients: Phytochemistry, pharmacology, and clinical evidence. *Nat Prod Commun* 14:1–12. <https://doi.org/10.1177/1934578X19880645>
- Gebreyohannes G (2014) Human herpes simplex virus categories, mode of transmission, treatment and preventive measures. *Int J Pharm Health Care Res* 2:211–226
- Gilbert L (2016) Louping ill virus in the UK: a review of the hosts, transmission and ecological consequences of control. *Exp Appl Acarol* 68:363–374. <https://doi.org/10.1007/s10493-015-9952-x>
- Gómez JD, Simón RD, Daniel AA, Zelenkova H (2012) Effectiveness of glycyrrhiginic acid (Glizigen) and an immunostimulant (Viusid) to treat anogenital warts. *Int Sch Res Notices*.: Article ID: 863692. <https://doi.org/10.5402/2012/863692>
- Gong L, Zou W, Zheng K, Shi B, Liu M (2020) The Herba Patriniae (Caprifoliaceae): a review on traditional uses, phytochemistry, pharmacology and quality control. *J Ethnopharmacol* 265:113264. <https://doi.org/10.1016/j.jep.2020.113264>
- Gould EA, Solomon T (2008) Pathogenic flaviviruses. *Lancet* 371:500–509. [https://doi.org/10.1016/S0140-6736\(08\)60238-X](https://doi.org/10.1016/S0140-6736(08)60238-X)
- Gu S, Li L, Huang H, Wang B, Zhang T (2019) Antitumor, antiviral, and anti-inflammatory efficacy of essential oils from *Atractylodes macrocephala* Koidz. Produced with different processing methods. *Molecules* 24:2956. <https://doi.org/10.3390/molecules24162956>
- Gundeti MS, Reddy RG, Muralidhar JV (2014) Subcutaneous intralesional Ksharodaka injection: a novel treatment for the management of Warts: a case series. *J Ayurveda Integr Med* 5:236–240. <https://doi.org/10.4103/0975-9476.146558>
- Guo W, Lu X, Wang D, Chen T, Fan Z, Song Y (2016) Effect of combination of Chinese herbal medicine versus Western medicine on mortality in patients after cardiopulmonary resuscitation: a systematic review and meta-analysis of randomized controlled trials. *Evid Based Complement Altern Med*: Article ID: 3506757. <https://doi.org/10.1155/2016/3506757>
- Guo Z, Lou Y, Kong M, Luo Q, Liu Z, Wu J (2019) A systematic review of phytochemistry, pharmacology and pharmacokinetics on *Astragali radix*: implications for *Astragali radix* as a personalized medicine. *Int J Mol Sci* 20:1463. <https://doi.org/10.3390/ijms20061463>

- Hamre D, Procknow JJ (1966) A new virus isolated from the human respiratory tract. *Proc Soc Exp Biol Med* 121:190–193. <https://doi.org/10.3181/00379727-121-30734>
- Han HY, Kim H, Son YH, Lee G, Jeong SH, Ryu MH (2014) Anti-cancer effects of *Kochia scoparia* fruit in human breast cancer cells. *Pharmacogn Mag* 10:S661–S667. <https://doi.org/10.4103/0973-1296.139812>
- Han HY, Lee HE, Kim HJ, Jeong SH, Kim JH, Kim H, Ryu MH (2016) *Kochia scoparia* induces apoptosis of oral cancer cells in vitro and in heterotopic tumors. *J Ethnopharmacol* 192:431–441. <https://doi.org/10.1016/j.jep.2016.09.019>
- Hardy ME (1999) Norwalk and “Norwalk-like viruses” in epidemic gastroenteritis. *Clin Lab Med* 19:675–690. [https://doi.org/10.1016/S0272-2712\(18\)30110-0](https://doi.org/10.1016/S0272-2712(18)30110-0)
- He JM, Huo DZ, Chen YM (2015) Influence of Yiqi Huashi Jiedu Decoction on TCM symptom score and HPV turn rate of cervicitis with HPV infection. *Chin Arch Tradit Chin Med* 33:2390–2392. (in Chinese)
- Heegaard ED, Brown KE (2002) Human parvovirus B19. *Clin Microbiol Rev* 15:485–505. <https://doi.org/10.1128/CMR.15.3.485-505.2002>
- Henrickson KJ (2003) Parainfluenza viruses. *Clin Microbiol Rev* 16:242–264. <https://doi.org/10.1128/CMR.16.2.242-264.2003>
- Hess IMR, Massey PD, Walker B, Middleton DJ, Wright TM (2011) Hendra virus: what do we know? *NSW Pub Health Bull* 22:118–122. <https://doi.org/10.1071/NB10077>
- Hien TT, Ha DT, Truong DM, Duc LV, Dao TT, Binh BT, Hai N (2016) Triolein from *Coix lacryma-jobi* induces cell cycle arrest through p53/p21 signaling pathway. *Biomed Pharmacol J* 9:519–524. <https://doi.org/10.13005/bpj/967>
- Hiramatsu M, Takahashi T, Komatsu M, Kido T, Kasahara Y (2009) Antioxidant and neuroprotective activities of Mogami-benibana (safflower, *Carthamus tinctorius* Linne). *Neurochem Res* 34:795–805. <https://doi.org/10.1007/s11064-008-9884-5>
- Hosseini A, Mirzaee F, Davoodi A, Jouybari HB, Azadbakh M (2018) The traditional medicine aspects, biological activity and phytochemistry of *Arnebia* spp. *Med Glas (Zenica)* 15:1–9. <https://doi.org/10.17392/926-18>
- Hou MJ, Fan RQ, Sun J, Xuan GW, Chi FH (1998) Effect of Chinese medicine Youdujing for human papilloma virus DNA. *J Guangzhou Univ Tradit Chin Med* 15:57–60. (in Chinese)
- Hou X-L, Hayashi-Nakamura E, Takatani-Nakase T, Tanaka K, Takahashi K, Komatsu K, Takahashi K (2011) Curdione plays an important role in the inhibitory effect of *Curcuma aromatica* on CYP3A4 in Caco-2 cells. *Evid Based Complement Altern Med: Article ID 913898*. <https://doi.org/10.1093/ecam/nep229>
- Hu S-h, Xiao X-n, Yi X, Zhang J-p (2009) Study progress of coix seed. *Lishizhen Med Mater Med Res* 20:1059–1060
- Hu J-F, Fan H, Wang L-J, Wu S, Zhao Y (2011) Limonoids from the fruits of *Melia toosendan*. *Phytochem Lett* 4:292–297. <https://doi.org/10.1016/j.phytol.2011.05.003>
- Hui DSC (2005) An overview on severe acute respiratory syndrome (SARS). *Monaldi Arch Chest Dis* 63:149–157. <https://doi.org/10.4081/monaldi.2005.632>
- Imran M, Rauf A, Abu-Izneid T, Nadeem M, Shariati MA, Khan IA, Imran A, Orhan IE, Rizwan M, Atif M, Gondal TA, Mubarak MS (2019) Luteolin, a flavonoid, as an anticancer agent: a review. *Biomed Pharmacother* 112:108612. <https://doi.org/10.1016/j.biopha.2019.108612>
- Jeong S-H, Kim B-B, Lee J-E, Ko Y, Park J-B (2015) Evaluation of the effects of *Angelicae dahuricae* radix on the morphology and viability of mesenchymal stem cells. *Mol Med Rep* 12:1556–1560. <https://doi.org/10.3892/mmr.2015.3456>
- Jiang EP, Sun XW, Kang HX, Sun LP, An WF, Yao YH, Hu XR (2015) Dehydrocostus lactone inhibits proliferation, antiapoptosis, and invasion of cervical cancer cells through PI3K/Akt signaling pathway. *Int J Gynecol Cancer* 25:1179–1186. <https://doi.org/10.1097/IGC.0000000000000474>
- Jiang SB, Lu YS, Zhang YS, Wu Y, Wang HX, Gao XH, Chen HD (2019) A retrospective study of a Chinese traditional medicine YIKEER in the treatment of Verruca patients in Liaoning District. *Evid Based Complement Altern Med: Article ID 9896148*. <https://doi.org/10.1155/2019/9896148>

- Jiang Y, Liu M, Liu H, Liu S (2020) A critical review: traditional uses, phytochemistry, pharmacology and toxicology of *Stephania tetrandra* S. Moore (fen fang Ji). *Phytochem Rev* 19:449–489. <https://doi.org/10.1007/s11101-020-09673-w>
- Jihe Z, Blagica A, Kristina K, Svetlana J (2020) Acupuncture and herbal treatment for human papillomavirus (HPV) and uterine myoma. *World J Adv Res Rev* 6:166–169. <https://doi.org/10.30574/wjarr>
- Jin Y-M, Xu T-M, Zhao Y-H, Wang Y-C, Cui M-H (2014) *In vitro* and *in vivo* anti-cancer activity of formononetin on human cervical cancer cell line HeLa. *Tumour Biol* 35:2279–2284. <https://doi.org/10.1007/s13277-013-1302-1>
- Jo H, Kim JW (2005) Implications of HPV infection in uterine cervical cancer. *Cancer Ther* 3:419–434
- Johnson C, Hargest V, Cortez V, Meliopoulos V, Schultz-Cherry S (2017) Astrovirus pathogenesis. *Viruses* 9:22. <https://doi.org/10.3390/v9010022>
- Kantala T, Maunula L (2018) Hepatitis E virus: zoonotic and foodborne transmission in developed countries. *Future Virol* 13:657–670. <https://doi.org/10.2217/fvl-2018-0062>
- Kelly TR, Hawkins MG, Sandrock CE, Boyce WM (2008) A review of highly pathogenic avian influenza in birds, with an emphasis on Asian H5N1 and recommendations for prevention and control. *J Avian Med Surg* 22:1–16. <https://doi.org/10.1647/2006-036R.1>
- Khanna N, Dalby R, Tan M, Arnold S, Stern J, Frazer N (2007) Phase I/II clinical safety studies of terameprocol vaginal ointment. *Gynecol Oncol* 107:554–562. <https://doi.org/10.1016/j.ygyno.2007.08.074>
- Kilgore PE, Ksiazek TG, Rollin PE, Mills JN, Villagra MR, Montenegro MJ, Costales MA, Paredes LC, Peters CJ (1997) Treatment of Bolivian hemorrhagic fever with intravenous ribavirin. *Clin Infect Dis* 24:718–722. <https://doi.org/10.1093/clind/24.4.718>
- Kim H, Jo S, Ryu J (2016) Anti-inflammatory effects of *Kochia scoparia* fruit on contact dermatitis in mice. *J Clin Exp Dermatol Res* 7:3. <https://doi.org/10.4172/2155-9554.C1.033>
- Kimura H, Tsukagoshi H, Ryo A, Oda Y, Kawabata T, Majima T, Kozawa K, Shimojima M (2015) Ebola virus disease: A literature review. *J Coastal Life Med* 3:85–90. <https://doi.org/10.12980/JCLM.3.201514J93>
- Kong ZL, Tzeng SC, Liu YC (2005) Cytotoxic neolignans: an SAR study. *Bioorg Med Chem Lett* 15:163–166. <https://doi.org/10.1016/j.bmcl.2004.10.011>
- Lakshmi S, Padmaja G, Remani P (2011) Antitumour effects of isocurcumenol isolated from *Curcuma zedoaria* rhizomes on human and murine cancer cells. *Int J Med Chem* 2011:253962. <https://doi.org/10.1155/2011/253962>
- Lara MV, Bonghi C, Famiani F, Vizzotto G, Walker RP, Drincovich MF (2020) Stone fruit as bio-factories of phytochemicals with potential roles in human nutrition and health. *Front Plant Sci* 11: Article 562252. <https://doi.org/10.3389/fpls.2020.562252>
- Le T-X, Ho AS-H, Mah S-H, Wong T-W, Ong H-C, Loh PH-M, Lim Y-M (2016) Determination of borneol and other chemical compounds of essential oil of *Dryobalanops aromatica* exudate from Malaysia. *Trop J Pharm Res* 15:1293–1297. <https://doi.org/10.4314/tjpr.v15i6.23>
- Leal É d S, Zanotto PM d A (2000) Viral diseases and human evolution. *Mem Inst Oswaldo Cruz* 95:193–200. <https://doi.org/10.1590/s0074-02762000000700033>
- Lee K-H, Morris-Natschke S, Qian K, Dong Y, Yang X, Zhou T, Belding E, Wu S-F, Wada K, Akiyama T (2011a) Recent progress of research on herbal products used in traditional Chinese medicine: the herbs belonging to The Divine Husbandman's Herbal Foundation Canon (神農本草經 Shén Nóng Běn Cǎo Jīng). *J Tradit Complement Med* 2:6–26. [https://doi.org/10.1016/s2225-4110\(16\)30066-9](https://doi.org/10.1016/s2225-4110(16)30066-9)
- Lee S, Kim H, Kang J-W, Kim J-H, Lee DH, Kim M-S, Yang Y, Woo E-R, Kim YM, Hong J (2011b) The biflavonoid amentoflavone induces apoptosis via suppressing E7 expression, cell cycle arrest at sub-G1 phase, and mitochondria-emanated intrinsic pathways in human cervical cancer cells. *J Med Food* 14:808–816. <https://doi.org/10.1089/jmf.2010.1428>
- Lee K-A, Lee S-H, Lee Y-J, Baeg SM, Shim J-H (2012) Hesperidin induces apoptosis by inhibiting Sp1 and its regulatory protein in MSTO-211H cells. *Biomol Ther (Seoul)* 20:273–279. <https://doi.org/10.4062/biomolther.2012.20.3.273>

- Li H-B, Chen F (2004) Preparative isolation and purification of bergapten and imperatorin from the medicinal plant *Cnidium monnieri* using high-speed counter-current chromatography by stepwise increasing the flow-rate of the mobile phase. *J Chromatogr A* 1061:51–54. <https://doi.org/10.1016/j.chroma.2004.10.091>
- Li GL, Xia Q, Chen SH, Gui SQ, Xu CJ (2012) Effects of Pinellia extract fraction on expressions of HPVE6 and P53 genes in cervical cancer cell lines. *China J Tradit Chin Med Pharm* 27:113–116. (in Chinese)
- Li Y, Cai W, Weng X, Li Q, Wang Y, Chen Y, Zhang W, Yang Q, Guo Y, Zhu X, Wang H (2015) Lonicerae japonicae flos and Lonicerae flos: a systematic pharmacology review. *Evid Based Complement Altern Med*. Article ID: 905063. <https://doi.org/10.1155/2015/905063>
- Li X, He Y, Zeng P, Liu Y, Zhang M, Hao C, Wang H, Ly Z, Zhang L (2019) Molecular basis for *Poria cocos* mushroom polysaccharide used as an antitumour drug in China. *J Cell Mol Med* 23:4–20. <https://doi.org/10.1111/jcmm.13564>
- Liang L-D, He T, Du T-W, Fan Y-G, Chen D-S, Wang Y (2015) Ginsenoside Rg-5 induces apoptosis and DNA damage in human cervical cancer cells. *Mol Med Rep* 11:940–946. <https://doi.org/10.3892/mmr.2014.2821>
- Lin X, Peng Z, Su C (2015) Potential anti-cancer activities and mechanisms of costunolide and dehydrocostuslactone. *Int J Mol Sci* 16:10888–10906. <https://doi.org/10.3390/ijms160510888>
- Lin J, Chen L, Qiu X, Zhang N, Guo Q, Wang Y, Wang M, Gober H-J, Li D, Wang L (2017) Traditional Chinese medicine for human papilloma virus (HPV) infections: a systematic review. *Biosci Trends* 11:267–273. <https://doi.org/10.5582/bst.2017.01056>
- Liu Y, Wang J, Wang W, Zhang H, Zhang X, Han C (2015) The chemical constituents and pharmacological actions of *Cordyceps sinensis*. *Evid Based Complement Altern Med*: Article ID 575063. <https://doi.org/10.1155/2015/575063>
- Liu P, Zhao H, Luo Y (2017) Anti-aging implications of *Astragalus Membranaceus* (Huangqi): a well-known Chinese tonic. *Aging Dis* 8:868–886. <https://doi.org/10.14336/AD.2017.0816>
- Luo K-w, Sun J-g, Chan J Y-w, Yang L, Wu S-h, Fung K-p, Liu F-Y (2011) Anticancer effects of imperatorin isolated from *Angelica dahurica*: induction of apoptosis in HepG2 cells through both death-receptor- and mitochondria-mediated pathways. *Chemotherapy* 57:449–459. <https://doi.org/10.1159/000331641>
- Ma XQ, Shi Q, Duan JA, Dong TT, Tsim KW (2002) Chemical analysis of radix Astragali (Huangqi) in China: a comparison with its adulterants and seasonal variations. *J Agric Food Chem* 50:4861–4866. <https://doi.org/10.1021/jf0202279>
- Ma XL, Xue XO, Li S, Wang Y, Xie W (2012) Clinical study of Zibai gel on treatment of cervical high-risk human papillomavirus infection. *Chin J Inform TCM* 19:9–12. (in Chinese)
- Ma ZQ, Hu H, He TT, Guo H, Zhang MY, Chen MW, Wang YT (2014) An assessment of traditional Uighur medicine in current Xinjiang region (China). *Afr J Tradit Complement Altern Med* 11:301–314. <https://doi.org/10.4314/ajtcam.v11i2.13>
- Mackenzie JS, Lindsay MDA, Smith DW, Imrie A (2017) The ecology and epidemiology of Ross River and Murray valley encephalitis viruses in Western Australia: examples of One Health in Action. *Trans R Soc Trop Med Hyg* 111:248–254. <https://doi.org/10.1093/trstmh/trx045>
- Madunić J, Madunić IV, Gajski G, Popić J, Garaj-Vrhovac V (2018) Apigenin: a dietary flavonoid with diverse anticancer properties. *Cancer Lett* 413:11–22. <https://doi.org/10.1016/j.canlet.2017.10.041>
- Mahata S, Bharti AC, Shukla S, Tyagi A, Husain SA, Das BC (2011) Berberine modulates AP-1 activity to suppress HPV transcription and downstream signaling to induce growth arrest and apoptosis in cervical cancer cells. *Mol Cancer* 10:39. <http://www.molecular-cancer.com/content/10/1/39>
- Manske RHF (1953) The alkaloids of fumariaceae plants. XLIX. Thalictricavine, a new alkaloid from *Corydalis tuberosa* DC. *J Am Chem Soc* 75(20):4928–4929. <https://doi.org/10.1021/ja01116a016>
- Maryam M, Te KK, Wong FC, Chai TT, Low GKK, Gan SC, Chee HY (2020) Antiviral activity of traditional Chinese medicinal plants *Dryopteris crassirhizoma* and *Morus alba* against dengue virus. *J Integr Agric* 19:1085–1096. [https://doi.org/10.1016/S2095-3119\(19\)62820-0](https://doi.org/10.1016/S2095-3119(19)62820-0)

- Máttar S, Teran CG, Figueiredo LT (2015) Diagnosis of hantavirus infection in humans. *Expert Rev Anti-Infect Ther* 13:939–946. <https://doi.org/10.1586/14787210.2015.1047825>
- McGeogh DJ, Cook S, Dolan A, Jamieson FE, Telford EAR (1995) Molecular phylogeny and evolutionary timescale for the family of mammalian herpes viruses. *J Mol Biol* 247:443–458. <https://doi.org/10.1006/jmbi.1995.0152>
- McGuffin M, Hobbs C, Upton R, Goldberg A (1997) American herbal products association. Botanical safety handbook. CRC Press, Boca Raton, pp 518–521
- Menéndez M, Hernández LÁ, vela de Oro OA, Chávez AT (2004) Actualización de la terapéutica del papilomavirus humano. Terapio convencional. *Rev Cubana Med* 43:34–41
- Meng J, Stobart CC, Hotard AL, Moore ML (2014) An overview of respiratory syncytial virus. *PLoS Pathog* 10:e1004016. <https://doi.org/10.1371/journal.ppat.1004016>
- Meyer-Hamme G, Beckmann K, Radtke J, Efferth T, Greten HJ, Rostock M, Schroder S (2013) A survey of Chinese medicinal herbal treatment for chemotherapy-induced oral mucositis. *Evid Based Complement Altern Med*: 284959. <https://doi.org/10.1155/2013/284959>
- Millman AJ, Nelson NP, Vellozzi C (2017) Hepatitis C: review of the epidemiology, clinical care, and continued challenges in the direct acting antiviral era. *Curr Epidemiol Rep* 4:174–185. <https://doi.org/10.1007/s40471-017-0108-x>
- Mishra A, Das BC (2015) Curcumin as an anti-human papillomavirus and anti-cancer compound. *Future Oncol* 11:1–3. <https://doi.org/10.2217/fon.15.166>
- Mlynarczyk-Bonikowska B, Majewska A, Malejczyk M, Mlynarczyk G, Majewski S (2013) Antiviral medication in sexually transmitted diseases. Part I: HSV, HPV. *Mini Rev Med Chem* 13:1837–1845. <https://doi.org/10.2174/13895575113136660088>
- Moga MA, Dimienescu OG, Arvatescu CA, Mironescu A, Dracea L, Ples L (2016) The role of natural polyphenols in the prevention and treatment of cervical cancer—an overview. *Molecules* 21:1055. <https://doi.org/10.3390/molecules21081055>
- Moga MA, Bălan A, Anastasiu CV, Dimienescu OG, Neculoiu CD, Gavris C (2018) An overview on the anticancer activity of *Azadirachta indica* (Neem) in Gynecological cancers. *Int J Mol Sci* 19:3898. <https://doi.org/10.3390/ijms19123898>
- Mortazavi HS, Monavari SHR, Ataei-Pirkooh A, Tavakoli A (2014) Middle East respiratory syndrome coronavirus (MERS-CoV): a review article. *Iranian J Virol* 8:59–68. <https://doi.org/10.21859/ivsv.8.2.3.59>
- Moutinho MSS, Aragão S, Carmo D, Casaca F, Silva S, Ribeiro J, Sousa H, Pires I, Queiroga F, Colaço B, Medeiros R, Oliveira PA, Lopes C, Bastos MMSM, Costa RMGD (2018) Curcumin and rutin down-regulate cyclooxygenase-2 and reduce tumor-associated inflammation in HPV16-transgenic mice. *Anticancer Res* 38:1461–1466. <https://doi.org/10.21873/anticancer.12371>
- Mulatu E, Feyisa A (2018) Review: yellow fever. *Res Rev J Med Health Sci* 7:15–21
- Munagala R, Aqil F, Jeyabalan J, Gupta RC (2015) Tanshinone IIA inhibits viral oncogene expression leading to apoptosis and inhibition of cervical cancer. *Cancer Lett* 356:536–546. <https://doi.org/10.1016/j.canlet.2014.09.037>
- Mustafa M, Elahee MI, Muniandy R, Sharifah AM, Mya N, Ramesh B (2016) Herpes simplex virus infections, pathophysiology and management. *IOSR J Dent Med Sci* 15:85–91. <https://doi.org/10.9790/0853-150738591>
- Mutombo AB, Tozin R, Simoens C, Lisbeth R, Bogers J, Geertruyden J-PV, Jacquemyn Y (2017) Efficacy of antiviral drug AV2 in the treatment of human papillomavirus-associated precancerous lesions of the uterine cervix: a randomized placebo-controlled clinical trial in Kinshasa, DR Congo. (KINVAV study). *Contemp Clin Trials Commun* 8:135–139. <https://doi.org/10.1016/j.conctc.2017.09.008>
- Nakamura K, Shinozuka K, Yoshikawa N (2015) Anticancer and antimetastatic effects of cordycepin, an active component of *Cordyceps sinensis*. *J Pharmacol Sci* 127:53–56. <https://doi.org/10.1016/j.jphs.2014.09.001>
- Newhouse VF, Burgdorfer W, McKiel JA, Gregson JD (1963) California encephalitis virus: serological survey of small wild mammals in northern United States and southern Canada and isola-

- tion of additional strains. *Am J Epidemiol* 78:123–129. <https://doi.org/10.1093/oxfordjournals.aje.a120326>
- Niu JJ, Song Y (2010) Effect of stichopus Japonicus acidic mucopolysaccharide on cell cycle of HeLa cells and its mechanism. *Med J Qilu* 25:386–388. (in Chinese)
- Ong CK, Chan SY, Campo SM, Fijnaga K, Mavromara-Nazos P, Labropoulou B, Pfister H, Tay SK, Meulen J, Villa LP, Bernard HU (1993) Evolution of human papilloma virus type 18: an ancient phylogenetic root in Africa and intratype diversity reflected co-evolution with human ethnic groups. *J Virol* 67:6424–6431
- Ortiz-Martínez Y, Vega-Useche L, Villamil-Gómez WE, Rodríguez-Morales AJ (2017) Saint Louis encephalitis virus, another re-emerging arbovirus: a literature review of worldwide research. *Infez Med* 1:77–79
- Park IU, Introcaso C, Dunne EF (2015) Human papillomavirus and genital warts: a review of the evidence for the 2015 centers for disease control and prevention sexually transmitted diseases treatment guidelines. *Clin Infect Dis* 61:S849–S855. <https://doi.org/10.1093/cid/civ813>
- Petersen LR, Brault AC, Nasci RS (2013) West Nile virus: review of the literature. *JAMA* 310:308–315. <https://doi.org/10.1001/jama.2013.8042>
- Poivre M, Duez P (2017) Biological activity and toxicity of the Chinese herb *Magnolia officinalis* Rehder & E. Wilson (Houpo) and its constituents. *J Zhejiang Univ Sci B* 18:194–214. <https://doi.org/10.1631/jzus.B1600299>
- Prakash O, Singh R, Kumar S, Srivastava S, Ved A (2017) *Gentiana lutea* Linn. (yellow gentian): a comprehensive review. *J Ayu Herb Med* 3:175–181
- Qian G-S, Wang Q, Leung KS-Y, Qin Y, Zhao Z, Jiang J-H (2007) Quality assessment of Rhizoma et Radix Notopterygii by HPTLC and HPLC and HPLC quantitative analysis. *J Pharm Biomed Anal* 44:812–817. <https://doi.org/10.1016/j.jpba.2007.03.029>
- Qin JL, Fu MX, Cheng JJ (2016) Clinical effect of Chinese medicine and Baofukang in treating high-risk human papilloma virus. *Modern J Integr Tradit Chin West Med* 25:1538–1540. (in Chinese)
- Rajesh ZM, Dhamande K, Bodla RB (2011) A review on study of swine flu. *Indo Glob Res J Pharm Sci* 1:47–51
- Ran X, Ma L, Peng C, Zhang H, Qin L-P (2011) *Ligusticum chuanxiong* Hort: a review of chemistry and pharmacology. *Pharm Biol* 49: 1180–1189. <https://doi.org/10.3109/13880209.2011.576346>
- Regea G (2017) Review on influenza virus and its prevention and control. *J Vet Med Res* 4:1089
- Rimkienė S, Ragažinskienė O, Savickienė N (2003) The cumulation of wild pansy (*Viola tricolor* L.) accessions: the possibility of species preservation and usage in medicine. *Medicina* 39:411–416
- Runowska M, Majewski D, Niklas K, Puszczewicz (2018) Chikungunya virus: a rheumatologist's perspective. *Clin Exp Rheumatol* 36:494–501
- Sadeghnia HR, Hesari TG, Mortazavian SM, Mousavi SH, Tayarani-Najaran Z, Ghorbani A (2014) *Viola tricolor* induces apoptosis in cancer cells and exhibits antiangiogenic activity on chicken chorioallantoic membrane. *Biomed Res Int: Article ID: 625792*. <https://doi.org/10.1155/2014/625792>
- Salehi B, Sener B, Kilic M, Sharifi-Rad J, Naz R, Yousaf Z, Mudau FN, Fokou PVT, Ezzat SM, El Bishbishy MH, Taheri Y, Lucariello G, Durazzo A, Lucarini M, Suleria HAR, Santini A (2019) *Dioscorea* plants: a genus rich in vital nutra-pharmaceuticals – a review. *Iran J Pharm Res* 18(Special Issue):68–89. <https://doi.org/10.22037/ijpr.2019.112501.13795>
- Sapp M, Volpers C, Müller M, Streeck RE (1995) Organization of the major and minor capsid proteins in human papillomavirus type 33 virus-like particles. *J Gen Virol* 76:2407–2412. <https://doi.org/10.1099/0022-1317-76-9-2407>
- Shen JJ, Liu ZH, Li J, Zhou YQ, Wang C, Wu RF (2013) The clinical observation for Baofukang and interferon in promoting the regression of low-grade cervical intraepithelial neoplasia. *Chin J Clin Obstet Gynecol* 14:509–512. (in Chinese)
- Shi H, Tan B, Ji G, Lu L, Cao A, Shi S, Xie J (2013) Zedoary oil (Ezhu You) inhibits proliferation of AGS cells. *Chin Med* 8:13. <https://doi.org/10.1186/1749-8546-8-13>

- Shin KM, Kim YH, Park WS, Kang I, Ha J, Choi JW, Park HJ, Lee KT (2004) Inhibition of methanol extract from the fruits of *Kochia scoparia* on lipopolysaccharide-induced nitric oxide, prostaglandin E2, and tumor necrosis factor- α production from murine macrophage RAW 264.7 cells. *Biol Pharm Bull* 27:538–543. <https://doi.org/10.1248/bpb.27.538>
- Sidh V, Sharma O (2019) Medicinal use of Makhanna (*Euryale ferox* Salisb): a review article. *World J Pharm Med Res* 5:103–104
- Silva DCM, dos Santos Moreira Silva EA, Gomes JAS, da Fonseca FG, Correa-Oliveira R (2010) Clinical signs, diagnosis, and case reports of Vaccinia virus infections. *Braz J Infect Dis* 14:129–134. <https://doi.org/10.1590/s1413-86702010000200003>
- Singh N, Shukla S, Gupta V, Tandia N, Singh P (2015) Mosquito borne zoonotic diseases: a review. *J Livestock Sci* 6:65–72
- Son ES, Kim S-H, Kim YO, Lee YU, Kyung SY, Jeong SH, Kim YJ, Park J-W (2019) *Coix lacryma-jobi* var. *ma-yuen* Stapf sprout extract induces cell cycle arrest and apoptosis in human cervical carcinoma cells. *BMC Complement Altern Med* 19:312. <https://doi.org/10.1186/s12906-019-2725-z>
- Song J-Z, Mo S-F, Yip Y-K, Qiao C-F, Han Q-B, Xu H-X (2007) Development of microwave assisted extraction for the simultaneous determination of isoflavonoids and saponins in radix Astragali by high performance liquid chromatography. *J Sep Sci* 30:819–824. <https://doi.org/10.1002/jssc.200600340>
- Song Y-C, Huang H-C, Chang CY-Y, Lee H-J, Liu C-T, Lo H-Y, Ho T-Y, Lin W-C, Yen H-R (2020) A potential herbal adjuvant combined with a peptide-based vaccine acts against HPV-related tumors through enhancing effector and memory Y-cell immune responses. *Front Immunol* 11:62. <https://doi.org/10.3389/fimmu.2020.00062>
- Stanley MA (2010) Pathology and epidemiology of HPV infection in females. *Gynecol Oncol* 117:S5–S10. <https://doi.org/10.1016/j.ygyno.2010.01.024>
- Stem PL, van der Burg SH, Hampson IN, Broker T, Fiander A, Lacey CJ, Kitchener HC, Einstein MH (2012) Therapy of human papillomavirus-related disease. *Vaccine* 30:F71–F82. <https://doi.org/10.1016/j.vaccine.2012.05.091>
- Sun H-L, Zhang Y-Q, Xie X-Y, Che Y-Y (2014) Studies on chemical constituents from seeds of *Euryale ferox*. *Zhong Yao Cai* 37:2019–2021
- Sun Y, Lenon GB, Yang AWH (2019) Phellodendri cortex: a phytochemical, pharmacological, and pharmacokinetic review. *Evid Based Complement Altern Med: Article ID: 7621929*. <https://doi.org/10.1155/2019/7621929>
- Tada K, Takido M, Kitanaka S (1999) Limonoids from fruit of *Melia toosendan* and their cytotoxic activity. *Phytochemistry* 51:787–791. [https://doi.org/10.1016/s0031-9422\(99\)00115-6](https://doi.org/10.1016/s0031-9422(99)00115-6)
- Taghipour M, Babamahmoodi F, Arashnia P, Taghipour S (2015) Orf virus infection in human (*Ecthyma Contagiosum*): a report of eight cases in the north of Iran. *Int J Med Invest* 4:183–186
- Temizel EM (2015) Outbreak of bovine papular stomatitis with concurrent cryptosporidiosis in a dairy herd in Turkey. *Bulg J Vet Med* 19:78–83. <https://doi.org/10.15547/bjvm.922>
- Timmons B, Akins M, Mahendroo M (2010) Cervical remodeling during pregnancy and parturition. *Trends Endocrinol Metab* 21:353–361. <https://doi.org/10.1530/REP-07-0032>
- Tong XQ, Lv W, Luo WQ, Lu WX, Li N (2016) Treatment of human cervical cancer cells with butein leads to apoptosis and DNA damage. *Int J Clin Exp Med* 9:11084–11089
- Tu Y, Xue Y, Guo D, Sun L, Guo M (2015) Carthami flos: a review of its ethnopharmacology, pharmacology and clinical applications. *Rev Bras Farmacogn* 25:553–566. <https://doi.org/10.1016/j.bjp.2015.06.001>
- Tu Y-F, Chien C-S, Yarmishyn AA, Lin Y-Y, Luo Y-H, Lin Y-T, Lai W-Y, Yang D-M, Chou S-J, Yang Y-P, Wang M-L, Chiou S-H (2020) A review of SARS-CoV-2 and the ongoing clinical trials. *Int J Mol Sci* 21:2657. <https://doi.org/10.3390/ijms21072657>
- Tyrell DAJ, Bynoe ML (1965) Cultivation of novel type of common-cold virus in organ cultures. *Br Med J* 1:1467–1470. <https://doi.org/10.1136/bmj.1.5448.1467>
- Ueng T-H, Kang J-J, Wang H-W, Lin P-C (1997) An overview of the toxicology of commonly used Traditional Chinese Medicine. *J Food Drug Anal* 5:241–264. <https://doi.org/10.38212/2224-6614.2932>

- van der Hoek L, Pyrc K, Jebbink MF, Vermeulen-Oost W, Berkhout RJM, Wolthers KC, Wertheim-van Dillen PME, Kaandorp J, Spaargaren J, Berkhout B (2004) Identification of a new human coronavirus. *Nat Med* 10:368–373. <https://doi.org/10.1038/nm1024>
- Waddell L, Pachal N, Mascarenhas M, Greig J, Harding S, Young I, Wilhelm B (2019) Cache Valley virus: a scoping review of the global evidence. *Zoonoses Public Health* 66:1–20. <https://doi.org/10.1111/zph.12621>
- Wang J, Yuan L, Xiao H, Wang C, Xiao C, Wang Y, Liu X (2014a) A novel mechanism for momordin Ic-induced HepG2 apoptosis: involvement of PI3K and MAPK-dependent PPAR γ activation. *Food Funct* 5:859–868. <https://doi.org/10.1039/c3fo60558b>
- Wang S-X, Zhang X-S, Guan H-S, Wang W (2014b) Potential anti-HPV and related cancer agents from marine resources: An overview. *Mar Drugs* 12:2019–2035. <https://doi.org/10.3390/md12042019>
- Wilkins T, Zimmerman D, Schade RR (2010) Hepatitis B: diagnosis and treatment. *Am Fam Physician* 81:965–972
- Woo PC, Lau SK, Chu C-M, Chan K-H, Tsoi H-W, Huang Y, Wong BHL, Poon RWS, Cai JJ, Luk W-K, Poon LLM, Wong SSY, Guan Y, Peiris JSM, Yuen K-Y (2005) Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *J Virol* 79:884–895. <https://doi.org/10.1128/JVI.79.2.884-895.2005>
- Woolhouse MEJ, Gaunt E (2007) Ecological origins of novel human pathogens. *Crit Rev Microbiol* 33:1–12. <https://doi.org/10.1080/10408410701647560>
- Woolhouse M, Scott F, Hudson Z, Howey R, Chase-Topping M (2012) Human viruses: discovery and emergence. *Philos Trans R Soc Lond B Biol Sci* 367:2864–2871. <https://doi.org/10.1098/rstb.2011.0354>
- Wu Y-C, Hsieh C-L (2011) Pharmacological effects of *Radix Angelica Sinensis* (*Danggui*) on cerebral infarction. *Chin Med* 6:32. <https://doi.org/10.1186/1749-8546-6-32>
- Wu X, Xiao F, Zhang Z, Li X, Xu Z (2005) Research on the analgesic effect and mechanism of bornyl acetate in volatile oil from *Amomum villosum*. *Zhong Yao Cai* 28:505–507
- Xiao J, Huang JL, Cai LE (2011) Study of Youdujing in treating HR-HPV infected cervical lesions. *Guangdong Med J* 32:2036–2039. (in Chinese)
- Xiao J, Wu J, Yu B (2012) Therapeutic efficacy of youdujing preparation in treating cervical high-risk human papilloma virus infection patients. *Zhongguo Zhong Xi Yi Jie He Za Zhi* 32:1212–1215
- Xiao Z, Xiao S, Zhang Y, Pan T, Ouyang B (2018) The anti-inflammatory effect of Fructus Kochiae on allergic contact dermatitis rats via pERK1/2/TLR4/NF- κ B pathway activation. *Evid Based Complement Altern Med: Article ID 1096920*. <https://doi.org/10.1155/2018/1096920>
- Xu YX, Yuan L (2016) Improvement of cervical microenvironment and effect of Erhuang powder on CIN I accompanied by human papillomavirus infection according to Th1/Th2 immune balance. *Liaoning J Tradit Chin Med* 43:962–965. +1118. (in Chinese)
- Yan F, Li F, Liu J, Ye S, Zhang Y, Jia J, Li H, Chen D, Mo X (2020) The formulae and biologically active ingredients of Chinese herbal medicines for the treatment of atopic dermatitis. *Biomed Pharmacother* 127:110142. <https://doi.org/10.1016/j.biopha.2020.110142>
- Yang X, Zhou S-L, Ma A-C, Xu H-T, Guan H-S, Liu H-B (2012) Chemical profiles and identification of key compound caffeine in marine-derived Traditional Chinese Medicine *Ostrea Concha*. *Mar Drugs* 10:1180–1191. <https://doi.org/10.3390/md10051180>
- Yang Z, Wang Y, Zheng Z, Zhao S, Zhao J, Lin Q, Li C, Zhu Q, Zhong N (2013) Antiviral activity of *Isatis indigotica* root-derived clemastanin B against human and avian influenza A and B viruses *in vitro*. *Int J Mol Med* 31:867–873. <https://doi.org/10.3892/ijmm.2013.1274>
- Yang L, Hou AJ, Yan ML, Xing XD, Guo XY, Jiang H, Yang BY, Chan K, Wang QH, Kuang HX (2019) Investigation of radix achyranthis bidentatae phytochemistry and pharmacology. *World J Tradit Chin Med* 5:50–60. <https://www.wjtcn.net/text.asp?2019/5/1/50/253495>
- Yang L, Li Q, Feng Y, Qiu D (2020) Simultaneous determination of three coumarins in *Angelica dahurica* by ^1H -qNMR method: a fast and validated method for crude drug quality control. *J Anal Methods Chem: Article ID 8987560*. <https://doi.org/10.1155/2020/8987560>

- Yao X-X, Jiang S-L, Tang Y-W, Yao D-M, Yao X (2005) Efficacy of Chinese medicine Yi-gan-kang granule in prophylaxis and treatment of liver fibrosis in rats. *World J Gastroenterol* 11:2583–2590. <https://doi.org/10.3748/wjg.v11.i17.2583>
- Yarnell E (2015) Herbs against Human Papillomavirus. *Altern Complement Ther* 21:71–76. <https://doi.org/10.1089/act.2015.21205>
- Zanotti KM, Belinson J (2002) Update on the diagnosis and treatment of human papillomavirus infection. *Cleve Clin J Med* 69:948–961. <https://doi.org/10.3949/ccjm.69.12.948>
- Zeng L, Zhen Y, Chen Y, Zou L, Zhang Y, Hu F, Feng J, Shen J, Wei B (2014) Naringin inhibits growth and induces apoptosis by a mechanism dependent on reduced activation of NFκB/COX-2-caspase-1 pathway in HeLa cervical cancer cells. *Int J Oncol* 45:1929–1936. <https://doi.org/10.3892/ijo.2014.2617>
- Zhang L-L, Tian K, Tang Z-H, Chen X-J, Bian Z-X, Wang Y-T, Lu J-J (2016) Phytochemistry and pharmacology of *Carthamus tinctorius* L. *Am J Chin Med* 44:197–226. <https://doi.org/10.1142/S0192415X16500130>
- Zhang Y, Liang Y, He C (2017) Anticancer activities and mechanisms of heat-clearing and detoxicating traditional Chinese herbal medicine. *Chin Med* 12:20. <https://doi.org/10.1186/s13020-017-0140-2>
- Zhao L, Li C, Zhang Y, Wen Q, Ren D (2014) Phytochemical and biological activities of an anti-cancer plant medicine: *Brucea javanica*. *Anti Cancer Agents Med Chem* 14:443–458. <https://doi.org/10.2174/18715206113136660336>
- Zhao Y, Wang Q-M, Yao L-T, Han X (2018) Effect of paiteling on the alteration of persistent HR-HPV infection after panhysterectomy. *Eur J Inflamm* 16:1–4. <https://doi.org/10.1177/2058739218819172>
- Zhou S, Zheng S, Zheng C, Qu F, Cai X, Xu J (2012) A thermosensitive gel formulation of an empirical traditional Chinese prescription for treating cervical erosion. *Acta Pharm Sin B* 2:495–501. <https://doi.org/10.1016/j.apsb.2012.05.005>
- Zhu HB, Wang YZ, Liu YX, Xia YI, Tang T (2010) Analysis of flavonoids in *Portulaca oleracea* L. by UV-vis spectrophotometry with comparative study on different extraction technologies. *Food Anal Method* 3:90–97. <https://doi.org/10.1007/s12161-009-9091-2>
- Zhu H-H, Huang G-H, Tate PL, Larcom LL (2012) Anti-cancer effect of *Angelica sinensis* on women's reproductive cancer. *Funct Foods Health Dis* 2:242–250. <https://doi.org/10.31989/ffhd.v2i6.86>

Chapter 9

Natural Products from Plants with Antimicrobial Action



Patrícia e S. Alves, Juliana S. de Figuerêdo, Felipe P. S. Santos,
Pedro V. O. S. Furtado, Teresinha J. A. S. Andrade, Joaquim S. C. Júnior,
Nerilson M. Lima, and Chistiane M. Feitosa

Abstract In recent years, there has been a continuous increase in resistance to traditional antibiotics developed by pathogenic bacteria. However, in many parts of the world, several medicinal plants are traditionally used to control infectious microorganisms. Because of this, antimicrobial agents derived from natural products have received a lot of attention, both for their effectiveness and also for being more economically accessible. Therefore, the research discussed in this chapter aims to conduct a systematic review on the use of medicinal plants and isolated compounds as a potential antimicrobial agent. The study focuses on an investigation of several electronic databases: Scopus, Web of Science, Academic Google, SciELO, PubMed, SciFinder, and ScienceDirect. The results may contribute to the increase of strategies for the treatment of infections caused by microorganisms. Since medicinal plants play an essential role in health, they may represent a significant source of new antimicrobial drugs to combat microorganisms resistant to multidrug.

Keywords Natural products · Antimicrobial action · Plants · Antimicrobial resistance

Abbreviations

CHNS	Carbon, hydrogen, nitrogen, sulfur
DW	Dry weight

P. e. S. Alves (✉) · J. S. de Figuerêdo · F. P. S. Santos · Pedro V. O. S. Furtado · C. M. Feitosa
Post-Graduation Department in Chemistry, Federal University of Piauí, Teresina, Brazil

Teresinha J. A. S. Andrade
Nucleus of Applied Research to Sciences (NIAC), Federal Institute of Maranhão, Campus
Presidente Dutra, São Luis, Brazil

J. S. C. Júnior
Federal Institute of Piauí (IFPI), Teresina, Brazil

N. M. Lima
Federal university of Juiz de Fora, Chemistry Department, Juiz de Fora, Brazil

EUCAST	European Committee for Antimicrobial Susceptibility Testing
FDA	Food and Drug Administration
FT-IR	Fourier transform-infrared
HPLC	High-performance liquid chromatography
HPLC-PCDA	High-efficiency liquid chromatography with photodiode arrangement detection
LC-MS	Liquid chromatography-mass spectrometry
MBC	Minimum bactericidal concentration
MIC	Minimum inhibitory concentration
NMR	Nuclear magnetic resonance
OEs	Oils Essenciais Naturais
UHPLC-ESI-MS/MS	High-efficiency liquid chromatography coupled with tandem mass spectrometry
UPLC-PDA	Ultra-performance liquid chromatography coupled with photodiode array
US	United States
UV-VIS	Ultraviolet spectroscopy-visible
WHO	World Health Organization

1 Introduction

Starting from the twentieth century, there were significant advances in the field of science with the use of antimicrobial drugs, such as penicillin which was discovered in 1928 by Sir Alexander Fleming, intending to improve human life expectancy and control microbial infections (Aminov 2010). Antimicrobials can be chemotherapy (synthetic) or antibiotics (natural substances) that inhibit the development of microorganisms or even destroy them. Antimicrobial drugs are considered the second-most used drug class globally (Saez-Llorens et al. 2000; Abushaheen et al. 2020).

As these drugs are more widely used, microorganisms develop resistance, quickly causing a global problem (Anand et al. 2020). The use of antimicrobial drugs without a medical prescription is a problem reported at least half a century ago (Scheckler and Bennett 1970; Kunin et al. 1973). This is due to the misdiagnosis between a bacterial infection and a viral infection, the absence of educational programs for the rational use of antimicrobials or the wide distribution of antimicrobials. In this way, the use of these drugs and improper self-medication exacerbate antimicrobial resistance (Mota et al. 2010).

This resistance to antibiotics has become one of the main problems of humanity since the end of the twentieth century. Therefore, the search for new antimicrobials effective in eliminating microbes becomes necessary, given that the traditional approaches to find new antimicrobial drugs are no longer effective due to the rapid resistance developed against them (Abreu et al. 2012).

One of the strategies applied for the treatment of infectious diseases would be natural products. For several years, medicinal plants derived from these natural products play an essential role in health. They could represent a significant source of new antimicrobial drugs to combat multidrug-resistant microorganisms (Aleksic Sabo and Knezevic 2019). Besides, antimicrobial resistance to plant extracts would be less likely, since a wide variety of their active compounds are found in these extracts that could reverse antibiotic resistance and minimize man's exposure to resistant bacteria (Gupta and Birdi 2017).

Bioactive compounds called natural products come from the biosynthesis of plants, lichens, fungi, bacteria, and microorganisms. Antibiotics, terpenoids, alkaloids, and polyketides are the main microbial natural products (Gunatilaka and Wijeratne 2012; Huang and Lin 2017; Newman and Cragg 2016).

It is noteworthy that approximately 50% of approved drugs are related to plants or natural products, and 10% of all antimicrobial agents are from natural plant products (Al-Marzoqi et al. 2016; Cowan 1999; Veeresham 2012). In this way, natural products are still used as a powerful therapy against pathogenic bacteria and are considered the pillars of discovering new antibiotics (Wright 2017).

According to Moore et al. (2017), there is an evident lack of new antimicrobial drugs. They are emphasizing the need to search for new bioactive therapeutic agents as treatment strategies for infections caused by microorganisms. In addition to medicinal plants, critical antimicrobial properties have been evidenced, demonstrating the enormous potential that does not control infectious diseases or that suggests as a valuable source of research for the removal of antimicrobial compost.

2 Antimicrobial Resistance

Antimicrobial resistance is a global public health problem, which has worsened over time, as infections caused by resistant microorganisms, in many cases, do not respond to treatment, which can increase hospital costs and increase the risk of death (Fair and Tor 2014). Some studies report that if there are no measures to neutralize this problem, there may be a beginning of a post-antibiotic era, with a shortage of effective treatments based on antimicrobials (Vasoo et al. 2015; Vanegas-Múnera and Jiménez-Quiceno 2020).

As these antimicrobials are indiscriminately used, microorganisms were developed by creating different forms of resistance, which led to a global problem (Davies and Davies 2010). Alexander Fleming, who received the Nobel Prize in Medicine in 1945, warned about the severe problem of antimicrobial resistance. Still, human being did nothing, and thus resistant bacteria emerged as a result of natural selection (Ventola 2015). This problem worsened over time due to the inappropriate use of antibiotics, was also favored by the lack of standards and control of their service, such as the ease of acquiring these drugs without a prescription, in addition to false marketing of antimicrobials (Organización de las Naciones Unidas para la Alimentación y la Agricultura 2016).

The first reports of antimicrobial resistance were published in 1944, three years after starting the use of penicillin. The isolates of *Staphylococcus aureus* were reported, these being the first isolates resistant to this antibiotic. Since then, the situation has only worsened; at times in the 1980s, there were few options of antibiotics available (Tang et al. 2014). Currently, the resistance rate is so high that some doctors use antibiotics that were previously discarded, because they have specific toxicity, such as colistin (Loho and Dharmayanti 2015).

Antimicrobial resistance can be of two types: natural or acquired. Natural or intrinsic, occur when there are bacteria of the same species naturally resistant to some families of antibiotics, which leads these bacteria to have a competitive advantage over other strains; therefore, the antibiotic has no effect. The acquired resistance can be attributed to mutations in chromosomes (stable, spontaneous, and transmitted vertically from generation to generation) or to changes in resistance genes (conjugation, translation or transformation and transmitted horizontally) (Guimarães et al. 2010; Pérez 2017).

The increase in antimicrobial resistance has severe consequences for public health, limiting the therapeutic variability of antimicrobial in combating pathologies in humans (Semret and Haraoui 2019; Costa et al. 2020). This increase in antimicrobial resistance is due to the misuse of these agents to resistant strains, such as methicillin to *Staphylococcus aureus*, vancomycin-resistant enterococci, resistance to drugs *Streptococcus pneumoniae* and *Mycobacterium tuberculosis*, enterobacteria, which produce beta-lactamase, extended-spectrum, and resistant to carbapenems, *Pseudomonas aeruginosa*, resistant to multiple drugs and *Acinetobacter baumannii* (Sabo and Knezevic 2019).

Thus, fungi became resistant to polyols, azoles, and echinocandins, which contributed to the emergence of drug-resistant strains and were found in all species of fungi (Robbins et al. 2017). An aggravating factor given the remarkable emergence of resistant protozoa and viruses is a limited number of antiprotozoal and antiviral agents (El-Taweel 2015; Irwin et al. 2016).

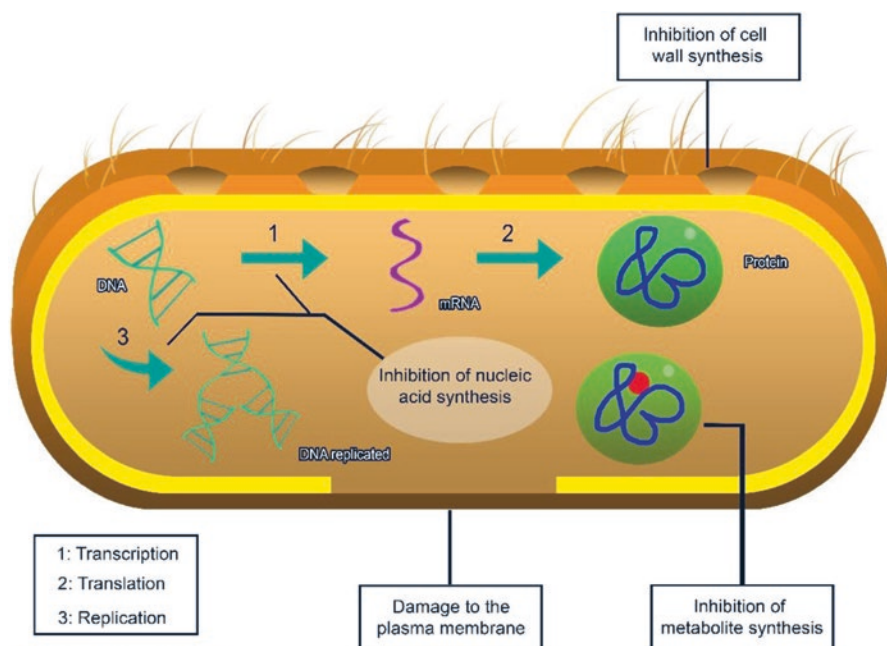
Plants are of great importance in modern medicine. Thus, the plant kingdom can be an alternative to combat antimicrobial resistance, so there is a search for new antimicrobial drugs that can combat resistant microorganisms. This can play an essential role in combating microbial resistance (Sabo and Knezevic 2019). However, there is little incentive from the pharmaceutical industries to produce new antimicrobial, mainly due to the low financial return, thus limiting research for this type of medicine (Aslam et al. 2018).

With the current antimicrobial resistance, a post-antibiotic era is getting closer and more concrete every day. This problem is due to not only the evolution and adaptation of these microorganisms but also by those who prescribe these drugs, by those who use them, by those responsible for the control and distribution of these drugs (Vanegas-Múnera and Jiménez-Quiceno 2020). Thus, public policies are needed to raise awareness and educate the population about the use of antimicrobial, as well as health professionals can limit the unnecessary use of antimicrobial (Hu et al. 2020).

3 Classification and Mechanism of Action

Antimicrobials are classified according to their chemical structure (derived from amino acids, sugars, acetates, propionates, among others), types of microorganisms on which they act, or even the effect of the organism (Manrique and Galvão 1997). Besides, they are classified through factors, such as the mechanism of action, bacterial activity, susceptible microorganisms, the spectrum of action, and mainly through the assessment of their natural or synthetic origin (Anvisa 2020).

The mechanism of action of an antimicrobial starts when it reaches an ideal concentration at the infection site, then actively or passively passes through the cell wall, showing an affinity for the binding site inside the bacteria and remaining long enough to exercise its inhibitory activity. Antibiotics can have bactericidal action, killing microorganisms directly, acting in strong reactions to the infecting cell, or just inhibiting bacterial growth through a bacteriostatic action, keeping the bacteria in a stationary phase. In bacteriophage, the host triggers its defenses, such as phagocytosis and the production of antibodies to control the invading microorganism. In this case, the inhibition can be reversible, as the bacteria can continue to produce toxins or become resistant to the drug if the host's defenses are not efficient Fig. 9.1. (Katzung 2007; Lago 2011; Brunton et al. 2012; Tortora et al. 2012; Pankey and Sabath 2004; Engleberg et al. 2013).



Source: Persona data.

Fig. 9.1 Mechanism of action of antibiotics. (Source: Personal data)

Regarding natural antibiotics, together with their semi-synthetic derivatives, they comprise most of the antibiotics used clinically and can be classified into β -lactams (penicillins, cephalosporins, carbapenems, oxapenins, and monobactams), tetracyclines, aminoglycosides, glycopeptides, lipodepsipeptides, streptogramins, lincosamines, chloramphenicol, among others. Antibiotics of synthetic origin are classified as sulfonamides, fluoroquinolones, and oxazolidinones (Abraham 2003; Patrick 2005; Pupo et al. 2006).

Other important examples of antimicrobial classes are Macrolides (Bologa et al. 2013), Glycopeptides (Guimarães et al. 2010), Amphenicols (Von 2004), and Quinolones (Emmerson and Jones 2003).

4 Herbal Medicines with Antimicrobial Action

The first antimicrobial drugs used successfully against potentially fatal infectious diseases were Salvarsan (arsphenamine) and its derivative nearsphenamine, used against syphilis (infection with *Treponema pallidum*) in 1911 (Mann 1984; Gaynes 2017). During the late 1930s and early 1940s, sulfamide antibiotic derivatives (e.g., prontosil, sulfamethazine) effective against lobar pneumonia (caused mainly by *Streptococcus pneumoniae*), meningococcal meningitis (caused by *Neisseria meningitidis*), and gonorrhea (caused by *Neisseria gonorrhoeae*) were introduced to the market (Kumar and Clark 1998; Gaynes 2017). However, until the discovery of penicillin (USA: 1945; United Kingdom 1946), these synthetic agents remained the only chemotherapeutic agents used for bacterial infections (Mann 1984).

However, the widespread use of antibiotics created an evolutionary adaptation of bacteria, leading to the so-called resistance to multiple drugs (Neu 1992). The consequence of the increased resistance of bacterial strains to biocidal agents is an increased risk of chronic infections and difficulties in their treatment (wound infections, osteomyelitis, septic arthritis, endocarditis, etc.) (Karam et al. 2016). However, there is a strong need to develop new compounds that are not only highly efficient but also that should not cause resistance to development in bacteria (Kyzioł et al. 2020).

In search of these new antibiotics, research on herbal medicines may prove promising since these medicines have been used for centuries to treat infectious diseases (Landers et al. 2012). They are readily available for patients to purchase themselves and appear to be increasingly popular, especially in the United Kingdom. This growing public interest in herbs can reduce dependence on antibiotics, especially for self-limited infections (Hu et al. 2020). A recent WHO report on traditional medicines noted that most of the world's population depends on traditional medicines for health care, including treatment of infections (WHO 2002).

Thus, compounds isolated from medicinal plants are preferable to synthetic compounds due to their use in conventional medicine (Rakholiya et al. 2013). Therefore, these compounds are considered a substituted source of antimicrobial drugs (Savoia 2012).

5 Plant Antimicrobial Activity

5.1 Extracts with Antimicrobial Action

Natural products are used since ancient times for the treatment and, or control of infectious diseases among them from fungi, bacteria, or pathogenic microorganisms (Dkhil et al. 2020; Frassinetti et al. 2020; Silva 2006). These products include extracts, fractions, essential oils, isolated compounds, among other medicinal plant derivatives, which may be promising for the advancement of new studies, therapies, and treatments for pathologies (Frassinetti et al. 2020; Mehlhorn 2014; Hayek et al. 2013). Thus, some tasks related to the development and progress of studies on prominent plant extracts, in potential, with antimicrobial action will be addressed.

Antimicrobial activities of the ethanolic extracts of the flowers and leaves of ten species of the genera *Senna* and *Cassia* were evaluated against seven bacteria and three strains of fungi, oral aerobic and anaerobic bacteria, and *Candida* spp. by the microdilution broth method. This investigation was due to the use of these species in treatments of infections in the Brazilian traditional medicine, used as laxative agents, analgesics, and antifungals for mycosis and other fungal infections of the skin. Nascimento et al. (2020) developed this work, showing that among the tested species, *Cassia fistula* L., *Senna macranthera* (Collad), *Cassia bakeriana* Craib, and *Senna spectabilis* DC., showed moderate activity against two bacterial strains with MIC values (minimum inhibitory concentration) varying between 200.0 and 400.0 $\mu\text{g}\cdot\text{mL}^{-1}$, while the ethanolic extract of *S. macranthera* flowers showed very low values of MIC (23.4, 11.7, and 5.9 $\mu\text{g}\cdot\text{mL}^{-1}$) in the antifungal test.

Thus, the ethanol extract of *S. macranthera* flowers was subjected to liquid-liquid extraction with solvents of different polarities, which include n-hexane, dichloromethane and ethyl acetate. The ethyl acetate fraction of *S. macranthera* flowers showed better antifungal activity (MIC values of 5.9 $\mu\text{g}\cdot\text{mL}^{-1}$ for *Candida glabrata*, 23.4 $\mu\text{g}\cdot\text{mL}^{-1}$ for *Candida albicans*, and (0.1 – 0.2 $\mu\text{g}\cdot\text{mL}^{-1}$) for *Candida tropicalis* using amphotericin B as a positive control, so this fraction indicated potential antifungal action and selectivity against strains evaluated. It is noteworthy that the MIC represents the lowest concentration of the extract capable of preventing microbial growth. From this fraction, analysis by UHPLC-ESI-MS/MS (High-Efficiency Liquid Chromatography Coupled by Tandem Mass Spectrometry) were performed, which identified different types of phenolic compounds, in particular, proanthocyanidins in several isomeric forms.

This class of secondary metabolites, proanthocyanidins (condensed tannins), are phenolic compounds (Kyraleou et al. 2020; Monteiro et al. 2005), active against species of *Candida* and thus may be associated with the antifungal action of the fraction cited (Piccinelli et al. 2016; Freitas et al. 2018). This study has collaborated to strengthen the traditional use of the species from these genera *Senna* and *Cassia*, particularly the flowers of *S. macranthera*, as a good and promising source of discoveries of compounds as antifungal agents.

Weremczuk-Jeřyna et al. (2019) evaluated the anti-pathogenic potential of the hydromethanolic extract of *Dracocephalum forrestii* sprouts cultivated in nutrient-sprinkled bioreactors. These antimicrobial evaluations were performed against selective strains of bacteria, six Gram-pathogenic, and four Gram-negative, and three pathogenic strains of fungi, which showed moderate activity limiting the growth of pathogens. The minimum bactericidal concentration (MBC) and MIC activity of the extract were analyzed, where most of the strains tested were within the range of 2.5–5 mg.mL⁻¹.

A greater effect was observed on *Bacillus cereus*, *Escherichia coli*, and *Pseudomonas aeruginosa* with MIC 2.5 mg mL⁻¹. The hydromethanolic extract at a concentration of 2.5 mg mL⁻¹. showed antifungal activity against *Candida albicans* and *Candida glabrata*. They were determined by the microdilution broth method according to the European Committee for Antimicrobial Susceptibility Testing (EUCAST). Rosmarinic acid, gentamicin, and fluconazole (all from Sigma-Aldrich) were used as reference antimicrobials with values lower than 1–5 mg.mL⁻¹.

In this work, the phenolic compounds present in the cultivated extract were evaluated qualitatively by UPLC-PDA-ESI-MS (ultra-performance liquid chromatography (UPLC) coupled to photodiode array detection (PDA) and electrospray ionization (ESI) mass spectrometry) and quantitatively by UPLC-PDA. Rosmarinic acid (17.90 mg g⁻¹ DW) and salvianolic acid B (6.50 mg g⁻¹ DW) were verified in larger quantities. Deba et al. (2008) and Estevinho et al. (2008) reported that plant material rich in polyphenols, bioactive phenolic compounds, may be associated with anti-pathogenic effects due to the interaction between these constituents. The author suggests further studies to improve the research and thus to estimate a possible synergistic effect of *D. forrestii* extract on a likely antibiotic therapy against multi-resistant bacteria.

Fei et al. (2018), in their studies, reveal that the potent antioxidant activity of two polyphenols can be considered as one of the ways this class of natural products inhibits the development of pathogenic microorganisms.

5.2 *Phytoconstituents with Antimicrobial Action*

The use of antibiotics and/or synthetic or natural compounds to treat infectious diseases is growing widely (Hemaiswarya et al. 2008; Bimani and Hossain 2020). Thus, scientists are increasingly improving their search for finding natural compounds bioactive that may be able to exercise biological activities to treat, improve, or resolve the resistance of microorganisms to synthetic drugs (Bimani and Hossain 2020; Vidhya et al. 2020).

Andrade et al. (2020) evaluated the antimicrobial activity of two isolated natural molecules, Braquidines BR-A and BR-B, against *S. aureus*, *E. coli*, and *C. albicans*. The isolation and analysis were performed from the purification of the dichloromethane fraction of *Arrabidaea brachypoda* flowers, by HPLC-PCDA

(High-Efficiency Liquid Chromatography with photodiode arrangement detection) and UV-VIS (Ultraviolet Spectroscopy-visible) revealing and identifying phenolic compounds, dimeric flavonoids differentiating only in the aromatic C ring by the presence of a hydroxyl group in BR-A, already in BR-B there is a methoxyl group, giving purity of 95 and 97%, respectively. BR-A showed no antimicrobial activity against the microbial strains tested, since MIC values above $1000 \mu\text{g.mL}^{-1}$, presenting a MIC of $1024 \mu\text{g.mL}^{-1}$, were determined by microdilution assay. Thus, according to the literature, these are considered clinically irrelevant results (Houghton et al. 2007). However, the BR-B showed antifungal activity against *C. albicans* of $161 \mu\text{g.mL}^{-1}$, showing promising future studies and improvements.

A phytochemical study on *Trianthema decandra* leaves was performed by Geethalakshmi and Sarada (2018), where two compounds of chloroform extract from the leaf were isolated and characterized using HPLC, UV, FT-IR, RMN, LC-MS, and CHNS techniques. Identifying a new sterol named as 17-(5-ethyl-6-methylheptan-2-yl) - 4, 4, 10, 13-tetramethyl-hexadecahydro-1H-cyclopenta (α) phenanthren-3-ol and the flavonoid as 2-(3',4'dihydroxyphenyl) 3,5,7-trihydroxychromen-4-one. The antimicrobial activity of the sterol and flavonoid isolates were analyzed by disc diffusion and broth dilution assays, showing good results against the tested microorganisms, in particular sterol presented an MIC of $39.05 \mu\text{g mL}^{-1}$ against the strain *Salmonella typhi* and the flavonoid presented MICs of 78.10 and $39.05 \mu\text{g mL}^{-1}$ against *Vibrio cholerae* and *P. aeruginosa*, respectively.

5.3 Essential Oils with Antimicrobial Action

A variety of oils essential (OEs), derived from aromatic plants, are used as insecticide, antiparasitic, fungicide, virucidal, bactericide, cosmetics, food, and agricultural industries (Dhifi et al. 2016; Atif et al. 2020). These OEs also act as preservatives, sedatives, antimicrobial, and spasmolytic analgesics. The chemical composition of OEs includes sesquiterpenes, monoterpenes, and their oxygenated compounds, such as oxides, phenols, ketones, ethers, esters, aldehydes, molecules containing nitrogen or sulfur (Ahmed et al. 2019).

With the presence of secondary metabolisms, OEs play a protective role against various microbes in plants. The actions generated by these metabolites can inhibit or slow the growth of bacteria, yeasts, and molds, whose components have a variety of targets, particularly on the microbial membrane and cytoplasm, and in some situations, they drastically alter cell morphology (Chorianopoulos et al. 2008; De Martino et al. 2009).

Because they have antimicrobial activity against several microorganisms, OEs are considered an alternative to conventional antibiotics (Silva-Santos et al. 2017).

Herbs such as cloves, cinnamon, oregano, peppers, rosemary, and thyme have OEs that generate intense antibacterial activity against *S. aureus*, *S. typhi*, and *P. aeruginosa* (Conner and Branen 1993). Among all the essential oils tested, clove

oil was the most effective. In addition, it was found that carvacrol has antimicrobial activity against a broad spectrum of bacterial strains (Fernández-Pan et al. 2015; Kristo et al. 2008).

Mohamed et al. (2013) reported effective inhibition in pathogenic bacterial strains such as *N. gonorrhoeae*, *E. coli*, *Bacillus subtilis*, *S. aureus*, and *P. aeruginosa*, developed by *Syzygium cumini* OE, containing 1,3,6 limonenoene, trans-carcinogenic-octatriene, β -pinene, δ -3-carene, α -pinene and α -caryophyllene; these compounds are responsible for the bactericidal action.

Because they come from plant sources, OEs can also develop synergistic activity, which effectively combat the growth of various microorganisms. Some constituents such as carvacrol, γ -terpinene, and p-cymene are more effective when combined (Elshafie and Camele 2017).

OEs are mentioned as potent antimicrobial agents, as they have substantial antibacterial activities against Gram-positive and Gram-negative pathogens (Yap et al. 2014). For example, OEs derived from medicinal aromatic plants, such as peppermint (*Mentha piperita*), thyme (*Thymus vulgaris*), and fennel (*Foeniculum vulgare*), are cataloged as effective against viruses, Gram-negative and Gram-positive bacteria, fungi, and yeast (Reichling 2018).

Various volatile, terpenic, and phenolic substances present in OEs show remarkable antimicrobial activity (Marchese et al. 2016). And according to the literature, OEs that contain these terpenes, phenolic, and aldehydes have an excellent application in biomedicine for properly eliminating many viral, fungal, and bacterial pathogens (Swamy et al. 2016).

6 Perspectives with the Use of Secondary Plant Metabolites

Antimicrobials have played an essential role in human health since their discovery. This relevance is reflected in the results obtained using antimicrobial pre-agents, which are also among the drugs most used by medicine. The discriminated and illicit use of antibiotics has resulted in the emergence of drug resistance among pathogens, which in many parts of the world, especially in developing countries, have reached critical levels (Ayukebong et al. 2017).

This practical and negligent use of antibiotics is a growing concern worldwide, fueling antimicrobial resistance. In the clinical context, particularly in the health care sector, it is vital to validate a prescription for antibiotics before administering the medication. Besides, the dosage of antibiotics must be such that pathogenic bacteria are eliminated in a complete cycle of antibiotics. However, there are few effective techniques for a quick discovery of susceptibility to drugs. Continuous unintended exposure of bacteria to antibiotics can promote antimicrobial resistance that can increase in the body for longer in most living organisms. The development of technologies to detect antibiotics (mainly used in the therapy of human infection)

is essential to containing the threat of antimicrobial resistance to guarantee its ideal use in the treatment of human bacterial infection (Nag et al. 2020).

Antimicrobial products characterized as drugs have a shelf life, changes in the metabolic and genetic level, a faster rate of evolution such as variable global temperature, a catalog of widely documented side effects. However, the main concerns of doctors and scientists are the prolonged use of antimicrobials, the high cost of clinical research and drug development. The main obstacle to antibiotic resistance is developing effective classes of antibiotics with a new mode of action (Anand et al. 2020).

It should be noted that more than 80% of the world population depends on conventional pharmaceutical products made up of medicinal plants to meet different conditions of human medicine (Ekor 2014; Oyeboode et al. 2016; Pan et al. 2014; Mahomoodally 2013). Worldwide interest in research aimed at discovering new drugs from plant sources is growing (Anand et al. 2020).

The natural products are characterized as a diverse group with different bioactivities for therapeutic purposes. Natural sources are privileged source in searching for new antimicrobial molecules (Agrawal et al. 2017). More than half of medicines in clinical use approved by the US Food and Drug Administration (FDA) are derived from natural products (Newman and Cragg 2016).

Therefore, the work appears with the perspective of contributing to the development of new antimicrobial drugs to combat microorganisms resistant to multidrug drugs, in addition to enabling positive actions about natural compounds derived from plants, which may later be the basis of pre-clinical studies in humans and in a future perspective, which will contribute to implementation of these compounds of natural origin in the treatment of pathologies.

7 Conclusions

This chapter pointed out that phytochemicals, extracts, and derivatives of natural compounds could be effective in the treatment of resistant microorganisms, since they have antimicrobial activity against microorganisms, especially in the therapeutic approach or acting as drugs. In this way, natural products may serve as a promising therapeutic source for various pathogenic microorganism, and therefore are pillars for discovering new antibiotics. However, the pharmacological utility of these products will require further studies. Thus, this review becomes extremely important, as it shows medicinal plants as a source of new therapies that can contribute to the discovery of potential candidates for the treatment of resistant microorganisms.

Acknowledgments We appreciate the support from UFPI.

References

- Abraham DJ (2003) *Burger's medicinal chemistry & drug discovery: chemotherapeutic agents*, 6th edn. Wiley, Hoboken
- Abreu AC, McBain AJ, Simoes M (2012) Plants as sources of new antimicrobials and resistance-modifying agents. *Nat Prod Rep* 29:1007–1021
- Abushaheen MA, Fatani AJ, Alosaimi M, Mansy W, George M, Acharya S, Rathod S, Divakar DD, Jhugroo P (2020) Antimicrobial resistance, mechanisms and its clinical significance. *Dis Mon* 66:1–21
- Agrawal S, Acharya D, Adholeya A, Barrow CJ, Deshmukh SK (2017) Nonribosomal peptides from marine microbes and their antimicrobial and anticancer potential. *Front Pharmacol* 8:1–26
- Ahmed AF, Attia FA, Liu Z, Li C, Wei J, Kang W (2019) Antioxidant activity and total phenolic content of essential oils and extracts of sweet basil (*Ocimum basilicum* L.) plants. *Food Sci Hum Well* 8:299–305
- Aleksic AS, Knezevic P (2019) Antimicrobial activity of *Eucalyptus camaldulensis* Dehn. plant extracts and essential oils: a review. *Ind Crop Prod* 132:413–429
- Al-Marzoqi AH, Al-Khafaji NMS, Hussein HJ (2016) In vitro antibacterial activity assessment of the crude phenolic, alkaloid and terpenoid compounds extracts of *Lepidium sativum* L. on human pathogenic bacteria. *Int J ChemTech Res* 9:529–532
- Aminov RI (2010) A brief history of the antibiotic era: lessons learned and challenges for the future. *Front Microbiol* 1:1–7
- Anand U, Nandy S, Mundhra A, Das N, Pandey DK, Dey A (2020) A review on antimicrobial botanicals, phytochemicals and natural resistance modifying agents from Apocynaceae family: possible therapeutic approaches against multidrug resistance in pathogenic microorganisms. *Drug Resist Updat* 51:1–8
- Andrade LMS, Oliveira ABM, Leal ALAB, Oliveira FAA, Portela AL, Neto JDSL, Siqueira-Júnior JP, Kaatz GW, Rocha CQ, Barreto HM (2020) Antimicrobial activity and inhibition of the NorA efflux pump of *Staphylococcus aureus* by extract and isolated compounds from Arrabidaea brachypoda. *Microb Pathog* 140:2–7
- ANVISA. Agência Nacional de Vigilância Sanitária. *Bulário Eletrônico da Anvisa*. Disponível em: <http://bulario.bvs.br>. Access in: Set/2020
- Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, Rasool MH, Nisae MA, Alvi RF, Aslam MA, Qamar UM, Salamat MKF, Baloch Z (2018) Antibiotic resistance: a rundown of a global crisis. *Infect Drug Resist* 11:1645–1658
- Atif M, Ilavenil S, Devanesan S, AlSalhi MS, Choi KC, Vijayaraghavan P, Alfuraydi AA, Alanazi NF (2020) Essential oils of two medicinal plants and protective properties of jack fruits against the spoilage bacteria and fungi. *Ind Crop Prod* 147:1–9
- Ayukekbong JA, Ntemgwa M, Atabe AN (2017) The threat of antimicrobial resistance in developing countries: causes and control strategies. *Antimicrob Resist Infect Control* 6:1–8
- Bimani BMH, Hossain MA (2020) A new antimicrobial compound from the leaves of *Dodonaea viscosa* for infectious diseases. *Bioact Mater* 5:602–610
- Bologa CG, Ursu O, Oprea TI, Melançon CE III, Tegos GP (2013) Emerging trends in the discovery of natural product antibacterials. *Curr Opin Pharmacol* 13:678–687
- Brunton LL, Chabner BA, Knollmann BC (2012) *As Bases Farmacológicas da Terapêutica*, 12th edn. McGraw-Hill, Brazil
- Chorianopoulos NG, Giaouris ED, Skandamis PN, Haroutounian SA, Nychas GJ (2008) Disinfectant test against monoculture and mixed-culture biofilms composed of technological, spoilage and pathogenic bacteria: bactericidal effect of essential oil and hydrosol of *Satureja thymbra* and comparison with standard acid–base sanitizers. *J Appl Microbiol* 104:1586–1596
- Conner DE, Davidson P, Branen AL (1993) *Naturally occurring compounds*, 1st edn. Marcel Dekker, New York
- Costa MDC, Cruz AIC, Bispo ASDR, Ferreira MA, Costa JA, Evangelista-Barreto NS (2020) Occurrence and antimicrobial resistance of bacteria in retail market spices. *Cienc Rural* 50:1–7

- Cowan MM (1999) Plant products as antimicrobial agents. *Clin Microbiol Rev* 12:564–582
- Davies J, Davies D (2010) Origins and evolution of antibiotic resistance. *Microbiol Mol Biol Rev* 74:417–433
- De Martino L, De Feo V, Nazzaro F (2009) Chemical composition and in vitro antimicrobial and mutagenic activities of seven Lamiaceae essential oils. *Molecules* 14:4213–4230
- Deba F, Xuan TD, Yasuda M, Tawata S (2008) Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from *Bidens pilosa* Linn. var. *Radiata*. *Food Control* 19:346–352
- Dhifi W, Bellili S, Jazi S, Bahloul N, Mnif W (2016) Essential oils' chemical characterization and investigation of some biological activities: a critical review. *Medicines* 3:1–16
- Dkhil MA, Zreiq R, Hafiz TA, Mubarak MA, Sulaiman S, Algahtani F, Abdel-Gaber R, Al-Shaebi EM, Al-Quraishy S (2020) Anthelmintic and antimicrobial activity of *Indigofera oblongifolia* leaf extracts. *Saudi J Biol Sci* 27:594–598
- Ekor M (2014) The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol* 4:1–10
- Elshafie HS, Camele I (2017) An overview of the biological effects of some mediterranean essential oils on human health. *Biomed Res Int* 2017:1–14
- El-Taweel HA (2015) Understanding drug resistance in human intestinal protozoa. *Parasitol Res* 114:1647–1659
- Emmerson A, Jones A (2003) The quinolones: decades of development and use. *J Antimicrob Chemother* 51:13–20
- Engleberg NC, Dirita V, Dermody T (2013) Schaechter's mechanisms of microbial disease, 5th edn. Lippincott Williams & Wilkins, Philadelphia
- Estevinho L, Pereira AP, Moreira L, Dias LG, Pereira E (2008) Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. *Food Chem Toxicol* 46:3774–3779
- Fair RJ, Tor Y (2014) Antibiotics and bacterial resistance in the 21st century. *Perspect Medicin Chem* 6:25–64
- Fei P, Ali MA, Gong S, Sun Q, Bi X, Liu S, Guo L (2018) Antimicrobial activity and mechanism of action of olive oil polyphenols extract against *Cronobacter sakazakii*. *Food Control* 94:289–294
- Fernández-Pan I, Maté JI, Gardrat C, Coma V (2015) Effect of chitosan molecular weight on the antimicrobial activity and release rate of carvacrol-enriched films. *Food Hydrocoll* 51:60–68
- Frassinetti S, Gabriele M, Moccia E, Longo V, Gioia DD (2020) Antimicrobial and antibiofilm activity of *Cannabis sativa* L. seeds extract against *Staphylococcus aureus* and growth effects on probiotic *Lactobacillus* spp. *LWT Food Sci Technol* 124:109–149
- Freitas ALD, Kaplum V, Rossi DCP, Silva LBR, Melhem MSC, Taborda CP, Mello JCP, Nakamura CV, Ishida K (2018) Proanthocyanidin polymeric tannins from *Stryphnodendron adstringens* are effective against *Candida* spp. isolates and for vaginal candidiasis treatment. *J Ethnopharmacol* 216:184–190
- Gaynes R (2017) The discovery of penicillin—new insights after more than 75 years of clinical use. *Emerg Infect Dis* 23:849–853
- Geethalakshmi R, Sarada VLD (2018) In vitro and in silico antimicrobial activity of sterol and flavonoid isolated from *Trianthema decandra* L. *Microb Pathog* 121:77–86
- Guimaraes DO, Momesso LS, Pupo MT (2010) Antibióticos: importância terapêutica e perspectivas para a descoberta e desenvolvimento de novos agentes. *Quim Nova* 33:667–679
- Gunatilaka A, Wijeratne E (2012) Natural products from bacteria and fungi. *J Pharmacogn Phytochem* 3:1–27
- Gupta PD, Birdi TJ (2017) Development of botanicals to combat antibiotic resistance. *J Ayurveda Integr Med* 8:266–275
- Hayek SA, Gyawali R, Ibrahim S (2013) Antimicrobial natural products, 1st edn. Formatex, Spain: Badajoz

- Hemaiswarya S, Kruthiventi AK, Doble M (2008) Synergism between natural products and antibiotics against infectious diseases. *Phytomedicine* 15:639–652
- Houghton PJ, Howes MJ, Lee CC, Steventon G (2007) Uses and abuses of in vitro tests in ethnopharmacology: visualizing an elephant. *J Ethnopharmacol* 110:391–400
- Hu XY, Logue M, Robinson N (2020) Antimicrobial resistance is a global problem—a UK perspective. *Eur J Integr Med* 36:1–4
- Huang T, Lin S (2017) Microbial natural products: a promising source for drug discovery. *Appl Microbiol Biochem* 1:1–3
- Irwin KK, Renzette N, Kowalik T, Jensen JD (2016) Antiviral drug resistance as an adaptive process. *Virus Evol* 2:1–10
- Karam G, Chastre J, Wilcox MH, Vincent JL (2016) Antibiotic strategies in the era of multidrug resistance. *Crit Care* 20:1–9
- Katzung BG (2007) *Farmacologia Básica e Clínica*, 9th edn. McGraw, Brasil
- Kristo E, Koutsoumanis KP, Biliaderis CG (2008) Thermal, mechanical and water vapor barrier properties of sodium caseinate films containing antimicrobials and their inhibitory action on *Listeria monocytogenes*. *Food Hydrocoll* 22:373–386
- Kumar P, Clark M (1998) *Clinical medicine*, 4th edn. W.B. Saunders, Edinburgh
- Kunin CM, Tupasi T, Craig WA (1973) Use of antibiotics: a brief exposition of the problem and some tentative solutions. *Ann Intern Med* 79:555–560
- Kyraleou M, Kallithraka S, Gkanidi E, Koundouras S, Mannion DT, Kilcawley KN (2020) Discrimination of five Greek red grape varieties according to the anthocyanin and proanthocyanidin profiles of their skins and seeds. *J Food Compost Anal* 92:1–9
- Kyziół A, Khan W, Sebastian V, Kyziół K (2020) Tackling microbial infections and increasing resistance involving formulations based on antimicrobial polymers. *Chem Eng J* 385:1–18
- Lago J (2011) *Mecanismos de Resistência e Seleção de Antibióticos*, 1st edn. Jornadas bioMérieux, Lisboa
- Landers TF, Cohen B, Wittum TE, Larson EL (2012) A review of antibiotic use in food animals: perspective, policy, and potential. *Public Health Rep* 127:4–22
- Loho T, Dharmayanti AC (2015) Um antibiótico e seu papel em infecções Gram-negativas multirresistentes. *Acta Med Indones* 47:157–168
- Mahomoodally MF (2013) Traditional medicines in Africa: an appraisal of ten potent African medicinal plants. *Evid Based Complement Alternat Med* 2013:2–14
- Mann RD (1984) *Modern drug use: an enquiry on historical principles*, 1st edn. MTP Press Limited, London
- Manrique EI, Galvão LL (1997) Racionalização e controle de antimicrobianos. *Infecções hospitalares Prevenção e Controle* 6:117–130
- Marchese A, Orhan IE, Daglia M, Barbieri R, Lorenzo AD, Nabavi SF, Gortzi O, Izadi M, Nabavi SM (2016) Antibacterial and antifungal activities of thymol: a brief review of the literature. *Food Chem* 210:402–414
- Mehlhorn H (2014) *Encyclopedic reference of parasitology*, 6th edn. Springer Press, Cham
- Mohamed AA, Ali SI, El-Baz FK (2013) Antioxidant and antibacterial activities of crude extracts and essential oils of *Syzygium cumini* leaves. *PLoS One* 8:1–7
- Monteiro JM, Albuquerque UP, Araújo EL, Amorim ELC (2005) Taninos: uma abordagem da química à ecologia. *Quim Nova* 28:892–896
- Moore BS, Carter GT, Brønstrup M (2017) Are natural products the solution to antimicrobial resistance? *Nat Prod Rep* 34:685–686
- Mota LM, Vilar FC, Dias LBA, Nunes TF, Moriguti JC (2010) Uso racional de antimicrobianos. *Medicina (Ribeirão Preto)* 43:164–172
- Nag P, Sadani K, Mukherji S, Mukherji S (2020) Beta-lactam antibiotics induced bacteriolysis on LSPR sensors for assessment of antimicrobial resistance and quantification of antibiotics. *Sens Actuators B Chem* 311:2–9

- Nascimento MNG, Martins MM, Cunha LCS, Santos PS, Goulart LR, Silva TS, Martins CHG, Morais SAL, Pivatto M (2020) Antimicrobial and cytotoxic activities of Senna and Cassia species (Fabaceae) extracts. *Ind Crop Prod* 148:1–11
- Neu HC (1992) The crisis in antibiotic resistance. *Science* 257:1064–1073
- Newman DJ, Cragg GM (2016) Natural products as sources of new drugs from 1981 to 2014. *J Nat Prod* 79:629–661
- Organización de las Naciones Unidas para la Alimentación y la Agricultura (2016) El Plan de acción de la FAO sobre la resistencia a los antimicrobianos 2016–2020. Organización de las Naciones Unidas para la Alimentación y la Agricultura, Roma. Disponible en: <http://www.fao.org/publications>
- Oyebode O, Kandala NB, Chilton PJ, Lilford RJ (2016) Use of traditional medicine in middle-income countries: a WHO-SAGE study. *Health Policy Plan* 31:984–991
- Pan SY, Litscher G, Gao SH, Zhou SF, Yu ZL, Chen HQ, Zhang SF, Tang MK, Sun JN, Ko KM (2014) Historical perspective of traditional indigenous medical practices: the current renaissance and conservation of herbal resources. *Evid Based Complement Alternat* 2014:1–20
- Pankey G, Sabath L (2004) Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram positives bacterial infections. *Clin Infect Dis* 38:864–870
- Patrick GL (2005) An introduction to medicinal chemistry, 1st edn. Oxford University Press, New York
- Pérez DQ (2017) Resistencia antimicrobiana: evolución y perspectivas actuales ante el enfoque "Una salud". *Rev Cubana Med Trop* 69:1–17
- Piccinelli AL, Pagano I, Esposito T, Mencherini T, Porta A, Petrone AM, Gazerro P, Picerno P, Sansone F, Rastrelli L, Aquino RP (2016) HRMS profile of a hazelnut skin proanthocyanidin-rich fraction with antioxidant and anti-candida albicans activities. *J Agric Food Chem* 64:585–595
- Pupo MT, Guimarães DO, Furtado NAJC, Borges WS (2006) Microbial natural products: a promising source of bioactive compounds, 2nd edn. Research Signpost, Thiruvananthapuram
- Rakholiya K, Kaneria M, Desai D, Chanda S (2013) Microbial pathogens and strategies for combating them: science, technology and education. *Microbiol Book Ser* 2:850–856
- Reichling J (2018) Plant-microbe interactions and secondary metabolites with antiviral, antibacterial and antifungal properties. *Ann Plant Rev* 39:189–279
- Robbins N, Caplan T, Cowen LE (2017) Molecular evolution of antifungal drug resistance. *Annu Rev Microbiol* 71:753–775
- Sabo VA, Knezevic P (2019) Antimicrobial activity of Eucalyptus camaldulensis Dehn. plant extracts and essential oils: a review. *Ind Crop Prod* 132:413–429
- Saez-Llorens X, Wong MMC, Castaño E, Suman OD, Moros DD, Atencio ID (2000) Impact of an antibiotic restriction policy on hospital expenditures and bacterial susceptibilities: a lesson from a pediatric institution in a developing country. *Pediatr Infect Dis J* 19:200–206
- Savoia D (2012) Plant-derived antimicrobial compounds: alternatives to antibiotics. *Future Microbiol* 7:979–990
- Scheckler WE, Bennett JV (1970) Antibiotic usage in seven community hospitals. *JAMA* 213:264–267
- Semret M, Haraoui LP (2019) Antimicrobial resistance in the tropics. *Infect Dis Clin* 33:231–245
- Silva MB (2006) Controle alternativo de pragas e doenças, 1st edn. EPAMIG-CTZM/UFV
- Silva-Santos C, Piccoli RH, Tebaldi VMR (2017) Atividade antimicrobiana de óleos essenciais e compostos isolados frente aos agentes patogênicos de origem clínica e alimentar. *Rev Inst Adolfo Lutz* 76:17–19
- Swamy MK, Akhtar MS, Sinniah UR (2016) Antimicrobial properties of plant essential oils against human pathogens and their mode of action: an updated review. *Evid Based Complement Alternat* 2016:1–21
- Tang SS, Apisarnthanarak A, Hsu LY (2014) Mechanisms of β -lactam antimicrobial resistance and epidemiology of major community- and healthcare-associated multidrug-resistant bacteria. *Adv Drug Deliv Ver* 78:3–13

- Tortora GJ, Funke BR, Case CL (2012) *Microbiologia*, 10th edn. Artmed Editora
- Vanegas-Múnera JM, Jiménez-Quiceno JN (2020) Resistencia antimicrobiana en el siglo XXI: ¿hacia una era postantibiótica? *Rev Fac Nac Salud Pública* 38:1–6
- Vasoo S, Barreto JN, Tosh PK (2015) Emerging issues in gram-negative bacterial resistance: an update for the practicing clinician. *Mayo Clin Proc* 90:395–403
- Veeresham C (2012) Natural products derived from plants as a source of drugs. *J Adv Pharm Technol Res* 3:200–201
- Ventola CL (2015) The antibiotic resistance crisis: part I: causes and threats. *Pharmacol Ther* 40:277–283
- Vidhya E, Vijayakumar S, Rajalakshmi S, Kalaiselvi S, Pandiyan P (2020) Antimicrobial activity and phytochemical screening of *Ocimum americanum* L extracts against pathogenic microorganisms. *Acta Ecol Sin* 40:214–220
- Von DH (2004) Protein science: a publication of the protein society. *Sci Phitochem* 13(1–25):2004
- Weremczuk-Jeśyna I, Kochan E, Szymczyk P, Lisiecki P, Kuzma L, Grzegorzczak-karolak I (2019) The antioxidant and antimicrobial properties of phenol-rich extracts of *Dracocephalum forrestii* W. W. Smith shoot cultures grown in the nutrient sprinkle bioreactor. *Phytochem Lett* 30:254–260
- WHO (2002) WHO traditional medicine strategy 2002–2005. <http://www.who.int/medicines/publications/traditionalpolicy/en/index.html>
- Wright GD (2017) Opportunities for natural products in 21st century antibiotic discovery. *Nat Prod Rep* 34:694–701
- Yap PSX, Yiap BC, Ping HC, Lim SHE (2014) Essential oils, a new horizon in combating bacterial antibiotic resistance. *Open Microbiol J* 8:6–14

Chapter 10

Volatile Organic Compounds and Their Capacity for Controlling Fungal Infection in Humans



Fernanda Achimón, Carolina Merlo, Romina P. Pizzolitto,
José S. Dambolena, Alejandra Omarini, and Julio A. Zygodlo

Abstract It is estimated that over 30% of the world's population has ever had a fungal infection. The most common fungal diseases are nail and skin infections, which are mainly caused by fungi of the genus *Trichophyton* spp., *Epidermophyton* spp., or *Microsporum* spp. Onychomycosis is a fungal infection that affects mainly nails, but can also cause foot and leg ulcerations, leading to extreme situations, such as limb amputation. Among dermatophytes, *T. rubrum* is mainly responsible for skin infections. Antifungal compounds, such as azoles, allylamines, and amorolfine (administered orally or topically), are usually used in the treatment of this disease. The oral mucosa and the genital tract are other targets of attack by fungal pathogens. In these cases, yeasts of the genus *Candida* spp. play a crucial role. It is estimated that about 75% of women have suffered *Candida* spp. vulvovaginitis at least once in their lives. Other opportunistic pathogenic fungi that frequently affect human health are some species belonging to the genus *Cryptococcus* spp. (cutaneous mycoses and opportunistic mycoses) and *Aspergillus* spp. (allergic reactions, keratitis and opportunistic onychomycosis). The usual problem for the treatment of these infections is

F. Achimón

Instituto Multidisciplinario de Biología Vegetal, Consejo Nacional de Investigaciones Científicas y Técnicas. IMBIV-CONICET, Córdoba, Argentina

C. Merlo · R. P. Pizzolitto

Instituto Multidisciplinario de Biología Vegetal, Consejo Nacional de Investigaciones Científicas y Técnicas. IMBIV-CONICET, Córdoba, Argentina

Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Córdoba, Argentina

J. S. Dambolena · J. A. Zygodlo (✉)

Instituto Multidisciplinario de Biología Vegetal, Consejo Nacional de Investigaciones Científicas y Técnicas. IMBIV-CONICET, Córdoba, Argentina

Facultad de Ciencias Exactas, Físicas y Naturales, Instituto de Ciencia y Tecnología de los Alimentos, Universidad Nacional de Córdoba, Córdoba, Argentina

e-mail: jzygodlo@unc.edu.ar

A. Omarini

Asociación para el Desarrollo de Villa Elisa y Zona, Villa Elisa, Entre Ríos, Argentina

the fungal resistance to azoles, polyenes, echinocandins, among other existing drugs. In this context, there is an urgent need to find out new alternatives for the treatment of fungal infections. Natural products, such as volatile organic compounds, essential oils, or their components, have shown promising antifungal activities. In this chapter, we will discuss the latest findings about the antifungal activity of volatile organic compounds, essential oils, and their individual components against fungi of clinical importance. Moreover, the structure related to the antifungal activity of these natural compounds, their mechanisms of action, and their synergistic properties will also be explored.

Keywords Dermatophytes · *Candida* · Opportunistic fungi · Volatile organic compounds · Terpenoids · Antifungal properties

Abbreviations

AIDS	Acquired immunodeficiency syndrome
EO(s)	Essential oil(s)
FICI	Fractional Inhibitory Concentration Index
ISO	International Organization for Standardization
ISOE	International Standard Organization on Essential Oils (ISOE)
LUMO	Lowest Unoccupied Molecular Orbital
MIC	Minimum inhibitory concentration
MLR	Multiple linear regression analyses
QSAR	Quantitative structure–activity relationship
RMSPE	Root mean square prediction error
SAPs	Secreted aspartic proteases
SAR	structure–activity relationship

1 Introduction

Fungal infections affect more than 30% of the world population (Hameed and Fatima 2019). The incidence of dermatophytosis, like onychomycosis, or superficial mycoses, such as tinea pedis or tinea cruris, has increased in recent years, and in many patients they manifest as chronic or recurrent diseases (Arora et al. 2020). Dermatophytosis and other fungal infections frequently occur in immunocompromised patients, such as those with acquired immunodeficiency syndrome (AIDS), immunologically impaired transplant patients, or patients that are being treated for more critical diseases like cancer (Cassella et al. 2002). Although dermatophytosis mainly affects adult men (Alessandrini et al. 2020), it shows a strong incidence with high morbidity and mortality in pediatric and geriatric populations of both genders (Khan et al. 2014; Gupta et al. 2020). Dermatophytosis is transmitted directly

between people (anthropophilic), by interaction with pets (zoophilic), soil material (geophilic), or indirectly through sharing clothes, brushes, and towels (Arora et al. 2020). Even when they are not life-threatening, superficial fungal infections have several psycho-social effects on patients, affecting their quality of life (Alessandrini et al. 2020). This reality has led to abandon the idea that superficial mycoses are a problem related to beauty; instead, they should be considered as an important public health problem (Bouyahya et al. 2018; Arora et al. 2020)

Onychomycosis is the most important dermatophytosis due to the large number of patients that suffer from this disease (Bouyahya et al. 2018; Alessandrini et al. 2020). Onychomycosis is caused by keratinophilic fungi producers of proteases that break keratin allowing infection of the stratum corneum of the skin, the hair shaft, and the nail (both toenails and fingernails) (Alessandrini et al. 2020). This disease requires long-term treatments, like other fungal diseases. Derivatives of morpholine, azoles, and allylamines are the most frequently used compounds for their control; however, they present dangerous hazardous side effects in many patients, such as high hepatotoxicity, alopecia, among other health problems (Alessandrini et al. 2020; Hameed and Fatima 2019; Marx-Stoelting et al. 2020). For these reasons, topical treatments are preferred since they are associated with low risk of adverse side effects (Alessandrini et al. 2020). The non-responsible use of antifungal drugs causes the raising of resistant strains, which leads to an increase in the treatment doses, hence producing stronger side effects (Baptista et al. 2015; Padovan et al. 2019; Bouyahya et al. 2020). Among the anthropophilic dermatophytes, we can find *Trichophyton rubrum* and *Trichophyton interdigitale* (Alessandrini et al. 2020), *Microsporium* spp., and *Epidermophyton* spp. (Arora et al. 2020). Among the non-dermatophyte fungi that cause mycosis in humans or animals we can find *Scopulariopsis brevicaulis* and species belonging to the genera *Fusarium* spp., *Aspergillus* spp., *Acremonium* spp., *Alternaria* spp., and *Scytalidium* spp. (Alessandrini et al. 2020).

In recent years, the number of scientific reports that assessed natural substances for the development of new antifungals has significantly increased, mainly because many of them produced no side effects (Bouyahya et al. 2020). Among natural products, essential oils (EOs) have shown promising results for the treatment of various diseases such as cancer (Najar et al. 2020), hypertension (Oboh et al. 2017), and diabetes (Lari et al. 2020). The EOs have also proved effective as antibacterial (Zygadlo et al. 2017), antifungal (Pizzolitto et al. 2020; Achimón et al. 2021), anti-parasitic (Azadbakht et al. 2020), anti-inflammatory (Yeh and Lin 2020), and anti-oxidants (Raspo et al. 2020). These medicinal and pharmacological properties are related to the chemical composition of EOs and to the lipophilic nature of the EO molecules (Baptista et al. 2015; Zygadlo et al. 2017). The combination of EOs or their bioactive components with synthetic antifungal drugs enable the reduction of drug concentration, thus decreasing its side effects (Lopes et al. 2017; Ali-Shtayeh et al. 2019). This combination of conventional antifungals and EOs or their components has shown many successful results in treating microorganisms that have developed multi-drug resistance (Lopes et al. 2017). This is very important in diseases such as dermatophytosis where the possibility of recurrence is very frequent, which is why treatments are prolonged over time. Decreasing doses is a main point in patient care to avoid chronic toxicity due to the action of the drugs.

2 Essential Oils and Their Components as Anti-dermatophytic Compounds

Antifungal drugs used for the control of dermatophytes comprise econazole, ketoconazole, fluconazole, itraconazole, miconazole, thioconazole, and clotrimazole. The most frequently used allylamines are terbinafine and naftifine, while the derivatives of morpholine amorolfine and butenafine are the third group of most widely used drugs (Gupta et al. 2020; Hameed and Fatima 2019). Combinations of these anti-dermatophytic drugs have also been performed to synergize their effects and break resistance in microorganisms, hence decreasing their dose (Martinez-Rossi et al. 2018; Padovan et al. 2019; Da Costa et al. 2020). Essential oils are plant-derived-metabolites, often lipophilic and liquid at room temperature, which are stored in specialized glands. The extraction of EOs can be carried out by using different techniques (Zygadlo 2011). The International Standard Organization on Essential Oils (ISOE) states that the extractive technique to obtain EOs from their vegetable sources must be by hydrodistillation of the aromatic plant (Hameed and Fatima 2019). Essential oils are a mixture of monoterpenes, phenylpropanoids, sesquiterpenes, and diterpenes. The functional groups of these compounds are usually diverse; we can find phenols, alcohols, ketones, ethers, aldehydes, and esters (Zygadlo 2011). This enormous chemical diversity of EOs can provide a broad spectrum of biological activities (Chan et al. 2016; Lari et al. 2020; Lira et al. 2020; Najjar et al. 2020). Although most bibliographic information indicates that the main action site of EOs or their components is the plasma membrane, the great chemical diversity of these mixtures leads us to think of a multi-target spectrum that involves many mechanisms of action (Buriani et al. 2020). The type of climate, the composition and structure of the soil, the age of the aromatic plants, the organ that produces the EOs, the vegetative state of the plant are some of the variables that also cause great diversity in the composition of EOs (Zygadlo 2011). This list still increases with the development of new cultivars of aromatic plants. For these reasons, to avoid inconveniences in the international trade when marketing and selecting an EO, they must be standardized and comply with quality and composition standards determined by the International Organization for Standardization (ISO) (Zygadlo 2011). This standardization does not exist for other natural compounds and must be performed under the governmental laws of each country, which regulate compounds with pharmacological properties. Hence, the EO that is marketed under ISO standards has its composition guaranteed, which would allow an almost immediate use in the development of antifungal formulations. Many EOs have been evaluated for their antifungal properties (Donato et al. 2020) and their bioactivity against resistant strains (Gallucci et al. 2014), showing strong synergizing properties when combined with synthetic antifungals (Amber et al. 2010; Da Costa et al. 2020; Nogueira Sobrinho et al. 2020). In any case, few studies have been carried out on dermatophytes (Lopes et al. 2017; Hameed and Fatima 2019). Table 10.1 shows the values of the minimum inhibitory concentration (MIC) of EOs against dermatophytes. The average MIC values for dermatophytes studied under the effect of citronellol is at least 50% higher than

Table 10.1 Minimal inhibitory concentration of EOs against dermatophytes

Essential oils (main components >18%)	Dermatophytes (MIC)	References
<i>Thymus pulegioides</i> (thymol, carvacrol)	<i>E. floccosum</i> FF9 (0.16 µL/mL), <i>T. rubrum</i> FF5 (0.32 µL/mL), <i>T. mentagrophytes</i> FF7 (0.16 µL/mL), <i>M. canis</i> FF1 (0.16 µL/mL), <i>M. gypseum</i> FF3 (0.16 µL/mL)	Pinto et al. (2006)
<i>Ocimum gratissimum</i> (thymol)	<i>T. mentagrophytes</i> (100 µL/mL), <i>T. interdigitale</i> (80 µL/mL), <i>T. rubrum</i> (80 µL/mL), <i>T. erinaceum</i> (80 µL/mL), <i>T. soudanense</i> (80 µL/mL), <i>T. violaceum</i> (150 µL/mL), <i>M. canis</i> (80 µL/mL), <i>M. gypseum</i> (100 µL/mL), <i>E. floccosum</i> (150 µL/mL)	Koba et al. (2009)
<i>Cinnamomum zeylanicum</i> (eugenol)	<i>M. canis</i> (4.5 µL/mL), <i>M. gypseum</i> (7.5 µL/mL), <i>T. mentagrophytes</i> (7.5 µL/mL), <i>T. terrestre</i> (10 µL/mL), <i>T. erinacei</i> (7.5 µL/mL).	Nardoni et al. (2015)
<i>Origanum majorana</i> (carvacrol)	<i>M. canis</i> (0.5 µL/mL), <i>M. gypseum</i> (2.0 µL/mL), <i>T. mentagrophytes</i> (1.0 µL/mL), <i>T. terrestre</i> (2.0 µL/mL), <i>T. erinacei</i> (1.5 µL/mL)	
<i>Origanum verum</i> (carvacrol)	<i>M. canis</i> (0.025 µL/mL), <i>M. gypseum</i> (0.025 µL/mL), <i>T. mentagrophytes</i> (0.5 µL/mL), <i>T. terrestre</i> (0.25 µL/mL), <i>T. erinacei</i> (0.5 µL/mL)	
<i>Satureja montana</i> (carvacrol)	<i>M. canis</i> (0.5 µL/mL), <i>M. gypseum</i> (2.0 µL/mL), <i>T. mentagrophytes</i> (2.0 µL/mL), <i>T. terrestre</i> (3.0 µL/mL), <i>T. erinacei</i> (2.0 µL/mL).	
<i>Thymus serpyllum</i> (thymol)	<i>M. canis</i> (0.025 µL/mL), <i>M. gypseum</i> (0.025 µL/mL), <i>T. mentagrophytes</i> (0.10 µL/mL), <i>T. terrestre</i> (0.10 µL/mL), <i>T. erinacei</i> (0.20 µL/mL)	
<i>Eucalyptus globulus</i> (1,8-cineole)	<i>M. canis</i> (0.1 µL/mL), <i>M. gypseum</i> (0.5 µL/mL), <i>T. mentagrophytes</i> (0.5 µL/mL), <i>T. terrestre</i> (1.5 µL/mL), <i>T. erinacei</i> (0.5 µL/mL)	
<i>Thymus capitellatus</i> (1,8-cineole)	<i>E. floccosum</i> (0.64 µL/mL), <i>T. rubrum</i> (0.64 µL/mL), <i>T. mentagrophytes</i> (0.64 µL/mL), <i>T. canis</i> (0.64 µL/mL), <i>M. gypseum</i> (1.25 µL/mL)	Salgueiro et al. (2006)
<i>Eucalyptus smithii</i> (1,8-cineole)	<i>M. canis</i> ATCC 32903 (500 µg/mL), <i>M. gypseum</i> ATCC 14683 (1000 µg/mL), <i>T. mentagrophytes</i> ATCC 9533 (250 µg/mL), <i>T. mentagrophytes</i> ATCC 11481 (125 µg/mL), <i>T. rubrum</i> CCT 5507 (62.5 µg/mL)	Baptista et al. (2015)
<i>Lavandula luisieri</i> (1,8-cineole)	<i>T. rubrum</i> (MUM 08.12, 08.13, 09.08, 09.25, 09.27, 09.29, 10.128, 10.132), <i>T. rubrum</i> ATCC MYA 4438 (All = 200 µg/mL), <i>T. mentagrophytes</i> ATCC MYA 4439 (200 µg/mL), <i>T. interdigitale</i> (MUM 09.21) (>400 µg/mL)	Dias et al. (2016)
<i>Mentha spicata</i> (carvone)	<i>E. floccosum</i> (2 µL/mL), <i>M. canis</i> (1 µL/mL), <i>T. mentagrophytes</i> (0.75 µL/mL), <i>T. rubrum</i> (1 µL/mL)	Ali-Shtayeh et al. (2019)

(continued)

Table 10.1 (continued)

Essential oils (main components >18%)	Dermatophytes (MIC)	References
<i>Cymbopogon citratus</i> (neral, geranial)	<i>T. rubrum</i> (MUM 08.12, 08.13, 09.08, 09.25, 09.27, 09.29, 10.128, 10.132), <i>T. rubrum</i> ATCC MYA 4438 (All = 200 µg/mL). <i>T. mentagrophytes</i> ATCC MYA 4439 (200 µg/mL). <i>T. interdigitale</i> (MUM 09.21) (>400 µg/mL)	Dias et al. (2016)
<i>Litsea cubeba</i> (neral, geranial)	<i>M. canis</i> (0.025 µL/mL), <i>M. gypseum</i> (0.25 µL/mL), <i>T. mentagrophytes</i> (0.25 µL/mL), <i>T. terrestre</i> (1.5 µL/mL), <i>T. erinacei</i> (0.25 µL/mL).	Nardoni et al. (2015)
<i>Illicium verum</i> ((E)-anethole)	<i>M. canis</i> (3.0 µL/mL), <i>M. gypseum</i> (1.0 µL/mL), <i>T. mentagrophytes</i> (0.2 µL/mL), <i>T. terrestre</i> (1.5 µL/mL), <i>T. erinacei</i> (3.5 µL/mL).	
<i>Foeniculum vulgare</i> ((E)-anethole)	<i>M. canis</i> (0.25 µL/mL), <i>M. gypseum</i> (0.5 µL/mL), <i>T. mentagrophytes</i> (1.5 µL/mL), <i>T. terrestre</i> (1.5 µL/mL), <i>T. erinacei</i> (3.0 µL/mL)	
<i>Mentha spicata</i> (menthol)	<i>M. canis</i> (2.0 µL/mL), <i>M. gypseum</i> (3.0 µL/mL), <i>T. mentagrophytes</i> (3.0 µL/mL), <i>T. terrestre</i> (3.0 µL/mL), <i>T. erinacei</i> (3.0 µL/mL)	
<i>Santalum album</i> ((Z)- α -santalol)	<i>M. canis</i> (7.5 µL/mL), <i>M. gypseum</i> (7.5 µL/mL), <i>T. mentagrophytes</i> (7.5 µL/mL), <i>T. terrestre</i> (10 µL/mL), <i>T. erinacei</i> (7.5 µL/mL)	
<i>Pelargonium graveolens</i> (citronellol)	<i>M. canis</i> (0.25 µL/mL), <i>M. gypseum</i> (1.5 µL/mL), <i>T. mentagrophytes</i> (0.75 µL/mL), <i>T. terrestre</i> (0.75 µL/mL), <i>T. erinacei</i> (0.75 µL/mL)	
<i>Ocimum basilicum</i> (linalool)	<i>M. canis</i> (1.0 µL/mL), <i>M. gypseum</i> (3.0 µL/mL), <i>T. mentagrophytes</i> (2.5 µL/mL), <i>T. terrestre</i> (3.0 µL/mL), <i>T. erinacei</i> (2.5 µL/mL)	
<i>Lippia alba</i> (linalool)	<i>T. rubrum</i> (39 µg/mL), <i>M. gypseum</i> (312 µg/mL), <i>E. floccosum</i> (156 µg/mL)	Costa et al. (2014)
<i>Cymbopogon martini</i> (trans-geraniol)	<i>M. gypseum</i> (200 ppm), <i>T. rubrum</i> (150 ppm)	Prasad et al. (2010)
<i>Thymus villosus</i> subsp. <i>lusitanicus</i> (geranyl acetate)	<i>T. rubrum</i> CECT 2794 (0.04 µL/mL), <i>T. mentagrophytes</i> FF7 (0.16 µL/mL), <i>T. interdigitale</i> CECT 2958 (0.16 µL/mL), <i>T. verrucosum</i> CECT 2992 (0.64 µL/mL), <i>M. canis</i> CECT 2905 (0.16 µL/mL), <i>E. floccosum</i> FF9 (0.08 µL/mL)	Pinto et al. (2013b)
<i>Helichrysum italicum</i> (neryl acetate)	<i>M. canis</i> (5.0 µL/mL), <i>M. gypseum</i> (10 µL/mL), <i>T. mentagrophytes</i> (10 µL/mL), <i>T. terrestre</i> (10 µL/mL), <i>T. erinacei</i> (10 µL/mL)	Nardoni et al. (2015)
<i>Citrus limon</i> (limonene)	<i>M. canis</i> (2.5 µL/mL), <i>M. gypseum</i> (2.5 µL/mL), <i>T. mentagrophytes</i> (5.0 µL/mL), <i>T. terrestre</i> (7.5 µL/mL), <i>T. erinacei</i> (5.0 µL/mL).	Nardoni et al. (2015)
<i>Citrus medica</i> (limonene)	<i>M. canis</i> (4.0 µL/mL), <i>M. gypseum</i> (5.0 µL/mL), <i>T. mentagrophytes</i> (7.5 µL/mL), <i>T. terrestre</i> (10.0 µL/mL), <i>T. erinacei</i> (8.0 µL/mL)	

(continued)

Table 10.1 (continued)

Essential oils (main components >18%)	Dermatophytes (MIC)	References
<i>Citrus lemon</i> (limonene)	<i>M. fulvum</i> MTCC 2837 (0.9 µg/mL), <i>T. tonsurans</i> MTCC 8475 (0.4 µg/mL), <i>M. canis</i> MTCC 2820 (1.2 µg/mL), <i>T. rubrum</i> MTCC 296 (0.8 µg/mL), <i>T. mentagrophytes</i> MTCC 7687 (0.8 µg/mL)	Jain and Sharma 2017
<i>Boswellia sacra</i> (α-thujene)	<i>M. canis</i> (5.0 µL/mL), <i>M. gypseum</i> (7.5 µL/mL), <i>T. mentagrophytes</i> (7.5 µL/mL), <i>T. terrestris</i> (10.0 µL / mL), <i>T. erinacei</i> (8.0 µL/mL)	Nardoni et al. (2015)
<i>Chenopodium ambrosioides</i> (<i>m</i> -cymene)	<i>M. gypseum</i> (700 ppm), <i>T. rubrum</i> (350 ppm)	Prasad et al. (2010)
<i>Cryptomeria japonica</i> (δ-cadinene)	<i>T. rubrum</i> (313 µg/mL)	Takao et al. (2012)
<i>Juniperus communis</i> ssp. <i>alpina</i> (α-pinene). Berries oil	<i>E. floccosum</i> FF9 (1.25 µL/mL), <i>T. rubrum</i> FF5 (1.25 µL/mL), <i>T. mentagrophytes</i> FF7 (1.25 µL/mL), <i>M. canis</i> FF1 (1.25 µL/mL), <i>M. gypseum</i> FF3 (2.5 µL/mL)	Cavaleiro et al. (2006)
<i>Juniperus oxycedrus</i> ssp. <i>oxycedrus</i> (α-pinene) Berries oil	<i>E. floccosum</i> FF9 (0.32 µL/mL), <i>T. rubrum</i> FF5 (0.32 µL/mL), <i>T. mentagrophytes</i> (0.32 µL/mL), <i>M. canis</i> FF1 (0.32 µL/mL), <i>M. gypseum</i> F F3 (0.32 µL/mL)	
<i>Juniperus oxycedrus</i> ssp. <i>oxycedrus</i> (α-pinene) Leaves oil	<i>E. floccosum</i> FF9 (0.08 µL/mL), <i>T. rubrum</i> FF5 (0.08 µL/mL), <i>T. mentagrophytes</i> FF7 (0.16 µL/mL), <i>M. canis</i> FF1 (0.08 µL/mL), <i>M. gypseum</i> FF3 (0.16 µL/mL).	
<i>Juniperus turbinata</i> (α-pinene) Berries oils	<i>E. floccosum</i> (0.64 µL/mL), <i>T. rubrum</i> FF5 (1.25 µL/mL), <i>T. mentagrophytes</i> (1.25 µL/mL), <i>M. canis</i> FF1 (0.32 µL/mL), <i>M. gypseum</i> FF3 (1.25 µL/mL)	
<i>Juniperus turbinata</i> (α-pinene) Leaves oils	<i>E. floccosum</i> (0.64 µL/mL), <i>T. rubrum</i> FF5 (0.64 µL/mL), <i>T. mentagrophytes</i> (1.25 µL/mL), <i>M. canis</i> FF1 (0.64 µL/mL), <i>M. gypseum</i> FF3 (1.25 µL/mL)	
<i>Myrtus communis</i> (tricyclene, 1,8-cineole)	<i>M. canis</i> (2.0 µL/mL), <i>M. gypseum</i> (3.0 µL/mL), <i>T. mentagrophytes</i> (1.5 µL/mL), <i>T. terrestris</i> (3.0 µL/mL), <i>T. erinacei</i> (2.0 µL/mL)	Nardoni et al. (2015)
<i>Rosmarinus officinalis</i> (α-pinene, 1,8-cineole)	<i>M. canis</i> (2.5 µL/mL), <i>M. gypseum</i> (2.5 µL/mL), <i>T. mentagrophytes</i> (5.0 µL/mL), <i>T. terrestris</i> (5.0 µL/mL), <i>T. erinacei</i> (1.5 µL/mL)	
<i>Vernonia chalybaea</i> (β-caryophyllene, bicyclogermacrene)	<i>T. rubrum</i> LABMIC 0201, <i>T. rubrum</i> LABMIC 0202, <i>T. rubrum</i> LABMIC 0203, <i>T. rubrum</i> LABMIC 0204 = (1.25 mg/mL)	Nogueira Sobrinho et al. (2020)
<i>Thapsia villosa</i> (limonene, methyleugenol)	<i>E. floccosum</i> FF9 (0.64 µL/mL), <i>T. rubrum</i> CECT 2794 (0.64 µL/mL), <i>T. mentagrophytes</i> FF7 (0.64 µL/mL), <i>T. mentagrophytes</i> var. <i>interdigitale</i> CECT 2958 (1.25 µL/mL), <i>T. verrucosum</i> CECT 2992 (1.25 µL/mL), <i>M. canis</i> FF1 (0.64 µL/mL), <i>M. gypseum</i> CECT 2908 (1.25 µL/mL)	Pinto et al. (2017)

(continued)

Table 10.1 (continued)

Essential oils (main components >18%)	Dermatophytes (MIC)	References
<i>Baccharis trimera</i> (β -pinene, carquejyl acetate)	<i>T. mentagrophytes</i> ATCC 9533 (31.25 $\mu\text{g/mL}$), <i>T. mentagrophytes</i> ATCC 11480 (125 $\mu\text{g/mL}$), <i>T. rubrum</i> CCT 5507 (0.03 $\mu\text{g/mL}$), <i>M. canis</i> ATCC 32903 (0.24 $\mu\text{g/mL}$), <i>M. gypseum</i> ATCC 14683 (125 $\mu\text{g/mL}$)	Caneschi et al. (2015)
<i>Citrus bergamia</i> (linalool acetate, limonene)	<i>M. canis</i> (4.0 $\mu\text{L/mL}$), <i>M. gypseum</i> (5.0 $\mu\text{L/mL}$), <i>T. mentagrophytes</i> (5.0 $\mu\text{L/mL}$), <i>T. terrestre</i> (5.0 $\mu\text{L/mL}$), <i>T. erinacei</i> (7.5 $\mu\text{L/mL}$)	Nardoni et al. (2015)
<i>Angelica major</i> (α -pinene, E- β -ocimene)	<i>T. mentagrophytes</i> FF7 (0.32 $\mu\text{L/mL}$), <i>T. mentagrophytes</i> var. <i>interdigitale</i> CECT 2958 (0.64 $\mu\text{L/mL}$), <i>T. rubrum</i> CECT 2794 (0.32 $\mu\text{L/mL}$), <i>T. verrucosum</i> CECT 2992 (1.25 $\mu\text{L/mL}$), <i>M. canis</i> FF1 (0.32 $\mu\text{L/mL}$), <i>M. gypseum</i> CECT 2908 (0.64 $\mu\text{L/mL}$), <i>E. floccosum</i> FF9 (0.32 $\mu\text{L/mL}$)	Cavaleiro et al. (2015)
<i>Ferulago capillaris</i> (α -pinene, limonene)	<i>T. mentagrophytes</i> FF7 (0.64 $\mu\text{L/mL}$), <i>M. canis</i> FF1 (0.32 $\mu\text{L/mL}$), <i>T. rubrum</i> CECT 2794 (0.32 $\mu\text{L/mL}$), <i>M. gypseum</i> CECT 2905 (0.64 $\mu\text{L/mL}$), <i>E. floccosum</i> FF9 (0.64 $\mu\text{L/mL}$)	Pinto et al. (2013a)

those of geraniol (Shin and Lim 2004; Pereira et al. 2015). The better antidermatophyte capacity of geraniol compared to citronellol could be related to its lower molar volume. Borneol, a secondary bicyclic alcohol, showed higher MIC values than linalool, a tertiary aliphatic alcohol against *Epidermophyton floccosum*, *T. rubrum*, and *Trichophyton mentagrophytes* (Salgueiro et al. 2006). The difference in the antidermatophytic activity could be related to a higher Log P value of linalool. However, linalool esters lose the antifungal capacity, and their MIC values ranged from 0.32 to 0.64 $\mu\text{L/mL}$ (Salgueiro et al. 2006). The aldehydes α/β unsaturated have a double bond between C₂-C₃, such as neral, geranial, and cinnamaldehyde. The conjugation of the carbonyl group with its α/β unsaturation transforms the C₃ of this molecule into the preferred site for a nucleophilic attack. This strong reactivity allows these molecules to form adducts with DNA, interact with electron-rich proteins, or act as thiol alkylators (Benigni 2005). The percentage of inhibition of *T. rubrum* at a concentration of 0.04% (v/v) is 95% for cinnamaldehyde and 69% for citral (Khan and Ahmad 2011a). The great antifungal activity of cinnamaldehyde with respect to citral is related to the Lowest Unoccupied Molecular Orbital (LUMO) which is an electrophilic descriptor that describes the total ability of a molecule to attack sites rich in electrons and is greater in cinnamaldehyde than in citral (Benigni 2005). The stereoisomers often exert different antifungal effects, with the *trans* isomers showing higher antifungal activity than the *cis* one (Miron et al. 2014). Among monoterpenes with an instilled α/β aldehyde group, geranial that exhibits the C₂-C₃ junction with isomerism (E) shows better anti-dermatophyte activity, that is, four times more active than neral (Miron et al. 2014). Among the primary alcohols with C₂-C₃ unsaturation, the (E) isomer geraniol shows antifungal properties superior to its (Z) isomer nerol (Miron et al. 2014). It is clear that the isomerism of the C₂-C₃ junction in aliphatic monoterpenes plays an important role in its antifungal potential, not so

much the functional group, alcohol, or aldehyde. α -Bisabolol, a sesquiterpenic monocyclic alcohol, shows 30 times more antifungal activity against *T. rubrum* and *T. interdigitale* than the sesquiterpenic acyclic alcohol nerolidol (De Oliveira et al. 2020). This effect could be due to the higher boiling point of α -bisabolol, which results in less loss of the compound in time, favoring the antifungal activity.

Among phenylpropanoids, an increase in the number of rings (comparing monocyclic with bi- and tricyclic) results in a better antifungal activity (Zacchino et al. 1999). Regarding monocyclic phenylpropanoids, the presence of a halogen in C₄ improves its antifungal activity more than ten times compared to ketoconazole (Zacchino et al. 1999).

3 Antifungal Mechanisms of Action of Essential Oils and Their Components

Among synthetic antifungals, allylamines act by inhibiting squalene epoxidase, which prevents the formation of squalene-2,3-epoxide, thus affecting ergosterol biosynthesis. On the other hand, azole drugs exert their effect through the inhibition of the enzyme 14- α -demethylase, which is responsible for the conversion of lanosterol to ergosterol. Likewise, morpholin-based antifungals also avoid ergosterol biosynthesis, by affecting C-14 sterol reductase and C-8 sterol isomerase, different enzymes that are targeted by allylamines and azoles (Hameed and Fatima 2019; Kumari and Singh 2020). Such mechanisms affect the integrity, permeability, and morphology of the fungal plasma membrane (De Oliveira Lima et al. 2017; Poojary 2017; Martínez-Matías and Rodríguez-Medina 2018). As explained before, the antifungal mechanism of an EO is multi-target and will depend to a large extent on the nature of its components and their concentration. However, the interaction with the plasma membrane and the alteration of its permeability could be considered as the first step in the mode of action of any EO (Swamy et al. 2016; Tariq et al. 2019). To determine the effect of EOs or their individual components on the fungal membrane, sorbitol is usually added along with the treatment compound. Sorbitol acts as an osmotic protective agent, managing to stabilize the fungal protoplasm against exogenous stressors. If the EOs or any of their components affect the plasma membrane, sorbitol would act as an osmotic stabilizer. Therefore, the MIC values of the treatment would increase compared to the MIC values in the absence of sorbitol. Experiments that evaluated the anti-dermatophyte properties of citral, geraniol, nerol, cinnamaldehyde, and eugenol in the presence of sorbitol have shown no difference in their MIC values compared to those where the osmotic protection compound is absent (Khan and Ahmad 2011a; Miron et al. 2014). Still, other scientific articles showed effects on the plasma membrane through the action of EOs or monoterpenes (Pereira et al. 2015; Flores et al. 2016; Ali-Shtayeh et al. 2019). Microscopic observations of different species of fungi exposed to EOs or their components clearly showed damage of varying magnitude on the hyphal morphology. The most frequent changes observed are grouping, wrinkling, and compression of

the hyphae, which loses their cylindrical shape. These changes produced by antifungal treatments of EOs, their components, or synthetic drugs are related to the loss of cellular cytoplasm (Baptista et al. 2015; Caneschi et al. 2015). Nerolidolol and, to a lesser extent, α -bisabolol interfere with the functionality of the fungal membrane, causing significant losses of K^+ (De Oliveira et al. 2020). The structural architecture of the fungal cell membrane depends in part on the ergosterol content, which in addition to having a structural role in the formation of the fungal membrane is related to the functional stability of membrane-bound enzymes (Pereira et al. 2015). The mode of action of azole-type heterocyclic antifungals is by blocking the biosynthetic pathway of ergosterol (De Oliveira Lima et al. 2017; Poojary 2017; Martínez-Matías and Rodríguez-Medina 2018). To assess the affinity of antifungal drugs, either synthetic or natural, exogenous ergosterol is added to the culture medium. If the antifungals interact with ergosterol, the MIC of that treatment is increased compared to the control MIC. Through this technique, it was possible to determine that citral, geraniol, and nerol form complexes with ergosterol. This observation leads us to suggest that the interaction of terpenes with ergosterol would be one of the possible mechanisms of antifungal action of EOs or their components (Miron et al. 2014). The morphogenesis of the fungus is one of the main factors in the pathogenesis of dermatophytes, and from its study, we can deduce their growth and infection capacities. The presence of geraniol stimulates the production of chlamydoconidia in *T. rubrum*. This resistance structure represents a defense mechanism that allows the fungus to survive in adverse environmental conditions or in stress caused by toxic agents (Pereira et al. 2015; Hay and Ashbee 2016). Citronellol with the reduced C_2 - C_3 bond does not induce the formation of chlamydoconidia; in fact, the configuration (E) of the C_2 - C_3 bond in geraniol would be the determinant of this effect (Pereira et al. 2015). During the development of dermatophytes, the presence of microconidia, macroconidia, arthroconidia, pseudophyphae, and true hyphae are usually observed. Microconidia are formed in the conidiophores located in the hyphae, while arthroconidia are formed by fragmentation of the hyphae (Hay and Ashbee 2016; Fajinmi et al. 2019). Adherence of arthroconidia to the host *stratum corneum* is the first step towards contagion and mycelial germination and development (Hay and Ashbee 2016; Jamin et al. 2020). Essential oils can also have a preventive action by inhibiting the formation of conidia (Liu et al. 2009; Flores et al. 2016; Fajinmi et al. 2019). Dermatophytes grow in the keratinized dead tissues, which explains why their development takes place inside the *stratum corneum* of the epidermis, in the keratinized nail bed, or in the nail plate. Within this keratinized environment, the dermatophyte is found as mycelium and arthroconidia; in this parasitic phase, there is no micro or macroconidia (Hay and Ashbee 2016). Due to these characteristics, variations of the effect of the antimycotic synthetic drugs are usually observed. The most vulnerable phase to antifungal products is the mycelial form, while arthroconidial is of greater resistance and is considered the main reason for the failure of many antifungal drugs during clinical treatments of the disease (Khan et al. 2014; Aneke et al. 2020). Arthroconidia is a type of conidia with high resistance to adverse environmental conditions, such as the application of an antifungal drug. In any case, EOs or their components can inhibit their development or

affect them structurally and physiologically (Liu et al. 2009; Khan et al. 2014). *Carum copticum* and *Thymus vulgaris* EOs showed MIC ($\mu\text{g/mL}$) values of 144 and 72 $\mu\text{g/mL}$, respectively against *T. rubrum* arthroconidia, while fluconazole, the positive control, had a MIC value of 1600 $\mu\text{g/mL}$ (Khan et al. 2014). Both EOs from *C. copticum* and *T. vulgaris* have p-cymene, thymol, and γ -terpinene as their main components, varying their percentages (Khan et al. 2014). The development of the fungal infection on the host cell will depend on the virulence factors that the fungus secretes into the environment. These secretions are formed by extracellular enzymes, mainly proteinases, among which we can find elastases, keratinases, and gelatinases, and lipases, such as phospholipases and esterases (Khan et al. 2014). The main function of this extracellular fungal complex is to degrade the structural barrier of the skin, nails, or hair and also obtain nutrients. In this way, inhibiting the virulence factors of dermatophytes is a strategy for the development of new antifungals (Khan et al. 2014). Keratin degradation is a process known as sulfitolysis, which involves breaking the disulfide bridges present in keratin (Mercer and Stewart 2019). Many EOs or their pure components are explored as antifungal agents through the evaluation of their protein inhibition capacity, such as keratinases or elastases, and in this way, they can be used to control superficial mycoses by reducing their pathogenicity (Bouyahya et al. 2020). Phenylpropanoids, such as eugenol and cinnamaldehyde, show a 77.9% and 96.6% reduction in elastase activity, respectively, while anti-keratinase activity was reduced by 97% by geraniol and 57% by citral (Khan and Ahmad 2011a). *Lippia alba* EO, with a high content of linalool, inhibits the peptidase activities of *E. floccosum* and *T. rubrum* by 25% and 85%, respectively, but was not efficient against the peptidases of *Microsporum gypseum* (Costa et al. 2014). The activity of *T. rubrum* elastases was inhibited 95% by thymol, while *T. vulgaris* EO with 44% of thymol inhibited 90.7% (Khan et al. 2014). The EO of myrrh obtained from *Commiphora molmol* has ruranoeudesma-1,3-diene and menthofuran as its main components. Myrrh EO reduced the elastase activity of dermatophytes by 64% (Mahboubi and Mohammad Taghizadeh Kashani 2016). The EOs from *Ziziphora clinopodioides* and *Ziziphora tenuior* present thymol and p-cymene as the major components, and both inhibited elastase at 0.5 $\mu\text{L/mL}$ in a dose-dependent manner (Mahboubi and Tabar 2018). *Artemisia sieberi* chemotypes with high content of α -thujone and β -thujone showed strong elastase inhibitory activity (>80%) against *M. gypseum*, *T. rubrum*, and *Microsporum canis* (Mahboubi and Kazempour 2015). *Zataria multiflora* EO has thymol and carvacrol as its main constituents and, at a dose of 1 $\mu\text{L/mL}$, inhibits 80% of the activity of the pancreatic poricin elastase and 100% of the activity of the elastase produced by dermatophytes (Mahboubi et al. 2017). Another virulent factor is the presence of fungal keratinases in the infection zone. These enzymes were inhibited between 30 and 56% by the EO of *L. alba* (Costa et al. 2014), while the EOs from *T. vulgaris* and *C. copticum* with high content of thymol and γ -terpinene were less effective in reducing the activity of keratinases in *E. floccosum*, *M. gypseum*, and *T. rubrum* (Khan et al. 2014).

Dermatophytes have developed resistance to azoles. The most important molecular mechanism in the resistance of dermatophytes are changes in target enzymes, changes in membrane permeability, greater efficiency in efflux pumps, and

difficulty in absorbing the antifungal compound (De Oliveira Lima et al. 2017). *Trichophyton* spp. strains when exposed to different anti-dermatophyte compounds produce an increase in mRNA transcription levels for the *mdr2* gene, which is related to the protein/ABC transporter complex (ATP-binding cassette). By this mechanism, *Trichophyton* spp. has managed to develop resistance to various antifungal compounds (De Oliveira et al. 2020; Ponte et al. 2020). The EOs and their components are cited in the literature as regulators of efflux pump, the main mechanism of multi-resistance in microorganisms to antifungal drugs (Roy et al. 2012; Limaverde et al. 2017; Martinez-Rossi et al. 2018; Ponte et al. 2020).

A very important problem in the topical treatments of superficial mycoses is the transdermal diffusion of the active ingredient. Nerolidol increases the diffusion for transdermal transport of different bioactive compounds; its aliphatic structure allows its alignment with the lipids of the *stratum corneum* and its passage (Chan et al. 2016). Although the bibliography states that monoterpenes facilitate the transport of active ingredients through the skin (Abdollahi et al. 2020), the transport of itraconazole through the nail was not improved by the use of monoterpenes (Abdollahi et al. 2020). Through QSAR studies it can be seen that terpenic hydrocarbons are the most potent enhancers for transdermal transport, while oxygenated terpenes are the weakest (Ghafourian et al. 2004). However, the high vaporization energy of terpenic hydrocarbons detracts from penetration capacity compared to the lower vaporization energies of alcohols and ketones. A third point is the molecular size, and among alcohols, the smallest size and the largest number of double bonds are indicators of better dermal penetration capacity (Ghafourian et al. 2004).

4 Synergism

Some examples of synergism between the components of sole EOs or EOs combined with synthetic antifungals are seen in Table 10.2. The combination of EOs or their components with conventional antifungal drugs aims to improve the bioactivity of synthetic compounds, minimizing their effective dose, thus reducing possible side effects. The compound β -caryophyllene has the ability to inhibit the growth of different dermatophyte strains and shows synergizing effects when mixed with ketoconazole (Nogueira Sobrinho et al. 2020). Likewise, citronellol and geraniol show strong antifungal synergism with ketoconazole against dermatophytes (Shin and Lim 2004). Nerolidol synergizes with griseofulvin decreasing its MIC value up to eight times, while α -bisabolol shows additive effect with griseofulvin (De Oliveira et al. 2020). Linalool depicts strong synergism with itraconazole and ketoconazole, but does not have any additive or synergistic effect with fluconazole (Ponte et al. 2020). The synergism of linalool with itraconazole and ketoconazole occurs through interference with the activity of ABC proteins/transporters (Ponte et al. 2020). *Otacanthus azureus* EO is characterized by a high concentration of the sesquiterpenes α -humulene and β -copaen-4- α -ol which show strong synergistic antifungal activity with ketoconazole, itraconazole, and fluconazole against *T. mentagrophytes*

(Houël et al. 2014). Ketoconazole combined with *Allium sativum* EO shows a strong synergizing effect while the allicin/ketoconazole combination exerts an additive effect (Pyun and Shin 2006).

Natural products can also be combined seeking to decrease MIC values. Thus, citral, a mixture of geranial and neral, shows greater anti-dermatophyte activity than each of its components separately (Miron et al. 2014). Ramsewak et al. (2003) studied the combination of various monoterpenes and EOs at a concentration of 5 mg/mL each against various dermatophytes, finding a strong synergistic effect in the mixture of camphor, menthol, thymol, and *Eucalyptus citriodora* EO (with α - and

Table 10.2 Minimal inhibitory concentration of monoterpenes and volatile organic compounds against dermatophytes

Monoterpenes	Dermatophytes (MIC)	Synthetic drug	References
β -Caryophyllene	<i>T. rubrum</i> LABMIC 0201 (1.25 mg/mL), <i>T. rubrum</i> LABMIC 0202 (0.62 mg/mL), <i>T. rubrum</i> LABMIC 0203 (1.25 mg/mL), <i>T. rubrum</i> LABMIC 0204 (1.25 mg/mL)	Ketoconazole. <i>T. rubrum</i> LABMIC 0201, <i>T. rubrum</i> LABMIC 0202, <i>T. rubrum</i> LABMIC 0203, <i>T. rubrum</i> LABMIC 0204 (1.0 μ g/mL)	Nogueira Sobrinho et al. (2020)
Myrcene	<i>T. mentagrophytes</i> FF7 (5.0 μ L/mL), <i>M. canis</i> FF1 (1.25 μ L/mL), <i>T. rubrum</i> CECT 2794 (2.5 μ L/mL), <i>M. gypseum</i> CECT 2905 (5.0 μ L/mL), <i>E. floccosum</i> FF9 (1.25 μ L/mL)	Fluconazole. <i>T. mentagrophytes</i> FF7 (16 μ g/mL), <i>M. canis</i> FF1 (128 μ g/mL), <i>T. rubrum</i> CECT 2794 (16 μ g/mL), <i>M. gypseum</i> CECT 2905 (128 μ g/mL), <i>E. floccosum</i> FF9 (16 μ g/mL)	Tavares et al. (2010)
Dillapiole	<i>T. mentagrophytes</i> FF7 (0.08 μ L/mL), <i>M. canis</i> FF1 (0.08 μ L/mL), <i>T. rubrum</i> CECT 2794 (0.08 μ L/mL), <i>M. gypseum</i> CECT 2905 (0.08 μ L/mL), <i>E. floccosum</i> FF9 (0.08 μ L/mL)	Fluconazole. <i>T. mentagrophytes</i> FF7 (16 μ g/mL), <i>M. canis</i> FF1 (128 μ g/mL), <i>T. rubrum</i> CECT 2794 (16 μ g/mL), <i>M. gypseum</i> CECT 2905 (128 μ g/mL), <i>E. floccosum</i> FF9 (16 μ g/mL)	Marongiu et al. (2007)
α -Bisabolol	<i>T. rubrum</i> LM4 (16 μ g/mL), <i>T. interdigitale</i> H6 (16 μ g/mL), <i>T. interdigitale</i> Dmdr ² (08 μ g/mL)	Griseofulvin. <i>T. rubrum</i> LM4 (8 μ g/mL), <i>T. interdigitale</i> H6 (8 μ g/mL), <i>T. interdigitale</i> Dmdr ² (8 μ g/mL)	De Oliveira et al. (2020)
Nerolidol	<i>T. rubrum</i> LM4 (512 μ g/mL), <i>T. interdigitale</i> H6 (512 μ g/mL), <i>T. interdigitale</i> Δ mdr ² (256 μ g/mL)	Chlorpromazine. <i>T. rubrum</i> LM4 (0.5 μ g/mL), <i>T. interdigitale</i> H6 (0.5 μ g/mL), <i>T. interdigitale</i> Δ mdr ² (1 μ g/mL)	

(continued)

Table 10.2 (continued)

Monoterpenes	Dermatophytes (MIC)	Synthetic drug	References
Chavicol	<i>M. canis</i> (625 µg/mL), <i>M. gypseum</i> (625 µg/mL), <i>T. mentagrophytes</i> (312.5 µg/mL), <i>T. rubrum</i> (312.5 µg/mL), <i>T. tonsurans</i> (312.5 µg/mL)		De Castro-Ontengco and Capal (2019)
1,8-cineole	<i>M. canis</i> (>2500 µg/mL), <i>M. gypseum</i> (>2500 µg/mL), <i>T. mentagrophytes</i> (>2500 µg/mL), <i>T. rubrum</i> (>2500 µg/mL), <i>T. tonsurans</i> (>2500 µg/mL)		
Eugenol	<i>M. canis</i> (<156 µg/mL), <i>M. gypseum</i> (312.5 µg/mL), <i>T. mentagrophytes</i> (<156 µg/mL), <i>T. rubrum</i> (<156 µg/mL), <i>T. tonsurans</i> (<156 µg/mL)		
Carvone	<i>E. floccosum</i> (0.5 µL/mL), <i>M. canis</i> (0.63 µL/mL), <i>T. mentagrophytes</i> (0.44 µL/mL), <i>T. rubrum</i> (0.5 µL/mL)		Ali-Shtayeh et al. (2019).
Methyl eugenol	<i>E. floccosum</i> FF9 (0.32 µL/mL), <i>T. rubrum</i> CECT 2794 (0.32 µL/mL), <i>T. mentagrophytes</i> FF7 (0.32 µL/mL), <i>T. mentagrophytes var. interdigitale</i> CECT 2958 (0.32 µL/mL), <i>T. verrucosum</i> CECT 2992 (0.32 µL/mL), <i>M. canis</i> FF1 (0.32 µL/mL), <i>M. gypseum</i> CECT 2908 (0.32 µL/mL)	Fluconazole. <i>E. floccosum</i> FF9 (16 µg/mL), <i>T. rubrum</i> CECT 2794 (16 µg/mL), <i>T. mentagrophytes</i> FF7 (16 µg/mL), <i>T. mentagrophytes var. interdigitale</i> CECT 2958 (128 µg/mL), <i>T. verrucosum</i> CECT 2992 (>128 µg/mL), <i>M. canis</i> FF1 (128 µg/mL), <i>M. gypseum</i> CECT 2908 (128 µg/mL)	Pinto et al. (2017)
(R)-(+)-limonene	<i>E. floccosum</i> FF9 (0.08 µL/mL), <i>T. rubrum</i> CECT 2794 (0.08 µL/mL), <i>T. mentagrophytes</i> FF7 (0.16 µL/mL), <i>T. mentagrophytes var. interdigitale</i> CECT 2958 (0.16 µL/mL), <i>T. verrucosum</i> CECT 2992 (0.16 µL/mL), <i>M. canis</i> FF1 (0.08 µL/mL), <i>M. gypseum</i> CECT 2908 (0.08 µL/mL)		

(continued)

Table 10.2 (continued)

Monoterpenes	Dermatophytes (MIC)	Synthetic drug	References
Anethole	<i>M. canis</i> (1%), <i>M. gypseum</i> (1%), <i>T. mentagrophytes</i> (2.5%), <i>T. terrestre</i> (2.5%), <i>T. erinacei</i> (2.5%)	Griseofulvin. <i>M. canis</i> (1 mg/L), <i>M. gypseum</i> (40 mg/L), <i>T. mentagrophytes</i> (160 mg/L), <i>T. terrestre</i> (40 mg/L), <i>T. erinacei</i> (2 mg/L). Terbinafine. <i>M. canis</i> (0.015 mg/L), <i>M. gypseum</i> (0.16 mg/L), <i>T. mentagrophytes</i> (16 mg/L), <i>T. terrestre</i> (0.16 mg/L), <i>T. erinacei</i> (0.01 mg/L) Itraconazole. <i>M. canis</i> (0.12 mg/L), <i>M. gypseum</i> (32 mg/L), <i>T. mentagrophytes</i> (32 mg/L), <i>T. terrestre</i> (0.8 mg/L), <i>T. erinacei</i> (0.25 mg/L)	Nardoni et al. (2015)
Carvacrol	<i>M. canis</i> (0.1%), <i>M. gypseum</i> (0.25%), <i>T. mentagrophytes</i> (2.5%), <i>T. terrestre</i> (2.5%), <i>T. erinacei</i> (2.5%)		
p-Cymene	<i>M. canis</i> (>10%), <i>M. gypseum</i> (>10%), <i>T. mentagrophytes</i> (>10%), <i>T. terrestre</i> (>10%), <i>T. erinacei</i> (>10%)		
1,8-Cineole	<i>M. canis</i> (5%), <i>M. gypseum</i> (5%), <i>T. mentagrophytes</i> (1%), <i>T. terrestre</i> (10%), <i>T. erinacei</i> (1%)		
Linalool	<i>M. canis</i> (1%), <i>M. gypseum</i> (1%), <i>T. mentagrophytes</i> (2.5%), <i>T. terrestre</i> (2.5%), <i>T. erinacei</i> (2.5%)		
Menthol	<i>M. canis</i> (0.5%), <i>M. gypseum</i> (2.5%), <i>T. mentagrophytes</i> (1%), <i>T. terrestre</i> (1%), <i>T. erinacei</i> (1%)		
Menthone	<i>M. canis</i> (1%), <i>M. gypseum</i> (1%), <i>T. mentagrophytes</i> (2.5%), <i>T. terrestre</i> (1%), <i>T. erinacei</i> (2.5%)		
α-Pinene	<i>M. canis</i> (>10%), <i>M. gypseum</i> (>10%), <i>T. mentagrophytes</i> (>10%), <i>T. terrestre</i> (>10%), <i>T. erinacei</i> (>10%)		
γ-Terpinene	<i>M. canis</i> (>10%), <i>M. gypseum</i> (>10%), <i>T. mentagrophytes</i> (>10%), <i>T. terrestre</i> (>10%), <i>T. erinacei</i> (>10%)		
Neral	<i>M. canis</i> (0.1%), <i>M. gypseum</i> (0.1%), <i>T. mentagrophytes</i> (0.25%), <i>T. terrestre</i> (0.25%), <i>T. erinacei</i> (0.25%)		
Eugenol	<i>M. canis</i> (0.1%), <i>M. gypseum</i> (0.25%), <i>T. mentagrophytes</i> (0.25%), <i>T. terrestre</i> (0.25%), <i>T. erinacei</i> (0.25%)		
Geraniol	<i>M. canis</i> (0.25%), <i>M. gypseum</i> (0.25%), <i>T. mentagrophytes</i> (1%), <i>T. terrestre</i> (0.5%), <i>T. erinacei</i> (0.5%)		
Geranial	<i>M. canis</i> (0.1%), <i>M. gypseum</i> (0.1%), <i>T. mentagrophytes</i> (0.25%), <i>T. terrestre</i> (1.5%), <i>T. erinacei</i> (0.25%)		
Citronellol	<i>M. canis</i> (0.25%), <i>M. gypseum</i> (0.25%), <i>T. mentagrophytes</i> (0.25%), <i>T. terrestre</i> (0.5%), <i>T. erinacei</i> (0.5%)		
Limonene	<i>M. canis</i> (>10%), <i>M. gypseum</i> (>10%), <i>T. mentagrophytes</i> (>10%), <i>T. terrestre</i> (>10%), <i>T. erinacei</i> (>10%)		
Thymol	<i>M. canis</i> (0.05%), <i>M. gypseum</i> (0.25%), <i>T. mentagrophytes</i> (0.12%), <i>T. terrestre</i> (0.25%), <i>T. erinacei</i> (0.25%)		
Fenchone	<i>M. canis</i> (0.25%), <i>M. gypseum</i> (0.5%), <i>T. mentagrophytes</i> (1%), <i>T. terrestre</i> (1%), <i>T. erinacei</i> (1.5%)		

(continued)

Table 10.2 (continued)

Monoterpenes	Dermatophytes (MIC)	Synthetic drug	References
Geraniol	<i>T. rubrum</i> ATCC 1683, LM98, LM130, LM 222, LM 309, LM 333, LM 422, LM 582, LM 600, LM 640, LM 710, LM 713, LM 720, LM 722. (MIC µg/mL = 32, 64, 64, 64, 16, 32,32, 16, 32, 256, 16,16, 64, 64.)		Pereira et al. (2015)
Citronellol	<i>T. rubrum</i> ATCC 1683, LM98, LM130, LM 222, LM 309, LM 333, LM 422, LM 582, LM 600, LM 640, LM 710, LM 713, LM 720, LM 722. (MIC µg/mL = 128, 128, 64, 32, 32, 8, 128, 64, 32, 128, 256, 32, 1024, 128.)		
β-Ocimene	<i>T. mentagrophytes</i> FF7 (0.64 µL/mL), <i>T. mentagrophytes</i> var. <i>interdigitale</i> CECT 2958 (0.32 µL/mL), <i>T. rubrum</i> CECT 2794 (0.08 µL/mL), <i>T. verrucosum</i> CECT 2992 (0.32 µL/mL), <i>M. canis</i> FF1 (0.16 µL/mL), <i>M. gypseum</i> CECT 2908 (0.64 µL/mL), <i>E. floccosum</i> FF9 (0.64 µL/mL)	Fluconazole. <i>T. mentagrophytes</i> FF7 (16 µg/mL), <i>T. mentagrophytes</i> var. <i>interdigitale</i> CECT 2958 (128 µg/mL), <i>T. rubrum</i> CECT 2794 (16 µg/mL), <i>T. verrucosum</i> CECT 2992 (>128 µg/mL), <i>M. canis</i> FF1 (128 µg/mL), <i>M. gypseum</i> CECT 2908 (128 µg/mL), <i>E. floccosum</i> FF9 (16 µg/mL)	Cavaleiro et al. (2015)
α-Pinene	<i>T. mentagrophytes</i> FF7 (0.32 µL/mL), <i>T. mentagrophytes</i> var. <i>interdigitale</i> CECT 2958 (0.32 µL/mL), <i>T. rubrum</i> CECT 2794 (0.08 µL/mL), <i>T. verrucosum</i> CECT 2992 (0.32 µL/mL), <i>M. canis</i> FF1 (0.32 µL/mL), <i>M. gypseum</i> CECT 2908 (0.64 µL/mL), <i>E. floccosum</i> FF9 (0.32 µL/mL)		
Neral	<i>T. rubrum</i> (32 µg/mL), <i>T. mentagrophytes</i> (128 µg/mL), <i>M. canis</i> (80 µg/mL), <i>M. gypseum</i> (>128 µg/mL)	Terbinafine. <i>T. rubrum</i> (0.25 µg/mL), <i>T. mentagrophytes</i> (2.0 µg/mL), <i>M. canis</i> (0.25 µg/mL), <i>M. gypseum</i> (0.25 µg/mL)	Miron et al. (2014)
Geraniol	<i>T. rubrum</i> (8.0 µg/mL), <i>T. mentagrophytes</i> (32 µg/mL), <i>M. canis</i> (48 µg/mL), <i>M. gypseum</i> (32 µg/mL)		
Citral	<i>T. rubrum</i> (4.0 µg/mL), <i>T. mentagrophytes</i> (32 µg/mL), <i>M. canis</i> (48 µg/mL), <i>M. gypseum</i> (16 µg/mL)		
Nerol	<i>T. rubrum</i> (64 µg/mL), <i>T. mentagrophytes</i> (128 µg/mL), <i>M. canis</i> (128 µg/mL), <i>M. gypseum</i> (128 µg/mL)		
Geraniol	<i>T. rubrum</i> (64 µg/mL), <i>T. mentagrophytes</i> (32 µg/mL), <i>M. canis</i> (40 µg/mL), <i>M. gypseum</i> (128 µg/mL)		

(continued)

Table 10.2 (continued)

Monoterpenes	Dermatophytes (MIC)	Synthetic drug	References
α -Pinene	<i>T. mentagrophytes</i> FF7 (0.32 μ L/mL), <i>M. canis</i> FF1 (0.16 μ L/mL), <i>T. rubrum</i> CECT 2794 (0.08 μ L/mL), <i>M. gypseum</i> CECT 2905 (0.16 μ L/mL), <i>E. floccosum</i> FF9 (0.16 μ L/mL)	Fluconazole. <i>T. mentagrophytes</i> FF7 (0.32 μ g/mL), <i>M. canis</i> FF1 (128 μ g/mL), <i>T. rubrum</i> CECT 2794 (16 μ g/mL), <i>M. gypseum</i> CECT 2905 (128 μ g/mL), <i>E. floccosum</i> FF9 (16 μ g/mL)	Pinto et al. (2013a)
Limonene	<i>T. mentagrophytes</i> FF7 (2.5 μ L/mL), <i>M. canis</i> FF1 (0.64 μ L/mL), <i>T. rubrum</i> CECT 2794 (0.64 μ L/mL), <i>M. gypseum</i> CECT 2905 (1.25 μ L/mL), <i>E. floccosum</i> FF9 (1.25 μ L/mL)		
Geranyl acetate	<i>T. rubrum</i> CECT 2794 (0.32 μ L/mL), <i>T. mentagrophytes</i> FF7 (0.32 μ L/mL), <i>T. interdigitale</i> CECT 2958 (0.32 μ L/mL), <i>T. verrucosum</i> CECT 2992 (0.64 μ L/mL), <i>M. canis</i> CECT 2905 (0.16 μ L/mL), <i>E. floccosum</i> FF9 (0.16 μ L/mL)	Fluconazole. <i>T. rubrum</i> CECT 2794 (16 μ g/mL), <i>T. mentagrophytes</i> FF7 (16 μ g/mL), <i>T. interdigitale</i> CECT 2958 (128 μ g/mL), <i>T. verrucosum</i> CECT 2992 (>128 μ g/mL), <i>M. canis</i> CECT 2905 (128 μ g/mL), <i>E. floccosum</i> FF9 (16 μ g/mL)	Pinto et al. (2013b)
Terpinen-4-ol	<i>T. rubrum</i> CECT 2794 (1.25 μ L/mL), <i>T. mentagrophytes</i> FF7 (2.5 μ L/mL), <i>T. interdigitale</i> CECT 2958 (1.25 μ L/mL), <i>T. verrucosum</i> CECT 2992 (1.25 μ L/mL), <i>M. canis</i> CECT 2905 (1.25 μ L/mL), <i>M. gypseum</i> CECT 2905 (2.5 μ L/mL), <i>E. floccosum</i> FF9 (1.25 μ L/mL)		
Linalool	<i>T. rubrum</i> CECT 2794 (1.25 μ L/mL), <i>T. mentagrophytes</i> FF7 (1.25 μ L/mL), <i>T. interdigitale</i> CECT 2958 (2.5 μ L/mL), <i>T. verrucosum</i> CECT 2992 (1.254 μ L/mL), <i>M. canis</i> CECT 2905 (2.5 μ L/mL), <i>M. gypseum</i> CECT 2905 (1.25 μ L/mL), <i>E. floccosum</i> FF9 (1.25 μ L/mL)		
Geraniol	<i>T. rubrum</i> CECT 2794 (0.16 μ L/mL), <i>T. mentagrophytes</i> FF7 (0.08 μ L/mL), <i>T. interdigitale</i> CECT 2958 (0.16 μ L/mL), <i>T. verrucosum</i> CECT 2992 (0.16 μ L/mL), <i>M. canis</i> CECT 2905 (0.16 μ L/mL), <i>M. gypseum</i> CECT 2905 (0.32 μ L/mL), <i>E. floccosum</i> FF9 (0.16 μ L/mL)		
δ -Cadinene	<i>T. rubrum</i> (500 μ g/mL)		
Epi-cubenol	<i>T. rubrum</i> (250 μ g/mL)		
β -Eudesmol	<i>T. rubrum</i> (1000 μ g/mL)		

(continued)

Table 10.2 (continued)

Monoterpenes	Dermatophytes (MIC)	Synthetic drug	References
Thymol	<i>T. mentagrophytes</i> (50 µL/mL), <i>T. interdigitale</i> (100 µL/mL), <i>T. rubrum</i> (50 µL/mL), <i>T. erinaceum</i> (50 µL/mL), <i>T. soudanense</i> (40 µL/mL), <i>T. violaceum</i> (30 µL/mL), <i>M. canis</i> (50 µL/mL), <i>M. gypseum</i> (50 µL/mL), <i>E. floccosum</i> (50 µL/mL)		Koba et al. (2009)
γ-Terpinene	<i>T. mentagrophytes</i> , <i>T. interdigitale</i> , <i>T. rubrum</i> , <i>T. erinaceum</i> , <i>T. soudanense</i> , <i>T. violaceum</i> , <i>M. canis</i> , <i>M. gypseum</i> , <i>E. floccosum</i> . All MIC >500 µL/mL		
p-Cymene	<i>T. mentagrophytes</i> , <i>T. interdigitale</i> , <i>T. rubrum</i> , <i>T. erinaceum</i> , <i>T. soudanense</i> , <i>T. violaceum</i> , <i>M. canis</i> , <i>M. gypseum</i> , <i>E. floccosum</i> . All MIC >500 µL/mL		
Carvacrol	<i>E. floccosum</i> FF9 (0.08 µL/mL), <i>T. rubrum</i> FF5 (0.08 µL/mL), <i>T. mentagrophytes</i> FF7 (0.04 µL/mL), <i>M. canis</i> FF1 (0.04 µL/mL), <i>M. gypseum</i> FF3 (0.04 µL/mL)	Fluconazole. <i>E. floccosum</i> FF9 (0.16 µg/mL), <i>T. rubrum</i> FF5 (0.32 µg/mL), <i>T. mentagrophytes</i> FF7 (0.32 µg/mL), <i>M. canis</i> FF1 (128 µg/mL), <i>M. gypseum</i> FF3 (>128 µg/mL)	Pinto et al. (2006)
p-Cymene	<i>E. floccosum</i> FF9 (5.0 µL/mL), <i>T. rubrum</i> FF5 (1.25 µL/mL), <i>T. mentagrophytes</i> FF7 (5.0 µL/mL), <i>M. canis</i> FF1 (2.5 µL/mL), <i>M. gypseum</i> FF3 (10 µL/mL)		
γ-Terpinene	<i>E. floccosum</i> FF9 (2.56 µL/mL), <i>T. rubrum</i> FF5 (5.0 µL/mL), <i>T. mentagrophytes</i> FF7 (10.0 µL/mL), <i>M. canis</i> FF1 (5.0 µL/mL), <i>M. gypseum</i> FF3 (10.0 µL/mL)		
Thymol	<i>E. floccosum</i> FF9 (0.16 µL/mL), <i>T. rubrum</i> FF5 (0.16 µL/mL), <i>T. mentagrophytes</i> FF7 (0.16 µL/mL), <i>M. canis</i> FF1 (0.08 µL/mL), <i>M. gypseum</i> FF3 (0.16 µL/mL)		
α-Pinene	<i>E. floccosum</i> FF9 (0.16 µL/mL), <i>T. rubrum</i> FF5 (0.16 µL/mL), <i>T. mentagrophytes</i> FF7 (0.32 µL/mL), <i>M. canis</i> FF1 (0.16 µL/mL), <i>M. gypseum</i> FF3 (0.32 µL/mL)	Fluconazole. <i>E. floccosum</i> FF9, <i>T. rubrum</i> FF5, <i>T. mentagrophytes</i> FF7 (16 µg/mL), <i>M. canis</i> FF1, <i>M. gypseum</i> FF3 (>128 µg/mL)	Cavaleiro et al. (2006)
δ-3-Carene	<i>E. floccosum</i> FF9 (0.16 µL/mL), <i>T. rubrum</i> FF5 (0.16 µL/mL), <i>T. mentagrophytes</i> FF7 (0.32 µL/mL), <i>M. canis</i> FF1 (0.16 µL/mL), <i>M. gypseum</i> FF3 (0.64 µL/mL)		

(continued)

Table 10.2 (continued)

Monoterpenes	Dermatophytes (MIC)	Synthetic drug	References
1.8-Cineole	<i>E. floccosum</i> (5.0 µL/mL), <i>T. rubrum</i> (2.5 µL/mL), <i>T. mentagrophytes</i> (5.0 µL/mL), <i>T. canis</i> (5.0 µL/mL), <i>M. gypseum</i> (10.0 µL/mL)	Fluconazole. <i>E. floccosum</i> (16 µg/mL), <i>T. rubrum</i> (16 µg/mL), <i>T. mentagrophytes</i> (16 µg/mL), <i>T. canis</i> (128 µg/mL), <i>M. gypseum</i> (>128 µg/mL)	Salgueiro et al. (2006)
Borneol	<i>E. floccosum</i> (2.5 µL/mL), <i>T. rubrum</i> (2.5 µL/mL), <i>T. mentagrophytes</i> (2.5 µL/mL), <i>T. canis</i> (2.5 µL/mL), <i>M. gypseum</i> (2.5 µL/mL)		
Linalool	<i>E. floccosum</i> (1.25.0 µL/mL), <i>T. rubrum</i> (1.25 µL/mL), <i>T. mentagrophytes</i> (1.25 µL/mL), <i>T. canis</i> (2.5 µL/mL), <i>M. gypseum</i> (2.5 µL/mL)		
Linalyl acetate	<i>E. floccosum</i> (0.32 µL/mL), <i>T. rubrum</i> (0.32 µL/mL), <i>T. mentagrophytes</i> (0.64 µL/mL), <i>T. canis</i> (0.32 µL/mL), <i>M. gypseum</i> (0.64 µL/mL)		
Benzoic acid	<i>T. erinacei</i> (<0.12 mg/mL), <i>T. mentagrophytes</i> (0.25 mg/mL), <i>T. rubrum</i> (0.25 mg/mL), <i>T. schoenleinii</i> (<0.25 mg/mL), <i>T. soudanense</i> (0.25 mg/mL), <i>T. tonsurans</i> (0.25 mg/mL)		
Citronellol	<i>T. erinacei</i> (0.5 mg/mL), <i>T. mentagrophytes</i> (1.0 mg/mL), <i>T. rubrum</i> (2.0 mg/mL), <i>T. schoenleinii</i> (1.0 mg/mL), <i>T. soudanense</i> (0.50 mg/mL), <i>T. tonsurans</i> (2.0 mg/mL)		
Geraniol	<i>T. erinacei</i> (0.5 mg/mL), <i>T. mentagrophytes</i> (0.5 mg/mL), <i>T. rubrum</i> (1.0 mg/mL), <i>T. schoenleinii</i> (0.5 mg/mL), <i>T. soudanense</i> (0.25 mg/mL), <i>T. tonsurans</i> (0.5 mg/mL)		
Thymol	<i>T. erinacei</i> (0.25 mg/mL), <i>T. mentagrophytes</i> (0.5 mg/mL), <i>T. rubrum</i> (0.5 mg/mL), <i>T. schoenleinii</i> (0.5 mg/mL), <i>T. soudanense</i> (0.5 mg/mL), <i>T. tonsurans</i> (0.5 mg/mL)		
Estragole	<i>T. mucoides</i> (5 mg/mL), <i>T. tonsurans</i> (10 mg/mL)	Ketoconazole. <i>T. mucoides</i> (12.5 µg/mL), <i>T. tonsurans</i> (25 µg/mL)	Shin and Kang (2003)

(continued)

β -pinenes as main components). The combination of *A. sativum* EO (diallyl sulfide, 57.1%)/*Cymbopogon martinii* EO (geraniol, 80.7%) showed MIC values lower than 0.06 mg/mL against *T. mentagrophytes* while the combination of *Pinus sylvestris* EO (monoterpenic hydrocarbons 79%)/*Origanum vulgare* EO (carvacrol/thymol 74%) showed an MIC of 0.13 mg/mL (Orchard et al. 2019). Other mixtures using *Santalum austrocaledonicum* EO (main component α -santolol) also showed to have an anti-dermatophyte potential (Orchard et al. 2019). On the other hand, many other combinations of EOs were reported to have no effect or simply were antagonistic (Prasad et al. 2010; Orchard et al. 2019).

The great potential of EOs or their components as antifungal agents has led to evaluation of Vicks VapoRub™ (a cream with a formulation based on monoterpenes and phenylpropanoids used for respiratory treatments) as an anti-dermatophyte product, showing encouraging results (Derby et al. 2011), even in immunosuppressed patients (Snell et al. 2016). These results have increased the available commercial topical formulations bearing EOs as bioactive components or as synergizing agents of synthetic antifungal drugs for the treatment of onychomycosis.

5 *Candida*

Candida spp is among the major causative agents of fungal diseases, with approximately 700,000 new cases of invasive candidiasis being reported each year (Góralaska et al. 2018; Silva et al. 2019). Relatively few species are pathogens, with most of the *Candida* infections (> 90%) being caused mainly by *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, and *C. tropicalis* (Köhler et al. 2017; Boral et al. 2018; Góralaska et al. 2018). They are commensal microorganisms of skin and mucosal surfaces such as oral cavity, gastrointestinal tract, and urogenital tract, but become opportunistic pathogens in the presence of medical vulnerabilities such as the decline of the host's immune defenses, systemic diseases, extensive wounds (burns and operations), poor oral and dental hygiene, smoking habits, and trauma (Borchers et al. 2017; Peixoto et al. 2017; Góralaska et al. 2018; Tedila et al. 2019; Singhi and Saini 2019).

Candida albicans is able to switch its phenotype according to the host environment and stress conditions, and the different morphologies are associated with its metabolism, pathogenesis, biofilm formation ability, and drug resistance (Köhler et al. 2017; Boral et al. 2018; Tedila et al. 2019). Furthermore, *C. albicans* secretes acid proteinases, phospholipases, and lipases that also contribute to its pathogenesis. These enzymes degrade the epithelial cells, and the hyphal extension produce damage by physical force (Köhler et al. 2017; Boral et al. 2018). Hence, species of the genus *Candida* can establish and form biofilms on ocular, oral, intestinal, and vaginal epithelial tissues (Sharifzadeh and Shokri 2016; Peixoto et al. 2017; Boral et al. 2018; Geddes-McAlister and Shapiro 2019; Song and Lee 2019). In addition, they are found forming biofilms in catheters producing invasive fungal infections

associated with high morbidity and mortality. The structure of the mature biofilm consists of yeast and hyphal and pseudohyphal elements immersed in a polysaccharide/protein matrix. The yeast form is essential for biofilm formation since biofilms that contain only hyphae can be easily disrupted (Manoharan et al. 2017; Boral et al. 2018). Biofilms are more resistant to antimicrobial agents and insensitive to host immune responses (Köhler et al. 2017; Manoharan et al. 2017). The increase of fungal infections is related to immunosuppression due to diseases like HIV/AIDS and cancer; pervasive dysbiosis of the human microbiome associated with exposure to broad-spectrum antimicrobials; the use of modern medical devices such as ventilators, stents, and catheters (Geddes-McAlister and Shapiro 2019). *Candida albicans* is the most frequently isolated species from patients with candidiasis (>50%), but infections caused by other non-*albicans Candida* are becoming more significant in different regions of the world (Allen et al. 2015; Góralaska et al. 2018; Silva et al. 2019). This is of great relevance since many non-*albicans Candida* species presented intrinsic resistance to fluconazole (Silva et al. 2019). For instance, the 10–15% of the *C. glabrata* clinical isolates are resistant to fluconazole and voriconazole, and this species also present high frequency of multidrug resistance which reduced susceptibility to both azoles and echinocandins (Geddes-McAlister and Shapiro 2019). Besides, the recent isolation of new strains such as *C. auris* with high rates of antifungal drug resistance is a new challenge to human health (Geddes-McAlister and Shapiro 2019). Therefore, new therapies are needed to successfully face these emerging fungal diseases, and the natural products as EOs could be an option due to their previously described properties.

5.1 Essential Oils and Their Components Against *Candida* sp.

The antimicrobial activity is associated with the chemical composition of the EO which depends on many factors, as mentioned above, and generally an EO with high concentrations of monoterpenic and sesquiterpene constituents is more effective as anticandidal agent (Bona et al. 2016; De Toledo et al. 2016; Benzaid et al. 2019; Dutta et al. 2020; El Mokni et al. 2020). Table 10.3 shows the MIC values of different EOs on *C. albicans*, *C. parapsilosis*, *C. glabrata*, and *C. krusei*. Although the literature does not present a standard MIC values (sensitive and resistant) for natural products against *Candida* spp., it was stated that values equal to or lower than 1000 µg/mL confirm sensitivity (De Toledo et al. 2016; Peixoto et al. 2017). Moreover, the EO activity is attributed to the synergic combination of its major and minor compounds, and some investigations found that an EO had better antifungal activity than their individual components. For instance, the major components such as citronellal, p-cymene, and thymol show lower effect on fungal growth compared with the EO (De Toledo et al. 2016; Dutta et al. 2020). In other study, thymol and the EO showed almost the same effects on strains of *C. albicans* and *C. krusei* (Mehriardestani et al. 2020). This discrepancy could be due to the different strains evaluated and the different methodologies used. The bibliography reports that

Table 10.3 Minimal inhibitory concentrations of EOs against *Candida* species

Plant EO	<i>C. albicans</i>	<i>C. glabrata</i>	<i>C. parapsilosis</i>	<i>C. tropicalis</i>	References
	MIC µg/mL				
<i>Pelargonium graveolens</i>	500	500	500	250	Essid et al. (2017)
	500	500	–	–	
	500	–	–	–	
	1000	–	–	–	
	1000	–	–	–	
<i>Cinnamomum verum</i>	625	62.5	62.5	312.5	
	312.5	62.5	–	–	
	625	–	–	–	
	625	–	–	–	
	625	–	–	–	
<i>Thymus capitatus</i>	125	125	125	125	
	125	125	–	–	
	125	–	–	–	
	125	–	–	–	
	125	–	–	–	
<i>Syzygium aromaticum</i>	250	250	250	250	
	125	250	–	–	
	250	–	–	–	
	250	–	–	–	
	250	–	–	–	
<i>Cymbopogon nardus</i>	1000	500	500	500	De Toledo et al. (2016)
	1000	500	1000	>1000	
	1000	500	–	1000	
	1000	1000	–	>1000	
<i>Trachyspermum ammi</i>	500	–	–	–	Mehriardestani et al. (2020)
	27	–	–	122.4	Dutta et al. (2020)
	22.4	–	–	127	
	13.8	–	–	–	
	24.2	–	–	–	
<i>Lavandula maroccana</i>	4604	4604	4604	–	Soulaimani et al. (2019)
<i>Lippia lasiocalycina</i>	512	–	–	–	De Almeida et al. (2018)
<i>Senecio anteuphorbium</i>	1024	2048	2048	–	Elhidar et al. (2019)
	2048	–	–	–	
<i>Plectranthus glandulosus</i>	5000	–	–	–	Ngo-Mback et al. (2019)
<i>Aeollanthus heliotropioides</i>	1250	–	–	–	

(continued)

Table 10.3 (continued)

Plant EO	<i>C. albicans</i>	<i>C. glabrata</i>	<i>C. parapsilosis</i>	<i>C. tropicalis</i>	References
	MIC µg/mL				
<i>Bubonium imbricatum</i>	750	370	180	–	Aghraz et al. (2016)
<i>Mentha arvensis</i>	2000	–	2000	–	Busato de Feiria et al. (2016)
<i>Mentha piperita</i>	2000	–	500	–	
<i>Laurus nobilis</i>	–	–	0.8	–	Córdoba et al. (2019)
	250	5000	–	500	Peixoto et al. (2017)
	250	–	–	250	(2017)
	–	–	0.8	–	Córdoba et al. (2019)
<i>Thymus vulgaris</i>	–	–	0.8	–	El Mokni et al. (2020)
<i>Cymbopogon citratus</i>	–	–	50	–	
<i>Lippia junelliana</i>	–	–	1.6	–	
<i>Calamintha officinalis</i>	–	–	200	–	
<i>Ballota nigra</i> subsp. <i>uncinata</i>	625	–	–	–	
<i>Ballota bullata</i>	625	–	–	–	
<i>Foeniculum vulgare</i>	25	–	–	–	
<i>Plantago afra</i>	312.5	–	–	–	Hammami et al. (2020)
<i>Eucalyptus globulus</i>	219	219	–	885	Quatrin et al. (2017)

species of *Candida* are more sensitive to different EOs than traditionally used drugs. The EOs from *Ocimum basilicum*, *Lavandula* spp., *Melaleuca alternifolia*, *Satureja montana*, and *Thymus capitatus* inhibited both growth and metabolic activity of *C. albicans* isolated from vaginal swab (resistant to three main azole antifungal drugs) more efficiently than clotrimazole, being *T. capitatus* EO the most effective (Bona et al. 2016). Also, *Cymbopogon nardus* EO was effective against almost all the *Candida* species tested including strains resistant to fluconazole and amphotericin-B, with MIC values that ranged from 250 to 1000 µg/mL (De Toledo et al. 2016). The continuous raising of antibiotic-resistant and less susceptible strains of pathogens to the widely prescribed antifungal drugs is a global public health concern. The EOs could act synergistically with currently used drugs, thus EOs and/or their components is an alternative strategy for the development of new antifungal agents. *Pelargonium graveolens*, *Cinnamomum verum*, *Piper mikanianum*, *Lavandula maroccana*, *Senecio anteuphorbium*, and *Vanillosmopsis arborea* EOs showed synergistic effect when combined with fluconazole against *Candida* strains (Essid et al. 2017; Rodrigues et al. 2018; Elhidar et al. 2019; Soulaïmani et al. 2019; Carneiro et al. 2020), whereas the combination of *L. maroccana* EO with

amphotericin B showed a total synergism against *C. krusei* and a partial synergism against *C. albicans*, *C. glabrata*, and *C. parapsilosis* (Soulaimani et al. 2019).

In general, antifungal agents that are effective against planktonic cell are often ineffective on biofilm, and the incomplete removal of biofilm may result in drug resistance or reinfection (Manoharan et al. 2017). The EOs also inhibit the morphological transition capacity of yeast to hypha, which is one of the greatest virulence factors of *Candida*. The EOs from *C. nardus*, *Mentha x piperita*, *P. mikanianum*, *Aeollanthus heliotropioides*, and *Plectranthus glandulosus* inhibit the formation of filamentous structures, contributing to the controlling of *Candida* infection (De Toledo et al. 2016; Benzaid et al. 2019; Ngo-Mback et al. 2019; Carneiro et al. 2020). The control and elimination of fungal biofilm is difficult, due to several types of molecular, structural, and specifically physiological interactions. In the biofilm, the fungi are more resistant to antimicrobial agents due to the barrier function of the extracellular matrix which contains polysaccharides, proteins, and nucleic acids (Song and Lee 2019). *Mentha piperita* EO decreases biofilm formation of *C. albicans* at concentrations of 10 $\mu\text{L/mL}$ and disrupts mature biofilm (Benzaid et al. 2019). The EOs of cascarilla bark (*Croton eluteria*), helichrysum, coriander, lemon eucalyptus, lemongrass, and lime at 0.01% reduced the formation of biofilm in *C. albicans* by more than 90% and also biofilm thickness (Manoharan et al. 2017). Moreover, *Laurus nobilis* EO reduced the formation of mature biofilm and inhibited the initial adhesion and biofilm formation at concentrations of 1000 $\mu\text{g/mL}$ of *C. albicans* (Peixoto et al. 2017). At concentrations of 2000 $\mu\text{g/mL}$ EO, three *Mentha* species inhibited biofilm formation (90%) and produced disruption of the *C. albicans* biofilm (80%) (Busato de Feiria et al. 2016).

5.2 Mechanisms of Action of Essential Oils in *Candida* sp.

The mechanism of action of EOs is frequently related to the cell wall biosynthesis and the ionic permeability of the cell membrane. Damage of the cell wall and membranes of the yeast was observed in *Candida* cells treated with EO from *T. capitatus*, *L. nobilis*, and *O. basilicum* (Bona et al. 2016; Peixoto et al. 2017; Miao et al. 2020). The first action of phenols compounds present in EOs is a nonspecific interaction with the mitochondrial or plasma membrane. Then, the alkylphenols with isopropyl substituents could be biotransformed into quinones methylene (highly toxic) during the metabolic processes. These could explain the different anticandidal activities observed among phenol compounds (Gallucci et al. 2014). *Pelargonium graveolens* EO acts decreasing the levels of major lipids (palmitic acid, stearic acid, and oleic acid) by 54.71% in cells of *C. albicans*, inhibiting the formation of long chain fatty acids. Consequently, the permeability barrier of the cell wall is disturbed, mainly oleic acid and fatty acid homeostasis. On the other hand, *C. verum* EO strongly reduces the ergosterol biosynthesis (83%) and was, indeed, more effective than fluconazole. The synergic effect of the combination of *C. verum* with fluconazole produced a total inhibition of ergosterol production causing a membrane damaging effect that could increase cell permeability and finally cell death (69.51% of killing rate) (Essid et al. 2017). The secreted aspartic proteases (SAPs) are produced during

the infection, facilitating the penetration of pathogenic *Candida* cells into the host organism. SAPs include 10 proteolytic enzymes that have the capacity to degrade immunoproteins, collagen, and fibronectin. Hence, the use of aspartyl proteinase inhibitors may in fact reduce the yeast virulence and pathogenesis (Köhler et al. 2017; Essid et al. 2017; Benzaid et al. 2019). *Pelargonium graveolens* and *C. verum* EO in combination with fluconazole inhibited 78.31% and 64.72% of SAPs activity, respectively (Essid et al. 2017), while *M. piperita* EO downregulated the expression of genes involved in SAPI, 2, 3, 9, synthesis, which may lead to reducing *C. albicans* virulence. Other mechanisms of this EO was a decreased expression of HWPI, a hyphal-specific adhesion gene that encodes the hyphal cell wall protein promoting *C. albicans* adhesion to different surface (Benzaid et al. 2019). Metabolomics analyses revealed that 34 metabolites changed their abundance in *C. albicans* cells under the sub lethal effect of *O. basilicum* EO. These metabolites were intermediates involved in the carbon, amino acids, polyamines, lipids and fatty acid metabolism, such as glycolysis/gluconeogenesis, pentose phosphate pathway, and TCA cycle, among others (Miao et al. 2020). In future research, metabolomic analyses could help to elucidate with greater detail the mechanisms of actions of EOs.

6 Cryptococcosis

Cryptococcus spp are encapsulated yeasts (3.5–8 μm) that reproduce by single budding, forming a narrow neck between the mother and daughter cells. Exceptionally, multiple budding, elongated shapes, and pseudohyphae are observed. These yeasts have a worldwide distribution (Heitman et al. 2011) and is frequently isolated from soil contaminated with pigeon or other bird droppings. It can be also isolated from dry avian manure accumulated in buildings but not from fresh manure. Birds do not suffer from *C. neoformans* infection but serve as vectors, because of their high body temperature which is approximately 42 °C, conditions that allow the microorganism to survive but not to develop (Vázquez Tsuji et al. 2005). The presence of nitrogenous products, humidity, and alkalinity permits the encapsulated yeast to remain viable for around two years in beard droppings. Additionally, *C. neoformans* is isolated from fruit and vegetables (Jorgensen et al. 2015). The genus *Cryptococcus* comprises many species. Different strains of *C. neoformans* have been grouped into two varieties that include three serotypes depending on their capsular structure: *C. neoformans* var. *neoformans*, A or D serotypes, which are isolated from bird droppings while *C. neoformans* var. *gattii* was isolated from the waste that surrounds the species *Eucalyptus camaldulensis* and *Eucalyptus tereticornis* and belong to B serotype (Heitman et al. 2011; Bandalizadeh et al. 2020).

The infection is acquired by inhalation of the dried yeasts, which easily reaches the alveolar spaces. The person-to-person transmission does not exist, but it has been reported that *Cryptococcus* can be transmitted through transplanted organs. No evidence of direct transmission from animals to humans has been reported (Vázquez Tsuji et al. 2005). The clinical course of cryptococcosis in a patient depends of the

inoculum quantity, the virulence of the infecting strain, and the immunological status of the patient (Gullo et al. 2013). The presence of *Cryptococcus* sp. in the pulmonary alveoli triggers a cellular and humoral immunity response of the host, which under normal conditions control the infection. Due to the increase of immunocompromised patients in the general population, *Cryptococcus* spp. have become habitual opportunistic agents. The clinical manifestation can be varied, from an asymptomatic colonization of the airways to a disseminated infection with a predilection for the central nervous system (Messina et al. 2015). Due to the increase of immunocompromised individuals that develop opportunistic infections of *C. neoformans* and the appearance of resistant strains, the development of safer treatments for the control of this emergent disease becomes necessary. Related to this, antifungal products from phytochemicals could be considered an alternative for *Cryptococcus* spp. control. In this context, EOs could be an alternative of safer treatments. Table 10.4 reports the MIC values and the main composition of EOs from aromatic plants against *C. neoformans*. Abu-Darwish et al. (2015) showed the anti-*Cryptococcus* activity (MIC = 0.64 mg/mL) of *Artemisia herba-alba* EO rich in oxygenated monoterpenes (86.8%), being β -thujone, 1,8-cineole, α -thujone, and camphor their main components. Moreover, the antifungal activity against *C. neoformans* (MIC = 0.11 mg/mL) of *T. vulgaris* was reported by Kamdem et al. (2015), with thymol as the main component (57.9%). Many authors have attributed the antifungal activity of some EOs to the presence of this monoterpene (Bektas et al. 2016; Scalas et al. 2018; Pizzolitto et al. 2020). Khoury et al. (2018) evaluated the chemical compositions and the effect of four EOs from different species belonging to Apiaceae on *C. neoformans*. The EOs from *Ferula elaeochytris* and *Prangos asperula* were mainly composed of monoterpene hydrocarbons (80.2% and 74.4%, respectively), being α -pinene (71.8%) and β -pinene (6.80%) the prevalent compounds of *F. elaeochytris* EO, and α -pinene (9.8%), sabinene (29.8%), α -phellandrene (8.0%), β -phellandrene (19.2%), and nerolidol (9.2%) the major constituents of *P. asperula* EO. A high content of sesquiterpenes (73.1%) was reported for *Smyrniololus olusatrum* EO. Moreover, *Daucus carota* EO was rich in hydrocarbons such as α -pinene (27.4%), myrcene (5.3%), α -humulene (9.8%), and D-germacrene (7.0%). The MIC values were 0.13 mg/mL for *F. elaeochytris*, *S. olusatrum*, and *D. carota* EOs, and 0.26 mg/mL for *P. asperula* EO. Lawson et al. (2019) attributed the antifungal effect of *Helianthus* spp. against *C. neoformans* to the dominance of pinene in EO composition. Sesquiterpenes have been suggested to be responsible for the observed anti-*Cryptococcus* activity (MIC = 0.27 mg/mL) of *Plectranthus* spp. EOs (Mothana et al. 2018). Do Prado et al. (2018) reported a high activity against *C. neoformans* (MIC = 0.65 mg/mL) of *Schinus molle* EO, which is characterized by the dominance of pinene (43%) and myrcene (11.5%). Moreover, as shown in Table 10.4, *Zanthoxylum monogynum* EO reported citronellal (9.6%), citronellol (43.3%), and farnesol (32%) as the major compounds of the EO, with significant antifungal effect on *C. neoformans* development (Da Silva et al. 2017). Dos Santos et al. (2015) determined monoterpenes (70.11%) and sesquiterpenes (27.24%) as the major constituents of *Plectranthus amboinicus*, a strong anti-*Cryptococcus* EO (MIC = 0.01 mg/mL). With regard to *L. nobilis* EO, Sousa

Table 10.4 Minimal inhibitory concentration of EOs against *Cryptococcus neoformans*

PLANT EO	Main components (%)	MIC	References
<i>Artemisia herba-alba</i>	β -Thujone (25.1), α -Thujone (22.9), 1,8-Cineole (20.1)	0.64 mg/mL	Abu-Darwish et al. (2015)
<i>Daucus carota</i> subsp. <i>maximus</i>	α -Pinene (27.4), Carotol (26.3), α -Humulene (9.8), D-germacrene (7.0)	0.13 mg/mL	Khoury et al. (2018)
<i>Ferula elaeochytris</i>	α -Pinene (71.8), β -Pinene (6.8)	0.13 mg/mL	
<i>Prangos asperula</i>	Sabinene (29.8), β -Phellandrene (19.2), α -Pinene (9.8), Nerolidol (9.2)	0.26 mg/mL	
<i>Smyrniium olusatrum</i>	Curzerene (31.5), Furanoteremophil-1-one (28.5), Furanodiene (13.1)	0.13 mg/mL	
<i>Apium graveolens</i>	Limonene (50.7), Myrcene (12.5)	4.37 mg/mL	Kamdem et al. (2015)
<i>Thymus vulgaris</i>	Thymol (57.9), p-Cymene (10.3), Linalool (6.9)	0.11 mg/mL	
<i>Plectranthus cylindraceus</i>	Maaliol (42.8), Camphor (7.2)	0.27 mg/mL	Mothana et al. (2018)
<i>Plectranthus asirensis</i>	β -Caryophyllene (13.3), Spathulenol (8.7), Bicyclogermacrene (7.4),	0.27 mg/mL	
<i>Plectranthus barbatus</i>	Borneol (20.7)	0.27 mg/mL	
<i>Helianthus annuus</i> "Chianti"	α -Pinene (50.6), Camphene (7.3), Limonene (7.2), Bornyl acetate (7.1)	0.08 mg/mL	Lawson et al. (2019)
<i>Helianthus annuus</i> "Mammoth"	α -Pinene (48.9), Sabinene (17.0), Limonene (7.1)	0.15 mg/mL	
<i>Helianthus strumosus</i>	α -Pinene (58.6), Myrcene (9.8)	0.08 mg/mL	
<i>Plectranthus amboinicus</i>	Carvacrol (37.7), γ -Terpinene (14.7), (Z)-Caryophyllene (14.1), p-Cymene (12.0), Trans- α -bergamotene (8.2)	0.01 mg/mL	Dos Santos et al. (2015)
<i>Laurus nobilis</i>	Isoeugenol (57.0), Myrcene (15.9), Chavicol (9.3)	0.26 mg/mL	Sousa Pinheiro et al. (2017)
<i>Artemisia stricta</i>	Capillene (41.6), Spathulenol (14.6), β -Caryophyllene (13.4)	5.00 mg/mL	Manika et al. (2016)
<i>Baccharis parvidentata</i>	Sabinene (15.2), Himachalol (10.3), α -Pinene (9.2)	1.25 mg/mL	Perera et al. (2016)
<i>Lippia origanoides</i>	(E)-Methyl cinnamate (40.0), Hedycaryl (8.0), β -Eudesmol (7.3), α -Eudesmol (7.6)	0.08 mg/mL	
<i>Zanthoxylum monogynum</i>	Farnesol (32.0), Citronellol (43.3), Citronellal (9.6)	1.50 mg/mL	Da Silva et al. (2017)
<i>Schinus molle</i>	β -Pinene (25.2), Epi- α -cadinol (21.3), α -Pinene (18.7)	0.65 mg/mL	Do Prado et al. (2018)

Pinheiro et al. (2017) reported a MIC value of 0.26 mg/mL being the isoeugenol (57.0%) and myrcene (15. %) the main constituents. *Artemisia stricta* EO reported an effect against *C. neoformans* where capillene, a non-terpenoid constituent, comprised the major portion (41.6%) followed by β -caryophyllene (13.4%), spathulenol (14.6%), and myrcene (6.3%) (Manika et al. 2016). Perera et al. (2016) reported that the main constituents of *Lippia origanoides* EO were sesquiterpenes (45.3%) and cinnamate derivatives (41.7%), being the most effective EO tested by the authors (MIC = 0.078 mg/mL). Finally, the antifungal activity of *Baccharis parvidentata* against *C. neoformans* was evaluated (MIC = 1.25 mg/mL) being characterized by a high content of monoterpenes (51.9%) and sesquiterpenes (37.9%).

On the other hand, the combined effect of EOs and conventional antifungal drugs has been evaluated against *C. neoformans*. Tullio et al. (2019) reported a synergistic effect between *M. piperita* EO and itraconazole against *C. neoformans*. Moreover, Cardoso et al. (2017) combined *O. basilicum* ethanolic extract with amphotericin B, showing an increase in their anti-*Cryptococcus* activity. Capsule size, pigmentation, and ergosterol synthesis were also reduced. Similar results were reported in *C. neoformans* when *O. basilicum* EO and its main components, geraniol and linalool, were combined with fluconazole (Cardoso et al. 2016). Additionally, the synergistic effect of EOs and their main components in combination with chemical drugs against *C. neoformans* strains was evaluated by Scalas et al. (2018), who revealed the potential use of thyme and oregano EOs and carvacrol in combination with azoles for cryptococcosis treatment.

7 Aspergillosis

Aspergillosis represents a large and heterogeneous group of non-contagious opportunistic diseases caused by filamentous fungi of the genus *Aspergillus*. *Aspergillus* spp. are ubiquitous in the environment and grow as saprophytes, independently of an animal host. The human is constantly exposed to spores of *Aspergillus* which are effectively cleared by the immune system. However, in individuals with impaired immune functions, this exposure leads to the occurrence of invasive infections. The severity of the infection is the outcome of complex host–pathogen interactions, as well as the efficiency of the administered therapy. Among known *Aspergillus* spp., the majority of human infections are caused by *A. fumigatus*, followed by *A. flavus*, *A. terreus*, *A. nidulans*, and *A. niger*. *Aspergillus fumigatus* is the most important aerial fungal pathogen that causes invasive pulmonary infections in immunocompromised patients that often results in death (Latge and Chamilos 2019). The most commonly used drugs for the treatment of aspergillosis consist of antifungal agents that either target ergosterol, the main component of fungal membranes, or the synthesis of 1,3-glucan, the major component of the fungal cell wall (Latge and Chamilos 2019). The survival rates of immunocompromised patients with invasive aspergillosis have improved drastically with the use of azole antifungal drugs. However, the development of resistance mechanisms in *A. fumigatus* has increased

the mortality rates of patients with azole-resistant invasive aspergillosis from 50% to 100%, thus restricting the use of azole drugs (Verweij et al. 2016). The raising of resistant strains has resulted in the need for alternative therapeutic strategies, such as the use of new antifungal agents alone or in combinations with existing drugs. In this context, plant EOs and their active components have shown promising antifungal activities (Natu and Tatke 2019). Many EOs and EO pure components have been investigated for their antifungal activity against *A. fumigatus*. The MICs values of different EOs against *A. fumigatus* are shown in Table 10.5. Pure oxygenated compounds of phenolic nature report great antifungal activity, which is explained by the free hydroxyl group available to form hydrogen bonds with the active sites of different enzymes. Several EOs with high content of phenolic compounds were evaluated by different authors (Khan and Ahmad 2011b; Horváth et al. 2016; Ebani et al. 2017). The EOs from *T. vulgaris*, *O. vulgare*, and *Syzygium aromaticum* exerted a strong antifungal effect – thymol (46.3%), carvacrol (65.9%), and eugenol (74.3%) being their major constituents, respectively. The MIC of eugenol was identical to the MIC of *S. aromaticum* EO (0.32 mg/mL), indicating that this compound is responsible for the activity of the EO. This was not the case for thymol that showed a slightly higher MIC (0.19 mg/mL) than the MIC of *T. vulgaris* EO (0.14 mg/mL), which would indicate the presence of minor constituents in the EO that synergize the toxic effect of thymol (Khan and Ahmad 2011b). The EOs from different species belonging to the genus *Cymbopogon* showed high antifungal activity – their main constituents being aldehydes and alcohols monoterpenes, such as geranial and neral in *C. citratus* (74.0%), citronellal and geraniol in *C. nardus* (61.5%) and geraniol in *C. martini* (50.7%). The antifungal activity of EOs is usually attributable to their major components. However, minor constituents should not be underestimated because their presence may lead to additive, synergistic, or antagonistic effects. For example, the EO from *Litsea cubeba* showed a neral and geranial content very similar to *C. citratus*, but their MIC values were quite different (1.77 and 0.89 mg/mL, respectively), suggesting that other compounds might be influencing the bioactivity (Ebani et al. 2018). This is also the case of the EO from *Aloysia triphylla* which has a strong antifungal activity. Its chemical composition consists of 37.7% limonene, 24.0% sabinene, and 12.0% citronellal (Ebani et al. 2018). The high bioactivity is probably due to the combination of sabinene and citronellal, which proved effective against *A. fumigatus* (Aguiar et al. 2014; Roh and Shin 2014). On the contrary, its major component limonene is known for its weak inhibitory activity (Mahdavi Omran et al. 2011). This is in agreement with the high MIC values registered for different species of the genus *Citrus* that have limonene as their predominant component (Ebani et al. 2018). Cinnamaldehyde is the predominant component of *Cinnamomum zeylanicum* (56.4%) and *C. verum* EOs (79.1%) (Li et al. 2013; Ebani et al. 2018). This compound, as well as geraniol and citral, targets certain virulence factors of pathogenic fungi, such as elastase and keratinase activities (Khan and Ahmad 2011a), that destroy structural barriers during the infective process. Additionally, a fourfold increase of the MIC value of cinnamaldehyde occurred in the presence of the osmotic protector sorbitol, suggesting that it might affect the fungal cell wall (Khan and Ahmad 2011a). Additionally, scanning and transmission

Table 10.5 Minimal inhibitory concentration of EOs against *Aspergillus fumigatus*

PLANT EO	Main components (%)	MIC	References
<i>Cymbopogon nardus</i>	Citronellal (36.2), Geraniol (25.3)	0.78 mg/mL	Horváth et al. (2016)
<i>Cinnamomum zeylanicum</i>	Cinnamaldehyde (74.0)	0.19 mg/mL	
<i>Thymus vulgaris</i>	Thymol (46.3), p-cymene (22.1)	0.39 mg/mL	
	–	0.50 mg/mL	Puškářová et al. (2017)
	–	0.14 mg/mL	Khan and Ahmad (2011b)
	Thymol (52.6)	5.72 mg/mL	Ebani et al. (2017)
<i>Syzygium aromaticum</i>	–	0.25 mg/mL	Puškářová et al. (2017)
	Eugenol (88.6), β -Caryophyllene (8.6)	1.56 mg/mL	Horváth et al. (2016)
	Eugenol (74.3), β -Caryophyllene (7.0)	0.32 mg/mL	Khan and Ahmad (2011b)
<i>Cymbopogon martini</i>	Geraniol (50.7), Geraniol acetate (19.2)	0.14 mg/mL	
<i>Cinnamomum verum</i>	Cinnamaldehyde (79.1)	0.20 mg/mL	Moussaid et al. (2019), Li et al. (2013)
<i>Cuminum cyminum</i>	–	1.66 mg/mL	Khosravi et al. (2011)
	–	1.50 mg/mL	
<i>Heracleum sphondylium</i>	(Z)- β -Ocimene (28.9)	1.00 mg/mL (root)	Ušjak et al. (2017)
	Germacrene D (11.0) β -esquiphellandrene (10.6)	2.00 mg/mL (leaf)	
	Apiole (16.8), α -Acorenol (9.0)	0.50 mg/mL (flower)	
	Octyl acetate (67.1) n-Octanol (16.6),	1.50 mg/mL (fruit)	
<i>Heracleum sibiricum</i>	β -Pinene (26.2), Elemicina (25.6) Methyl eugenol (22.3)	0.30 mg/mL (root)	
	(Z)-Isoelemicin (16.6), Elemicina (14.9)	0.60 mg/mL (leaf)	
	Methyl eugenol (22.9), Elemicina (22.7), (Z)-Isoelemicin (18.5)	0.30 mg/mL (flower)	
	Octyl acetate (64.3) n-Octanol (21.1)	0.15 mg/mL (fruit)	
<i>Heracleum montanum</i>	(Z)- β -Ocimene (20.4)	0.50 mg/mL (root)	
	(E)- β -Farnesene (18.4), (E)-Caryophyllene (12.4)	2.00 mg/mL (leaf)	
	(E)- β -Farnesene (11.4), Sabinene (8.0)	0.50 mg/mL (flower)	
	Octyl acetate (57.5), n-Octanol (15.7)	3.00 mg/mL (fruit)	

(continued)

Table 10.5 (continued)

PLANT EO	Main components (%)	MIC	References	
<i>Abies holophylla</i>	Bornyl acetate (19.4), Limonene (16.8), 3-carene (13.65), camphene (10.7), α -Pinene (10.4)	0.12 mg/mL	Jang et al. (2012)	
<i>Aloysia tryphilla</i>	Limonene (36.7), Sabinene (24.0), Citronellal (12.0)	0.85 mg/mL	Ebani et al. (2018)	
<i>Citrus aurantium</i>	Limonene (94.7)	8.50 mg/mL		
<i>Citrus bergamia</i>	Limonene (33.2), Linalyl acetate (31.7)	8.70 mg/mL		
<i>Citrus limon</i>	Limonene (65.7), γ -Terpinene (9.3)	4.25 mg/mL		
<i>Citrus reticulata</i>	Limonene (72.1), γ -Terpinene (19.2)	4.25 mg/mL		
<i>Melaleuca alternifolia</i>	4-Terpineol (30.2), γ -Terpinene (16.9)	1.78 mg/mL		
<i>Eucalyptus globulus</i>	1,8-Cineole (89.8)	4.58 mg/mL		
<i>Cymbopogon citratus</i>	Geranial (38.4), Neral (35.2)	0.89 mg/mL		
<i>Litsea cubeba</i>	Geranial (36.4), Neral (32.5)	1.77 mg/mL		
<i>Pelargonium graveolens</i>	Citronellol (44.5), Geraniol (13.7)	8.90 mg/mL		
<i>Mentha piperita</i>	Menthol (32.4), Menthone (26.6)	9.12 mg/mL		
<i>Boswellia sacra</i>	α -Thujene (54.2)	8.50 mg/mL		
<i>Ocimum basilicum</i>	Linalool (46.0), Eugenol (11.5)	2.29 mg/mL		Ebani et al. (2017)
<i>Illicium verum</i>	(E)-anethol (89.8)	0.59 mg/mL		
<i>Rosmarinus officinalis</i>	α -Pinene (37.9), 1,8-cineole (22.0)	0.29 mg/mL		
<i>Salvia sclarea</i>	Linalyl acetate (54.7), (Z)-8-Hydroxylinalool (15.8)	2.23 mg/mL		
<i>Lavandula hybrida</i>	Linalool (31.5), Linalyl acetate (26.8)	8.50 mg/mL		
<i>Origanum vulgare</i>	Carvacrol (65.9)	0.19 mg/mL		
	–	0.25 mg/mL	Puškárová et al. (2017)	
<i>Thuja plicata</i>	–	0.75 mg/mL		
<i>Lavandula viridis</i>	1,8-Cineole (34.5), Camphor (13.4), α -Pinene (9.0)	2.50 μ L/mL	Zuzarte et al. (2011)	
<i>Lavandula stoechas</i>	Fenchone (37.0), Camphor (27.3)	1.25 μ L/mL	Zuzarte et al. (2013)	
<i>Thymus herba barona</i>	Carcacrol (54.0), Thymol (30.2)	0.16 μ L/mL		
<i>Oenanthe crocata</i>	Trans- β -Ocimene (31.3), Sabinene (29.0)	1.25 μ L/mL	Valente et al. (2013)	
<i>Seseli tortuosum</i>	α -Pinene (24.9), β -Pinene (23.9), (Z)- β -Ocimene (13.3)	5.00 μ L/mL	Gonçalves et al. (2012)	
<i>Seseli montanum</i>	α -Pinene (36.0), β -Pinene (22.5), Limonene (8.8)	10.00 μ L/mL		

electron micrographs of *A. fumigatus* treated with cinnamaldehyde showed a loss of integrity of the cell wall and membrane, expansion of endoplasmic reticulum, degeneration of mitochondria, autolysis, and degradation of cytoplasm content and an irregular distribution of polysaccharides. The pattern of ultrastructural alterations reported for this compound suggests multiple sites of action in fungi. The treatment of invasive pulmonary aspergillosis with cinnamaldehyde was highly effective in immunosuppressed mice (Deng et al. 2018). The survival rate of mice treated orally with cinnamaldehyde was significantly higher (80%) than the survival rate of mice treated with the positive control, voriconazole (60%). Additionally, the content of 1,3-glucan in lung tissues was significantly lower in mice treated with cinnamaldehyde compared to voriconazole, suggesting that cinnamaldehyde either interferes with the synthesis or destroys the integrity of the fungal cell wall (Deng et al. 2018).

As mentioned above, EOs can be obtained from different parts of the plants. The EOs from flowers, leaves, fruits, and roots from three different species belonging to the genus *Heracleum* were evaluated against *A. fumigatus* (Ušjak et al. 2017). Different major compounds were identified among species (and plant parts), being the EO from *Heracleum sibiricum* the one with lower MIC values. Particularly, the fruit of EOs was two- to four-fold more effective than the EOs from other plant parts of *H. sibiricum*, and 10- to 20-fold better than the fruit EO extracted from the other species (Ušjak et al. 2017).

Different interactions can occur among the components of an EO. Synergism represents a dynamic interplay of two or more compounds to enhance a bioactive effect. For example, the EO from *Abies holophylla* presents similar amounts of borneol, α -bisabolol, limonene, α -pinene, β -pinene, bornyl acetate, α -humulene, camphene, and caryophyllene (Jang et al. 2012). The MIC value of each pure compound was 0.25 mg/mL for borneol and α -bisabolol, and 0.5 mg/mL for the remaining compounds. The MIC value of the EO was significantly lower than 0.12 mg/mL, revealing a strong synergistic effect (Jang et al. 2012). Indeed, the mix of borneol and α -bisabolol reported a MIC of 0.12 mg/mL, lower than each single constituent, evidencing a synergistic effect. This pattern highlights the importance of hydroxyl groups-containing terpenes as antifungals. The sesquiterpene α -bisabolol inhibits *A. fumigatus* growth by affecting $\Delta 24$ -sterol methyltransferase, a crucial enzyme in ergosterol biosynthesis pathway (Jahanshiri et al. 2017), and borneol causes severe damages to the cell wall (Lee et al. 2013). Not all the compounds of an EO are effective antifungal agents. In many cases, pure compounds show higher antifungal activity (lower MIC values) than the EO. For example, *Seseli tortuosum* and *Seseli montanum* EOs have modest antifungal activities (MIC = 5 μ L/L and 10 μ L/L respectively). However, antifungal assays using their major component, α -pinene reported a MIC value of 1.25 μ L/L (Gonçalves et al. 2012).

The emergence of resistant strains has resulted in the need for novel antimycotic agents. Combinations of two or more antifungal agents have been performed to assess possible synergy and achieve a better therapeutic action. The degree of synergy between antimicrobial agents is often expressed in terms of the Fractional Inhibitory Concentration Index (FICI). According to Odds (2003), FICI ≤ 0.5

indicates synergy, FICI >0.5–4.0 indicates no interaction, and FICI >4.0 indicates an antagonistic effect. The EOs from *S. aromaticum* and *T. vulgaris* exhibit synergistic interactions with fluconazole against *A. fumigatus* (FICI = 0.250) (Khan and Ahmad 2011b). Regarding pure active compounds, eugenol shows a moderate synergism (FICI = 0.375) while thymol and cinnamaldehyde exhibit a stronger synergy with fluconazole (FICI = 0.187) against *A. fumigatus*. Fluconazole is one of the most efficient and safest antifungal drugs that affect the activity of the enzyme 14- α -demethylase, interrupting ergosterol biosynthesis (Khan and Ahmad 2011b). The synergistic interactions of EOs or their active compounds with fluconazole may be related to the simultaneous effects on different target sites by new agents and fluconazole, which is the desirable effect of combination therapies. Further experiments are needed to evaluate the therapeutic potential of these natural antifungal agents in combination with existing drugs against *A. fumigatus*.

8 Conclusion

The incidence of fungal infections has been continuously increasing over the last decades, with a high rate of death among patients with impaired immune systems. Furthermore, the rising of resistant strains has encouraged the development of new therapeutic strategies. Essential oils are composed of volatile organic compounds of different chemical nature mainly monoterpenes, sesquiterpenes, or phenylpropanoids, which proved to be effective for the treatment of invasive and superficial mycoses that affect human health. In addition, the synergistic activity of EOs and their pure components with existing antifungal drugs has been widely reported. The discovery of synergistic mechanisms to be exploited in combinational therapies will decrease the doses of synthetic drugs, thus reducing the development of resistance.

Acknowledgments The authors thank CONICET, FONCYT, and SECyT-UNC for the financial support.

References

- Abdollahi D, Jafariaraz Z, Afshar M (2020) Effect of monoterpenes on ex vivo transungual delivery of itraconazole for the management of onychomycosis. *J Cosmet Dermatol* 19(10):2745–2751
- Abu-Darwish M, Cabral C, Gonçalves M, Cavaleiro C Cruz MT, Efferth T, Salgueiro L (2015) *Artemisia herba-alba* essential oil from Buseirah (South Jordan): chemical characterization and assessment of safe antifungal and anti-inflammatory doses. *J Ethnopharmacol* 174:153–160
- Achimón F, Brito VD, Pizzolitto RP, Ramirez Sánchez A, Gómez EA, Zygadlo JA (2021) Chemical composition and antifungal properties of commercial essential oils against the maize phytopathogenic fungus *Fusarium verticillioides*. *Rev Argent Microbiol* S0325-7541(21):00001–00008
- Aghraz A, Wanner J, Schmidt E, Aitdra L, Aitsidibrahim M, Tabanca N, Larhsini M (2016) Chemical composition, antioxidant, antimicrobial and insecticidal activities of essential oil from a Moroccan endemic plant: *Bubonium imbricatum*. *Nat Prod Commun* 11:1717–1720

- Aguiar RWDS, Ootani MA, Ascencio SD, Ferreira TP, Santos MMD, Santos GRD (2014) Fumigant antifungal activity of *Corymbia citriodora* and *Cymbopogon nardus* essential oils and citronellal against three fungal species. *Sci World J* 2014:1–8
- Alessandrini A, Starace M, Bruni F, Piraccini BM (2020) An open study to evaluate effectiveness and tolerability of a nail oil composed of vitamin E and essential oils in mild to moderate distal subungual onychomycosis. *Ski Appendage Disord* 6(1):14–18
- Ali-Shtayah MS, Jamous RM, Abu-Zaitoun SY, Khasati AI, Kalbouneh SR (2019) Biological properties and bioactive components of *Mentha spicata* L. essential oil: focus on potential benefits in the treatment of obesity, Alzheimer's disease, dermatophytosis, and drug-resistant infections. *Evid Based Complement Alternat Med* 2019:3834265
- Allen D, Wilson D, Drew R, Perfect J (2015) Azole antifungals: 35 years of invasive fungal infection management. *Expert Rev Anti-Infect Ther* 13:787–798
- Amber K, Aijaz A, Immaculata X, Luqman KA, Nikhat M (2010) Anticandidal effect of *Ocimum sanctum* essential oil and its synergy with fluconazole and ketoconazole. *Phytomedicine* 17(12):921–925
- Aneke CI, Rhimi W, Pellicoro C, Cantacessi C, Otranto D, Cafarchia C (2020) The best type of inoculum for testing the antifungal drug susceptibility of *Microsporum canis*: in vivo and in vitro results. *Mycoses* 63(7):711–716
- Arora T, Oberoi L, Malhotra A (2020) Mycological pattern of dermatophytes and non-dermatophytes in a tertiary care hospital. *Int J Health Sci Res* 10(4):37–41
- Azadbakht M, Saeedi Akbarabadi A, Motazedian MH, Monadi T, Akbari F (2020) Anti-parasitic activity of some medicinal plants essential oils on *Giardia lamblia* and *Entamoeba histolytica*, in vitro. *Res J Pharmacogn* 7(1):41–47
- Bandalizadeh Z, Javidnia J, Hosseini SA, Moosazadeh M, Amouei A, Kermani F, Shokohi T (2020) *Cryptococcus* and cryptococcosis in Iran during 1969 – 2019: a systematic review and meta-analysis. *J Med Mycol* 30:100917
- Baptista EB, Zimmermann-Franco DC, Lataliza AAB, Raposo NRB (2015) Chemical composition and antifungal activity of essential oil from *Eucalyptus smithii* against dermatophytes. *Rev Soc Bras de Med Trop* 48(6):746–752
- Bektas E, Serdar G, Sokmen M, Sokmen A (2016) Biological activities of extracts and essential oil of *Thymus transcaucasicus* Ronniger. *J Essent Oil Bear Plants* 19:444–453
- Benigni R (2005) Structure–activity relationship studies of chemical mutagens and carcinogens: mechanistic investigations and prediction approaches. *Chem Rev* 105(5):1767–1800
- Benzaid C, Belmadani A, Djeribi R, Rouabhia M (2019) The effects of *Mentha × piperita* essential oil on *C. Albicans* growth, transition, biofilm formation, and the expression of secreted aspartyl proteinases genes. *Antibiotics (Basel)* 8:10
- Bona E, Cantamessa S, Pavan M, Novello G, Massa N, Rocchetti A, Gamalero E (2016) Sensitivity of *Candida albicans* to essential oils: are they an alternative to antifungal agents? *J Appl Microbiol* 121:1530–1545
- Boral H, Metin B, Döğen A, Seyedmousavi S, Ilkit M (2018) Overview of selected virulence attributes in *Aspergillus fumigatus*, *Candida albicans*, *Cryptococcus neoformans*, *Trichophyton rubrum*, and *Exophiala dermatitidis*. *Fungal Genet Biol* 111:92–107
- Borchers AT, Chang C, Eric Gershwin M (2017) Mold and human health: a reality check. *Clin Rev Allergy Immunol* 52:305–322
- Bouyahya A, Dakka N, Lagrouh F, Abrini J, Bakri Y (2018) In vitro antiproliferative and antidermatophyte activities of essential oils from three moroccan medicinal plants. *J Biolog Active Prod Nat* 8(3):144–153
- Bouyahya A, Lagrouh F, El Omari N, Bourais I, El Jemli M, Marmouzi I, Bakri Y (2020) Essential oils of *Mentha viridis* rich phenolic compounds show important antioxidant, antidiabetic, dermatoprotective, antidermatophyte and antibacterial properties. *Biocatal Agric Biotechnol* 23:101471

- Buriani A, Fortinguerra S, Sorrenti V, Caudullo G, Carrara M (2020) Essential oil phytocomplex activity, a review with a focus on multivariate analysis for a network pharmacology-informed phylogenomic approach. *Molecules* 25(8):1833
- Busato de Feiria SN, Santana P d L, Boni GC, Borioallo MFG, Figueira GM, Höfling JF (2016) Essential oil composition of *Mentha* spp. extracted seasonally and their effects against *Candida* yeast growth and biofilm formation. *Adv Med Plant Res* 4:106–115
- Caneschi CA, Martins FJ, Larrudé DG, Romani EC, Brandão MAF, Raposo NRB (2015) In vitro antifungal activity of *Baccharis trimera* less (DC) essential oil against dermatophytes. *Trop J Pharm Res* 14(11):2083–2089
- Cardoso NNR, Alviano CS, Blank AF, Romanos MTV, Fonseca BB, Rozental S, Alviano DS (2016) Synergism effect of the essential oil from *Ocimum basilicum* var. *maria bonita* and its major components with fluconazole and its influence on ergosterol biosynthesis. *Evid Based Complement Alternat Med* 2016:5647182
- Cardoso NNR, Alviano CS, Blank AF, Arrigoni-Blank MDF, Romanos MTV, Cunha MM, Alviano DS (2017) Anti-cryptococcal activity of ethanol crude extract and hexane fraction from *Ocimum basilicum* var. *maria bonita*: mechanisms of action and synergism with amphotericin B and *Ocimum basilicum* essential oil. *Pharm Biol* 55:1380–1388
- Carneiro JNP, da Cruz RP, Campina FF, do Socorro Costa M, Dos Santos ATL, Sales DL, Rebelo RA (2020) GC/MS analysis and antimicrobial activity of the *Piper mikanianum* (Kunth) Steud. essential oil. *Food Chem Toxicol* 135:110987
- Cassella S, Cassella JP, Smith I (2002) Synergistic antifungal activity of tea tree (*Melaleuca alternifolia*) and lavender (*Lavandula angustifolia*) essential oils against dermatophyte infection. *Int J Aromather* 12(1):2–15
- Cavaleiro C, Pinto E, Gonçalves MJ, Salgueiro L (2006) Antifungal activity of *Juniperus* essential oils against dermatophyte, *Aspergillus* and *Candida* strains. *J Appl Microbiol* 100(6):1333–1338
- Cavaleiro C, Salgueiro L, Gonçalves MJ, Hrimpeng K, Pinto J, Pinto E (2015) Antifungal activity of the essential oil of *Angelica major* against *Candida*, *Cryptococcus*, *Aspergillus* and dermatophyte species. *J Nat Med* 69(2):241–248
- Chan WK, Tan LTH, Chan KG, Lee LH, Goh BH (2016) Nerolidol: a sesquiterpene alcohol with multi-faceted pharmacological and biological activities. *Molecules* 21(5):529
- Córdoba S, Vivot W, Szusz W, Albo G (2019) Antifungal activity of essential oils against *Candida* species isolated from clinical samples. *Mycopathologia* 184:615–623
- Costa DCM, Vermelho AB, Almeida CA, Dias EPDS, Cedrola SML, Arrigoni-Blank MDF, Alviano DS (2014) Inhibitory effect of linalool-rich essential oil from *Lippia alba* on the peptidase and keratinase activities of dermatophytes. *J Enzyme Inhib Med Chem* 29(1):12–17
- Da Costa B, Pippi B, Andrzejewski Kaminski TF, Andrade SF, Fuentesfria AM (2020) In vitro anti-dermatophytic synergism of double and triple combination of clioquinol with ciclopirox and terbinafine. *Mycoses* 63(9):993–1001
- Da Silva FB, dos Santos NO, Pascon RC, Vallim MA, Figueiredo CR, Martins RCC, Sartorelli P (2017) Chemical composition and in vitro cytotoxic and antimicrobial activities of the essential oil from leaves of *Zanthoxylum monogynum* St. Hill (Rutaceae). *Medicines (Basel)* 4:25–31
- De Almeida WS, de Lima SG, Barreto HM, de Sousa Andrade LM, Fonseca L, Sobrinho CA, Muratori MCS (2018) Chemical composition and antimicrobial activity of the essential oil of *Lippia lasiocalycina* Cham. (Verbenaceae). *Ind Crop Prod* 125:236–240
- De Castro-Ontengco D, Capal T (2019) The activity of the leaf essential oil of Philippine *Piper betel* against dermatophytes and *Candida albicans*. *Philipp J Syst Biol* 13(2):15–18
- De Oliveira Lima MI, de Medeiros AA, Silva KS, Cardoso GN, de Oliveira LE, de Oliveira PF (2017) Investigation of the antifungal potential of linalool against clinical isolates of fluconazole resistant *Trichophyton rubrum*. *J Mycol Med* 27(2):195–202
- De Oliveira JC, de Vasconcelos PÁ, de Medeiros CAC, Ponte HAS, de Oliveira PF (2020) The sensitivity modifying activity of nerolidol and α -bisabolol against *Trichophyton* spp. *Indian J Microbiol* 60:1–6

- De Toledo LG, Dos Santos Ramos MA, Spósito L, Castilho EM, Pavan FR, Lopes ÉDO, Bauab TM (2016) Essential oil of *Cymbopogon nardus* (L.) Rendle: a strategy to combat fungal infections caused by *Candida* species. *Int J Mol Sci* 17(8):1252
- Deng J, Wang G, Li J, Zhao Y, Wang X (2018) Effects of cinnamaldehyde on the cell wall of *A. fumigatus* and its application in treating mice with invasive pulmonary aspergillosis. *Evid Based Complement Alternat Med* 2018:5823209
- Derby R, Rohal P, Jackson C, Beutler A, Olsen C (2011) Novel treatment of onychomycosis using over-the-counter mentholated ointment: a clinical case series. *J Am Board Fam Med* 24(1):69–74
- Dias N, Dias MC, Cavaleiro C, Sousa MC, Lima N, Machado M (2016) Oxygenated monoterpenes-rich volatile oils as potential antifungal agents for dermatophytes. *Nat Prod Res* 31(4):460–464
- Do Prado AC, Garces HG, Bagagli E, Rall VLM, Furlanetto A, Fernandes Junior A, Furtado FB (2018) *Schinus molle* essential oil as a potential source of bioactive compounds: antifungal and antibacterial properties. *J Appl Microbiol* 126:516–522
- Donato R, Sacco C, Pini G, Bilia AR (2020) Antifungal activity of different essential oils against *Malassezia* pathogenic species. *J Ethnopharmacol* 249:112376
- Dos Santos N, Mariane B, Lago J, Sartorelli P, Rosa W, Soares MG, Pascon RC (2015) Assessing the chemical composition and antimicrobial activity of essential oils from Brazilian plants—*Eremanthus erythropappus* (Asteraceae), *Plectranthus barbatus* and *P. amboinicus* (Lamiaceae). *Molecules* 20:8440–8452
- Dutta S, Kundu A, Saha S, Prabhakaran P, Mandal A (2020) Characterization, antifungal properties and in silico modelling perspectives of *Trachyspermum ammi* essential oil. *LWT Food Sci Technol* 131:109786
- Ebani VV, Nardoni S, Bertelloni F, Najari B, Pistelli L, Mancianti F (2017) Antibacterial and antifungal activity of essential oils against pathogens responsible for otitis externa in dogs and cats. *Medicines (Basel)* 4(2):21
- Ebani VV, Najari B, Bertelloni F, Pistelli L, Mancianti F, Nardoni S (2018) Chemical composition and in vitro antimicrobial efficacy of sixteen essential oils against *Escherichia coli* and *Aspergillus fumigatus* isolated from poultry. *Vet Sci* 5(3):62
- El Mokni R, Majdoub S, Jlassi I, Joshi RK, Hammami S (2020) Gas chromatography–mass spectrometry profile and antimicrobial activities of *Ballota bullata* Pomel and *B. nigra* L. subsp. *uncinata* (Fiori & Bég.): a comparative analysis. *Int J Mass Spectrom* 450:116305
- Elhidi N, Nafis A, Kasrati A, Goehler A, Bohnert JA, Abbad A, Mezrioui NE (2019) Chemical composition, antimicrobial activities and synergistic effects of essential oil from *Senecio antephorbium*, a Moroccan endemic plant. *Ind Crop Prod* 130:310–315
- Essid R, Hammami M, Gharbi D, Karkouch I, Hamouda TB, Elkahoui S, Tabbene O (2017) Antifungal mechanism of the combination of *Cinnamomum verum* and *Pelargonium graveolens* essential oils with fluconazole against pathogenic *Candida* strains. *Appl Microbiol Biotechnol* 101:6993–7006
- Fajinmi OO, Kulkarni MG, Benická S, Zeljković SĆ, Doležal K, Tarkowski P, Van Staden J (2019) Antifungal activity of the volatiles of *Agathosma betulina* and *Coleonema album* commercial essential oil and their effect on the morphology of fungal strains *Trichophyton rubrum* and *T. mentagrophytes*. *S Afr J Bot* 122:492–497
- Flores FC, Beck RC, da Silva CDB (2016) Essential oils for treatment of onychomycosis: a mini-review. *Mycopathologia* 181(1–2):9–15
- Gallucci MN, Carezzano ME, Oliva MM, Demo MS, Pizzolitto RP, Zunino MP, Zygodlo JA, Dambolena JS (2014) In vitro activity of natural phenolic compounds against fluconazole-resistant *Candida* species: a quantitative structure-activity relationship analysis. *J Appl Microbiol* 116:795–804
- Geddes-McAlister J, Shapiro RS (2019) New pathogens, new tricks: emerging, drug-resistant fungal pathogens and future prospects for antifungal therapeutics. *Ann N Y Acad Sci* 1435:57–78
- Ghafourian T, Zandasrar P, Hamishekar H, Nokhodchi A (2004) The effect of penetration enhancers on drug delivery through skin: a QSAR study. *J Control Release* 99(1):113–125

- Gonçalves MJ, Tavares AC, Cavaleiro C, Cruz MT, Lopes MC, Canhoto J, Salgueiro L (2012) Composition, antifungal activity and cytotoxicity of the essential oils of *Seseli tortuosum* L. and *Seseli montanum* subsp. *peixotoanum* (Samp.) M. Laínz from Portugal. *Ind Crop Prod* 39:204–209
- Góralaska K, Blaszkowska J, Dzikowiec M (2018) Neuroinfections caused by fungi. *Infection* 46:443–459
- Gullo F, Rossi S, Sadi J, Teodoro VLI, Mendes-Giannini MJS, Fusco-Almeida AM (2013) Cryptococcosis: epidemiology, fungal resistance, and new alternatives for treatment. *Eur J Clin Microbiol Infect Dis* 32:1377–1391
- Gupta AK, Stec N, Summerbell RC, Shear NH, Piguet V, Tosti A, Maria Piraccini B (2020) Onychomycosis: a review. *J Eur Acad Dermatol Venereol* 34(9):1972–1990
- Hameed S, Fatima Z (2019) Pathogenicity and drug resistance of human pathogens. Springer, Singapore
- Hammani S, Debbabi H, Jlassi I, Joshi RK, El Mokni R (2020) Chemical composition and antimicrobial activity of essential oil from the aerial parts of *Plantago afra* L. (Plantaginaceae) growing wild in Tunisia. *S Afr J Bot* 132:410–414
- Hay RJ, Ashbee HR (2016) Fungal infections. Rook's textbook of dermatology, 9th edn. Wiley-Blackwell, Oxford, pp 1–110
- Heitman J, Kozel TR, Kwon-Chung KJ, Perfect JR, Casadevall A (2011) *Cryptococcus* from human pathogen to model yeast, 1st edn. American Society for Microbiology, Washington, DC
- Horváth G, Jenei JT, Vágvölgyi C, Böszörményi A, Krisch J (2016) Effects of essential oil combinations on pathogenic yeasts and moulds. *Acta Biol Hung* 67(2):205–214
- Houël E, Rodrigues AMS, Jahn-Oyac A, Bessière JM, Eparvier V, Deharo SD (2014) In vitro antidermatophytic activity of *Otacanthus azureus* (Linden) Ronse essential oil alone and in combination with azoles. *J Appl Microbiol* 116(2):288–294
- Ilić DP, Stanojević LP, Troter DZ, Stanojević JS, Danilović BR, Nikolić VD, Nikolić LB (2019) Improvement of the yield and antimicrobial activity of fennel (*Foeniculum vulgare* Mill.) essential oil by fruit milling. *Ind Crop Prod* 142:11854
- Jahanshiri Z, Shams-Ghahfarokhi M, Asghari-Paskiabi F, Saghiri R, Razzaghi-Abyaneh M (2017) α -Bisabolol inhibits *Aspergillus fumigatus* Af239 growth via affecting microsomal Δ 24-sterol methyltransferase as a crucial enzyme in ergosterol biosynthesis pathway. *World J Microbiol Biotechnol* 33(3):55
- Jain N, Sharma M (2017) Evaluation of *Citrus lemon* essential oil for its chemical and biological properties against fungi causing dermatophytic infection in human beings. *Anal Chem Lett* 7(3):402–409
- Jamin F, Estuningsih S, Pribadi ES, Handharyani E (2020) Mechanisms of dermatophyte invasion on New Zealand rabbit skin models. In E3S Web of conferences 151: 01055. EDP Sciences
- Jang SK, Lee SY, Kim SH, Hong CY, Park MJ, Choi IG (2012) Antifungal activities of essential oils from six conifers against *Aspergillus fumigatus*. *J Korean Wood Sci Technol* 40(2):133–140
- Jorgensen J, Carroll K, Funke G, Pfaller MA (2015) Manual of clinical microbiology, 11th. edn. ASM Press, Washington, DC
- Kamdem M, Sameza ML, Dongmo PMJ, Boyom FF, Bakargna-Via I, Fokou JBH, Menut C (2015) Antiradical, anti-inflammatory and antifungal activities of essential oils of two aromatic plants: *Apium graveolens* (Apiaceae) and *Thymus vulgaris* (Lamiaceae). *J Life Sci* 9:51–64
- Khan MSA, Ahmad I (2011a) In vitro antifungal, anti-elastase and anti-keratinase activity of essential oils of *Cinnamomum*-, *Syzygium*- and *Cymbopogon*-species against *Aspergillus fumigatus* and *Trichophyton rubrum*. *Phytomedicine* 19(1):48–55
- Khan MSA, Ahmad I (2011b) Antifungal activity of essential oils and their synergy with fluconazole against drug-resistant strains of *Aspergillus fumigatus* and *Trichophyton rubrum*. *Appl Microbiol Biotechnol* 90(3):1083–1094
- Khan MSA, Ahmad I, Cameotra SS (2014) *Carum copticum* and *Thymus vulgaris* oils inhibit virulence in *Trichophyton rubrum* and *Aspergillus* spp. *Braz J Microbiol* 45(2):523–531

- Khosravi AR, Minoeianhaghighi MH, Shokri H, Emami SA, Alavi SM, Asili J (2011) The potential inhibitory effect of *Cuminum cyminum*, *Ziziphora clinopodioides* and *Nigella sativa* essential oils on the growth of *Aspergillus fumigatus* and *Aspergillus flavus*. *Braz J Microbiol* 42(1):216–224
- Khoury M, El Beyrouthy M, Eparvier V et al (2018) Chemical diversity and antimicrobial activity of the essential oils of four Apiaceae species growing wild in Lebanon. *J Essent Oil Res* 30:25–31
- Koba K, Poutouli PW, Raynaud C, Sanda K (2009) Antifungal activity of the essential oils from *Ocimum gratissimum* L. grown in Togo. *J Sci Res* 1(1):164–171
- Köhler JR, Hube B, Puccia R, Casadevall A, Perfect JR (2017) Fungi that infect humans. *Fung Kingdom* 5:813–843
- Kumari A, Singh RK (2020) Morpholine as ubiquitous pharmacophore in medicinal chemistry: deep insight into the structure-activity relationship (SAR). *Bioorg Chem* 96:103578
- Lari ZN, Hajimonfarednejad M, Riasatian M, Abolhassanzadeh Z, Iraj A, Vojoud M, Shams M (2020) Efficacy of inhaled *Lavandula angustifolia* Mill. Essential oil on sleep quality, quality of life and metabolic control in patients with diabetes mellitus type II and insomnia. *J Ethnopharmacol* 251:112560
- Latge JP, Chamilos G (2019) *Aspergillus fumigatus* and aspergillosis in 2019. *Clin Microbiol Rev* 33(1):1–75
- Lawson SK, Sharp LG, Satyal P, Setzer WN (2019) Volatile components of the aerial parts of *Conoclinium coelestinum* from North Alabama. *Am J Essent Oils Nat Prod* 7:8–10
- Lee SY, Kim SH, Hong CY, Park MJ, Choi IG (2013) Effects of (–)-borneol on the growth and morphology of *Aspergillus fumigatus* and *Epidermophyton floccosum*. *Flavour Frag J* 28(2):129–134
- Li YQ, Kong DX, Wu H (2013) Analysis and evaluation of essential oil components of cinnamon barks using GC–MS and FTIR spectroscopy. *Ind Crop Prod* 41:269–278
- Limaverde PW, Campina FF, da Cunha FA, Crispim FD, Figueredo FG, Lima LF, Balbino VQ (2017) Inhibition of the TetK efflux-pump by the essential oil of *Chenopodium ambrosioides* L. and α -terpinene against *Staphylococcus aureus* IS-58. *Food Chem Toxicol* 109:957–961
- Lira MHPD, Andrade Júnior FPD, Moraes GFQ, Macena GDS, Pereira FDO, Lima IO (2020) Antimicrobial activity of geraniol: an integrative review. *J Essent Oil Res* 32(3):187–197
- Liu X, Wang LP, Li YC, Li HY, Yu T, Zheng XD (2009) Antifungal activity of thyme oil against *Geotrichum citri-aurantii* in vitro and in vivo. *J Appl Microbiol* 107(5):1450–1456
- Lopes G, Pinto E, Salgueiro L (2017) Natural products: an alternative to conventional therapy for dermatophytosis? *Mycopathologia* 182(1–2):143–167
- Mahboubi M, Kazempour N (2015) The antifungal activity of *Artemisia sieberi* essential oil from different localities of Iran against dermatophyte fungi. *J Mycol Med* 25(2):65–71
- Mahboubi M, Mohammad Taghizadeh Kashani L (2016) The anti-dermatophyte activity of *Commiphora molmol*. *Pharm Biol* 54(4):720–725
- Mahboubi M, Tabar RH (2018) Chemical composition and antifungal activities of *Ziziphora tenuir* and *Z. clinopodioides* essential oils against dermatophytes. *Herba Pol* 64(2):37–45
- Mahboubi M, Heidarytabar R, Mahdizadeh E, Hosseini H (2017) Antimicrobial activity and chemical composition of *Thymus* species and *Zataria multiflora* essential oils. *Agric Nat Resour* 51(5):395–401
- Mahdavi Omran S, Moodi MA, Norozian Amiri SMB, Mosavi S, Ghazi Mir Saeed SAM, Jabbari Shiade SM, Salehi M (2011) The effects of limonene and orange peel extracts on some spoilage fungi. *Int J Mol Clin Microbiol* 1(2):82–86
- Manika N, Singh Chanotiya C, Darokar M, Singh S, Bagchi GD (2016) Compositional characters and antimicrobial potential of *Artemisia stricta* Edgew f. *stricta* Pamp. essential oil. *Rec Nat Prod* 10:40–46
- Manoharan RK, Lee JH, Kim YG, Kim SI, Lee J (2017) Inhibitory effects of the essential oils α -longipinene and linalool on biofilm formation and hyphal growth of *Candida albicans*. *Biofouling* 33:143–155

- Marongiu B, Maxia A, Piras A, Porcedda S, Tuveri E, Gonçalves MJ, Salgueiro L (2007) Isolation of *Crithmum maritimum* L. volatile oil by supercritical carbon dioxide extraction and biological assays. *Nat Prod Res* 21(13):1145–1150
- Martínez-Matías N, Rodríguez-Medina JR (2018) Fundamental concepts of azole compounds and triazole antifungals: a beginner's review. *P R Health Sci J* 37(3):135–142
- Martinez-Rossi NM, Bitencourt TA, Peres NT, Lang EA, Gomes EV, Quaresimin NR, Rossi A (2018) Dermatophyte resistance to antifungal drugs: mechanisms and prospectus. *Front Microbiol* 9:1108
- Marx-Stoelting P, Knebel C, Braeuning A (2020) The connection of azole fungicides with xeno-sensing nuclear receptors, drug metabolism and hepatotoxicity. *Cell* 9(5):1192
- Mehriardestani M, Aliahmadi A, Toliati T, Dalimi A, Momeni Z, Rahimi R (2020) Evaluation of antimicrobial activity of *Trachyspermum ammi* (L.) Sprague essential oil and its active constituent, thymol, against vaginal pathogens. *Tradit Integr Med* 5:49–58
- Mercer DK, Stewart CS (2019) Keratin hydrolysis by dermatophytes. *Med Mycol* 57(1):13–22
- Messina FA, Maiolo E, Negroni R, Arechavala A, Santiso G, Bianchi M (2015) Alternativas terapéuticas de la criptococosis meníngea. *Actual Infectol* 23:25–32
- Miao Q, Zhao L, Wang Y, Hao F, Sun P, He P, Deng G (2020) Microbial metabolomics and network analysis reveal fungistatic effect of basil (*Ocimum basilicum*) oil on *Candida albicans*. *J Ethnopharmacol* 260:113002
- Miron D, Cornelio R, Troleis J, Mariath J, Zimmer AR, Mayorga P, Schapoval EES (2014) Influence of penetration enhancers and molecular weight in antifungals permeation through bovine hoof membranes and prediction of efficacy in human nails. *Eur J Pharm Sci* 51:20–25
- Mothana RA, Khaled JM, Noman OM, Kumar A, Alajmi MF, Al-Rehaily AJ, Kurkcuoglu M (2018) Phytochemical analysis and evaluation of the cytotoxic, antimicrobial and antioxidant activities of essential oils from three *Plectranthus* species grown in Saudi Arabia. *BCM Complement Med Ther* 18:1–10
- Moussaid F, El Atki Y, El Barnossi A, Abdellaoui A, Housseini AI (2019) In vitro antifungal activity of *Cinnamum burmannii* and *Cuminum cyminum* essential oils against two nosocomial strains of *Aspergillus fumigatus*. *J Pharm Sci Res* 11(3):718–720
- Najar B, Shortrede JE, Pistelli L, Buhagiar J (2020) Chemical composition and in vitro cytotoxic screening of sixteen commercial essential oils on five cancer cell lines. *Chem Biodivers* 17(1):e1900478
- Nardoni S, Giovanelli S, Pistelli L, Mugnaini L, Profili G, Pisseri F, Mancianti F (2015) In vitro activity of twenty commercially available, plant-derived essential oils against selected dermatophyte species. *Nat Prod Commun*. <https://doi.org/10.1177/1934578X1501000840>
- Natu KN, Tatke PA (2019) Essential oils—prospective candidates for antifungal treatment? *J Essent Oil Res* 31(5):347–360
- Ngo-Mback MNL, Famewo EB, MubarakAli D, Eke P, Thajuddin N, Afolayan AJ, Boyom FF (2019) An investigation of chemical composition and antimicrobial activity of essential oils extracted from *Aeollanthus* and *Plectranthus* species. *Biocatal Agric Biotechnol* 22:101412
- Nogueira Sobrinho AC, Morais SMD, Souza EBD, Albuquerque MRJR, Santos HSD, Cavalcante CSDP, Fontenelle RODS (2020) Antifungal and antioxidant activities of *Vernonia chalybaea* Mart. ex DC. essential oil and their major constituent β -caryophyllene. *Braz Arch Biol Technol* 63:1–11
- Oboh G, Olasehinde TA, Ademosun AO (2017) Inhibition of enzymes linked to type-2 diabetes and hypertension by essential oils from peels of orange and lemon. *Int J Food Prop* 20(sup1):586–594
- Odds FC (2003) Synergy, antagonism, and what the checkerboard puts between them. *J Antimicrob Chemother* 52(1):1–1
- Orchard A, van Vuuren SF, Viljoen AM (2019) Commercial essential oil combinations against topical fungal pathogens. *Nat Prod Commun* 14(1). <https://doi.org/10.1177/1934578X1901400139>

- Padovan ACB, da Silva Rocha WP, de Moraes Toti AC, de Jesus DFF, Chaves GM, Colombo AL (2019) Exploring the resistance mechanisms in *Trichosporon asahii*: Triazoles as the last defense for invasive trichosporonosis. *Fungal Genet Biol* 133:103267
- Peixoto LR, Rosalen PL, Ferreira GLS, Freires IA, de Carvalho FG, Castellano LR, de Castro RD (2017) Antifungal activity, mode of action and anti-biofilm effects of *Laurus nobilis* Linnaeus essential oil against *Candida* spp. *Arch Oral Biol* 73:179–185
- Pereira FDO, Mendes JM, Lima IO, Mota KSDL, Oliveira WAD, Lima EDO (2015) Antifungal activity of geraniol and citronellol, two monoterpenes alcohols, against *Trichophyton rubrum* involves inhibition of ergosterol biosynthesis. *Pharm Biol* 53(2):228–234
- Perera WH, Bizzo HR, Gama PE, Alviano CS, Salimena FRG, Alviano DS, Leitão SG (2016) Essential oil constituents from high altitude Brazilian species with antimicrobial activity: *Baccharis parvidentata* Malag., *Hyptis monticola* Mart. ex Benth. and *Lippia origanoides* Kunth. *J Essent Oil Res* 29:109–116
- Pinto E, Pina Vaz C, Salgueiro L, Gonçalves MJ, Costa-de-Oliveira S, Cavaleiro C, Martinez de Oliveira J (2006) Antifungal activity of the essential oil of *Thymus pulegioides* on *Candida*, *Aspergillus* and dermatophyte species. *J Med Microbiol* 55(10):1367–1373
- Pinto E, Hrimpeng K, Lopes G, Vaz S, Gonçalves MJ, Cavaleiro C, Salgueiro L (2013a) Antifungal activity of *Ferulago capillaris* essential oil against *Candida*, *Cryptococcus*, *Aspergillus* and dermatophyte species. *Eur J Clin Microbiol Infect Dis* 32(10):1311–1320
- Pinto E, Gonçalves MJ, Hrimpeng K, Pinto J, Vaz S, Vale-Silva LA, Salgueiro L (2013b) Antifungal activity of the essential oil of *Thymus villosus* subsp. *lusitanicus* against *Candida*, *Cryptococcus*, *Aspergillus* and dermatophyte species. *Ind Crop Prod* 51:93–99
- Pinto E, Gonçalves MJ, Cavaleiro C, Salgueiro L (2017) Antifungal activity of *Thapsia villosa* essential oil against *Candida*, *Cryptococcus*, *Malassezia*, *Aspergillus* and dermatophyte species. *Molecules* 22(10):1595
- Pizzolitto RP, Jacquat AG, Usseglio VL, Achimón F, Cuello AE, Zygodlo JA, Dambolena JS (2020) Quantitative-structure-activity relationship study to predict the antifungal activity of essential oils against *Fusarium verticillioides*. *Food Control* 108:106836
- Ponte HAS, Lima MIDO, Lima EDO, Pereira FDO (2020) Linalool modulates dermatophyte susceptibility to azole drugs. *Med Mycol* 58(2):272–274
- Poojary SA (2017) Topical antifungals: a review and their role in current management of dermatophytes. *Clin Dermatol Rev* 1(3):24
- Prasad CS, Shukla R, Kumar A, Dubey NK (2010) In vitro and in vivo antifungal activity of essential oils of *Cymbopogon martini* and *Chenopodium ambrosioides* and their synergism against dermatophytes. *Mycoses* 53(2):123–129
- Pušárová A, Bučková M, Kraková L, Pangallo D, Kozics K (2017) The antibacterial and antifungal activity of six essential oils and their cyto/genotoxicity to human HEL 12469 cells. *Sci Rep* 7(1):1–11
- Pyun MS, Shin S (2006) Antifungal effects of the volatile oils from *Allium* plants against *Trichophyton* species and synergism of the oils with ketoconazole. *Phytomedicine* 13(6):394–400
- Quatrin PM, Verdi CM, de Souza ME, de Godoi SN, Klein B, Gundel A, Santos RCV (2017) Antimicrobial and antibiofilm activities of nanoemulsions containing *Eucalyptus globulus* oil against *Pseudomonas aeruginosa* and *Candida* spp. *Microb Pathog* 112:230–242
- Ramsewak RS, Nair MG, Stommel M, Selanders L (2003) In vitro antagonistic activity of monoterpenes and their mixtures against ‘toe nail fungus’ pathogens. *Phytother Res* 17(4):376–379
- Raspo MA, Vignola MB, AndreattaAE JHR (2020) Antioxidant and antimicrobial activity of citrus essential oils from Argentina and the United States. *Food Biosci* 36:100651
- Rodrigues FFG, Colares AV, Nonato C d FA, Galvão-Rodrigues FF, Mota ML, Braga MFBM, da Costa JGM (2018) In vitro antimicrobial activity of the essential oil from *Vanillosmopsis arborea* Barker (Asteraceae) and its major constituent, α -bisabolol. *Microb Pathog* 125:144–149
- Roh J, Shin S (2014) Antifungal and antioxidant activities of the essential oil from *Angelica koreana* Nakai. *Evid Based Complement Alternat Med*. <https://doi.org/10.1155/2014/398503>

- Roy SK, Pahwa S, Nandanwar H, Jachak SM (2012) Phenylpropanoids of *Alpinia galanga* as efflux pump inhibitors in *Mycobacterium smegmatis* mc2 155. *Fitoterapia* 83(7):1248–1255
- Salgueiro LR, Pinto E, Gonçalves MJ, Costa I, Palmeira A, Cavaleiro C, Martinez-de-Oliveira J (2006) Antifungal activity of the essential oil of *Thymus capitellatus* against *Candida*, *Aspergillus* and dermatophyte strains. *Flavour Fragr J* 21(5):749–753
- Scalas D, Mandras N, Roana J, Tardugno R, Cuffini AM, Ghisetti V, Tullio V (2018) Use of *Pinus sylvestris* L. (Pinaceae), *Origanum vulgare* L. (Lamiaceae), and *Thymus vulgaris* L. (Lamiaceae) essential oils and their main components to enhance itraconazole activity against azole susceptible / not-susceptible *Cryptococcus neoform*. *BMC Complement Altern Med* 18:1–13
- Sharifzadeh A, Shokri H (2016) Antifungal activity of essential oils from Iranian plants against fluconazole-resistant and fluconazole-susceptible *Candida albicans*. *Avicenna J Phytomed* 6:215–222
- Shin S, Kang CA (2003) Antifungal activity of the essential oil of *Agastache rugosa* Kuntze and its synergism with ketoconazole. *Lett Appl Microbiol* 36(2):111–115
- Shin S, Lim S (2004) Antifungal effects of herbal essential oils alone and in combination with ketoconazole against *Trichophyton* spp. *J Appl Microbiol* 97(6):1289–1296
- Silva LN, de Mello TP, de Souza RL, Branquinha M, dos Santos ALS (2019) Current challenges and updates on the therapy of fungal infections. *Curr Top Med Chem* 19:495–499
- Singhi P, Saini AG (2019) Fungal and parasitic CNS infections. *Indian J Pediatr* 86:83–90
- Snell M, Klebert M, Önen NF, Hubert S (2016) A novel treatment for onychomycosis in people living with HIV infection: Vicks VapoRub™ is effective and safe. *J Assoc Nurses AIDS Care* 27(1):109–113
- Song YG, Lee SH (2019) Efficacy of newly developed denture cleaning device on physical properties of denture material and *Candida* biofilm. *J Dent Sci* 14:248–254
- Soulaimani B, Nafis A, Kasrati A, Rochdi A, Mezrioui NE, Abbad A, Hassani L (2019) Chemical composition, antimicrobial activity and synergistic potential of essential oil from endemic *Lavandula maroccana* (Mill.). *South African J Bot* 125:202–206
- Sousa Pinheiro L, Alves de Oliveira A, Queiroga Sarmiento Guerra F (2017) Antifungal activity of the essential oil isolated from *Laurus nobilis* L. against *Cryptococcus neoformans* strains. *J Appl Pharm Sci* 7:115–118
- Swamy MK, Akhtar MS, Sinniah UR (2016) Antimicrobial properties of plant essential oils against human pathogens and their mode of action: an updated review. *Evid Based Complement Alternat Med*. <https://doi.org/10.1155/2016/3012462>
- Takao Y, Kuriyama I, Yamada T, Mizoguchi H, Yoshida H, Mizushima Y (2012) Antifungal properties of Japanese cedar essential oil from waste wood chips made from used sake barrels. *Mol Med Rep* 5(5):1163–1168
- Tariq S, Wani S, Rasool W, Shafi K, Bhat MA, Prabhakar A, Rather MA (2019) A comprehensive review of the antibacterial, antifungal and antiviral potential of essential oils and their chemical constituents against drug-resistant microbial pathogens. *Microb Pathogen* 134:103580
- Tavares AC, Gonçalves MJ, Cruz MT, Cavaleiro C, Lopes MC, Canhoto J, Salgueiro LR (2010) Essential oils from *Distichoselinum tenuifolium*: chemical composition, cytotoxicity, antifungal and anti-inflammatory properties. *J Ethnopharmacol* 130(3):593–598
- Tedila H, Assefa A, Haji F (2019) Candidiasis and opportunistic mycosis in human. *Res Microbiol* 3:190–203
- Tullio V, Roana J, Scalas D, Mandras N (2019) Evaluation of the antifungal activity of *Mentha x piperita* (Lamiaceae) of Pancalieri (Turin, Italy) essential oil and its synergistic interaction with azoles. *Molecules* 24:3148
- Ušjak L, Petrović S, Drobac M, Soković M, Stanojković T, Ćirić A, Niketić M (2017) Essential oils of three cow parsnips—composition and activity against nosocomial and foodborne pathogens and food contaminants. *Food Funct* 8(1):278–290

- Valente J, Zuzarte M, Gonçalves MJ, Lopes MC, Cavaleiro C, Salgueiro L, Cruz MT (2013) Antifungal, antioxidant and anti-inflammatory activities of *Oenanthe crocata* L. essential oil. *Food Chem Toxicol* 62:349–354
- Vázquez Tsuji O, Martínez Barbabosa I, Campos Rivera T (2005) Criptococosis. Historia natural y estado actual del tratamiento. *Acta Pediatr Mex* 26:18–28
- Verweij PE, Chowdhary A, Melchers WJ, Meis JF (2016) Azole resistance in *Aspergillus fumigatus*: can we retain the clinical use of mold-active antifungal azoles? *Clin Infect Dis* 62(3):362–368
- Yeh TH, Lin JY (2020) *Acorus gramineus* and *Euodia ruticarpa* steam distilled essential oils exert anti-inflammatory effects through decreasing Th1/Th2 and pro-/anti-inflammatory cytokine secretion ratios in vitro. *Biomol Ther* 10(2):338
- Zacchino SA, López SN, Pezzenati GD, Furlán RL, Santecchia CB, Muñoz L, Enriz RD (1999) In vitro evaluation of antifungal properties of phenylpropanoids and related compounds acting against dermatophytes. *J Nat Prod* 62(10):1353–1357
- Zuzarte M, Gonçalves MJ, Cavaleiro C, Canhoto J, Vale-Silva L, Silva MJ, Salgueiro L (2011) Chemical composition and antifungal activity of the essential oils of *Lavandula viridis* L'Hér. *J Med Microbiol* 60(5):612–618
- Zuzarte M, Gonçalves MJ, Cavaleiro C, Cruz MT, Benzarti A, Marongiu B, Salgueiro L (2013) Antifungal and anti-inflammatory potential of *Lavandula stoechas* and *Thymus herba-barona* essential oils. *Ind Crop Prod* 44:97–103
- Zygodlo JA (2011) Aceites esenciales. Química, ecología, comercio, producción y salud. Universitas. (9789871457632), Córdoba, p 192
- Zygodlo JA, Zunino MP, Pizzolitto RP, Merlo C, Omarini AB, Dambolena JS (2017) Antibacterial and anti-biofilm activities of essential oils and their components including modes of action. Essential oils and nanotechnology for treatment of microbial diseases. CRC Press, Taylor & Francis Group, Boca Raton

Chapter 11

Promising Antimicrobial Agents from Some Latin American Medicinal Plants Against Disease-Causing Pathogens in Humans and Animals



Amner Muñoz-Acevedo, María C. González, Osnaider J. Castillo, Cindy P. Guzmán, Sandra Rodríguez-Acosta, Martha Cervantes-Díaz, Ricardo D. D. G. De Albuquerque, Bettina M. Ruppelt, Ninoska Flores, Alberto Giménez-Turba, Feliza Ramón-Farias, Leticia M. Cano-Asseleih, Elsa Rengifo, Gabriel Vargas-Arana, and Mahendra Rai

A. Muñoz-Acevedo (✉) · M. C. González · O. J. Castillo · C. P. Guzmán
Departamento de Química y Biología, Universidad del Norte, Colombia
e-mail: amnerm@uninorte.edu.co

S. Rodríguez-Acosta
Grupo de Análisis Económico, Instituto de Estudios Económicos del Caribe, Departamento de Economía, Universidad del Norte, Barranquilla, Colombia

M. Cervantes-Díaz
Grupo Investigaciones Ambientales para el Desarrollo Sostenible, Universidad Santo Tomás, Bucaramanga, Colombia

R. D. D. G. De Albuquerque
Laboratório de Tecnologia em Produtos Naturais, Universidade Federal Fluminense, Brasil

B. M. Ruppelt
Departamento de Tecnologia Farmacêutica, Universidade Federal Fluminense, Niterói, Brasil

N. Flores · A. Giménez-Turba
Instituto de Investigaciones Fármaco Bioquímicas, Universidad Mayor de San Andrés, La Paz, Bolivia

F. Ramón-Farias
Facultad de Ciencias Biológicas y Agropecuarias, Universidad Veracruzana, Veracruz, México

L. M. Cano-Asseleih
Centro de Investigaciones Tropicales, Universidad Veracruzana, Xalapa-Enríquez, México

E. Rengifo · G. Vargas-Arana
Instituto de Investigaciones de la Amazonía Peruana, Iquitos, Perú

M. Rai
Department of Biotechnology, Sant Gadge Baba Amravati University, Amravati, India

Abstract According to WHO and FAO, the current global problem in public health/environmental which is of high economic impact is the lack of effectiveness/resistance to “antimicrobials” by many pathogens (e.g., *Staphylococcus* spp., *Mycobacterium* spp., *Klebsiella* spp., *Pseudomonas* spp., *Helicobacter* spp., *Listeria* spp., *Salmonella* spp., *Acinetobacter* spp., *Aspergillus* spp., *Candida* spp.) that cause disease in humans (e.g., septicemia, nosocomial, respiratory, STD) and food-producing animals (e.g., cattle, goats, poultry), which could be related to food security. Nonetheless, since 1990s, new antimicrobials (new chemical libraries or structures/scaffolds) have not been found in the pharmaceutical industry, but their “new agents” (commercially available drugs) were redesigned from earlier times and prospecting for new discoveries was no longer relevant. In that sense, the WHO has mentioned/emphasized the need to research/develop new antimicrobials since the available therapeutic options are limited, due to the low investment in development and research of new drugs, as well as the few incentives to search/isolate/synthesize new molecules that allow to combat/control/reduce the problem of resistance. Among this search for therapeutic options, the WHO itself has recommended the inclusion of *traditional and complementary medicine* as a promising alternative that, if it does not completely solve the problem of resistance, at least temporarily contributes to the solution as a new treatment. Thus, nature has provided from some plants certain constituents (isolated/in mixture) with a high biological potential against particular pathogenic microorganisms that cause human/animal diseases. In this chapter, specific cases of molecules/essential oils/extracts of certain medicinal plants from some Latin American countries (Bolivia, Brazil, Colombia, Mexico, and Peru) that have been effective against disease-causing pathogens in humans and/or animals are described.

Keywords Promising antimicrobial agents · Latin American medicinal plants · Disease-causing pathogens · Treatment for humans/animals

1 Introduction

The antimicrobial resistance (AMR) arises when pathogenic microorganisms (e.g., bacteria/fungi, viruses, parasites) decrease their response/susceptibility (growth/reproduction/replication) to drugs that counteract them (inhibit/kill), possibly through mechanisms of genetic plasticity [modification/mutation (phenotypical criterion), although for some authors other additional criteria like pharmacological and clinical could be involved], making infectious processes difficult to treat, thereby increasing the risk of disease spread, severity, and death. Three main factors have been considered to be responsible for the emergence of resistance: (i) abuse and misuse of antimicrobial agents, i.e., nonprescription (developing countries) or overprescription (developed countries), self-medication, and noncompliance with treatments; (ii) healthcare-acquired infections (nosocomial); and (iii) the use of antimicrobials in food-producing animals. As a consequence, the AMR is

considered one of the greatest public health problems/challenges of the twenty-first century. At present, the situation has worsened as some pathogenic strains have become multi-resistant to the drugs available for treatments; according to WHO the main strains are *Mycobacterium tuberculosis* (global priority), *Acinetobacter baumannii* (critical priority), *Pseudomonas aeruginosa* (critical priority), Enterobacteriaceae (critical priority), *Enterococcus faecium* (high priority), *Staphylococcus aureus* (high priority), *Klebsiella pneumoniae* (high priority), *Helicobacter pylori* (high priority), *Campylobacter* spp. (high priority), nontyphoidal *Salmonella* (high priority), *Neisseria gonorrhoeae* (high priority), *Candida* spp. and *Aspergillus* spp. (WHO 2020a, CDC 2020, Mukherjee 2019, Parish 2019, Siddiqui 2019, Butaye et al. 2015, Prestinaci et al. 2015, Okeke et al. 2003).

The term *antimicrobial agent* refers to any substance that is capable of killing/inhibiting the growth of microorganisms (bacteria, fungi, viruses, or parasites), whether of natural (antibiotics, produced by bacteria/fungi) or synthetic (antibacterial drugs, purely chemically derived products) origin and applies to the agent intended for use in humans, veterinary, or agricultural (Butaye et al. 2015; ITFAMR 1999). Based on this definition, plants (e.g., *Ageratina adenophora*, *Artemisia vulgaris*, *Berberis fremontii*, *Camellia sinensis*, *Cassia tora*, *Cinnamomum tamala*, *C. verum*, *C. zeylanicum*, *Curcuma longa*, *Hemidesmus indicus*, *Hypericum perforatum*, *Indigofera suffruticosa*, *Leucas aspera*, *Lythrum salicaria*, *Melaleuca alternifolia*, *Mikania glomerata*, *Momordica charantia*, *Origanum vulgare*, *Oxalis corniculata*, *Piper nigrum*, *Plumbago zeylanica*, *Rabdosia rubescens*, *Rosa rugosa*, *Rosmarinus vulgaris*, *Sambucus nigra*, *Scutellaria baicalensis*, *Syzygium aromaticum*, *S. cumini*, *Terminalia chebula*, *Thymus vulgaris*, among others) have the capacity to produce secondary metabolites [e.g., phenols (eugenol, carvacrol, thymol, curcumin), phenolic acids (benzoic and cinnamic acids), flavonoids, quinones, coumarins, tannins, alkaloids, as well as some essential oils or extracts enriched with them] with a great potential as antimicrobial agents (Chassagne et al. 2021; Manandhar et al. 2019; dos Santos et al. 2016; Chouhan et al. 2015; dos Santos et al. 2015a; dos Santos et al. 2015b; Magi et al. 2015; Saritha et al. 2015; Tiwari et al. 2015; Monte et al. 2014; Pandey and Kumar 2013; Rai et al. 2013; Abreu et al. 2012; Savoia 2012; Dorman and Deans 2000; Nascimento et al. 2000; Cowan 1999).

On the other hand, since 1999, a *Public Health Action Plan to Combat Antimicrobial Resistance* has been proposed by Interagency Task Force on AMR, which is constituted by different Federal Agencies (e.g., Centers for Disease Control and Prevention, or CDC, Food and Drug Administration, or FDA, National Institutes of Health, or NIH, etc.). This “action plan” contains four focus areas: surveillance, prevention and control, research, and product development (ITFAMR 2011). The fourth area, which is of greatest interest for the pharmaceutical industry, promotes the development of new antimicrobial agents to aid/improve the diagnosis, prevention, and treatment of infections whether or not caused by resistant microorganisms, because new active molecules are neither being discovered nor developed as quickly as the increasing microbial resistance requires (ITFAMR 2011; 1999). However, due to certain realities of the pharmaceutical industry [time (15–20 years) and

financial (> USD 1000 billion)/scientific constraints)], few new (for Gram-positive bacteria) or old (Gram-negative bacteria for more than 40 years) antimicrobial drugs have been marketed for several years, and those that were marketed during this time are the result of modifications made to existing antimicrobial drugs (Buckland 2017; Rai et al. 2013). Therefore, there is an imperative need to find any antimicrobial substance, preferably of natural origin, that can contribute to solving this deficit. This situation created an opportunity that has been exploited for biological prospecting from medicinal plants; only 3% of the antibacterial drugs (approved by FDA) are produced from plants (97% derived from microorganisms, i.e., 51% from bacteria and 46% from fungi) (Patridge et al. 2016; Quave 2016; Kirst 2013).

This chapter deals with some Latin American medicinal plants (12) containing promising antimicrobial agents (specifically antibacterial/antifungal substances) against disease-causing pathogens in humans and animals.

2 Text Mining

A query was performed on the Scopus database (Elsevier BV, 2020) using the general search equation TITLE-ABS-KEY (antimicrobial*) AND PUBYEAR>1999 to carry out the scientometric analysis; 252, 294 records indexed in this database were obtained in the 1999–2020 timeline: ~ 80% of the registers corresponded mainly to scientific articles and ~ 11% to reviews. Respecting to fields of application, ~ 24% were in medicine; ~ 14% in biochemistry, genetics, and molecular biology; ~ 12% in pharmacology, toxicology, and pharmaceuticals; ~ 9% in immunology and microbiology, as well as in chemistry, specifically.

In order to identify research trends on promising antimicrobial agents obtained from Latin American medicinal plants and their action against pathogens that cause disease in humans and animals (veterinary use), an additional text-mining analysis was carried out on this topic based on scientific articles published during the period 2000–2020 (to date), according to the following search equation (TITLE-ABS-KEY (antimicrobial*) AND TITLE-ABS-KEY (“medicinal plant*”)) AND DOCTYPE (ar) AND PUBYEAR >1999, which was limited considering application areas such as pharmaceuticals, medicine, biochemistry, among others. 5,044 indexed records were retrieved from the Scopus database, which corresponds to 2% of all reported on antimicrobials (1999–2020).

The scientific dynamic (number of articles per year) is presented in Fig. 11.1. Consequently, an exponential growth in the number of publications generated in the 2000–2011 period was observed, while in the following years this trend was downward, with the exception of 2016 when the number of records (>400) increased considerably. The growth rate (percentage value/year) of the number of publications during this same period was calculated using De Solla Price’s Law (De Solla Price 1976), whose value was 26.1%/year with a high data correlation ($R^2 = 0.90$).

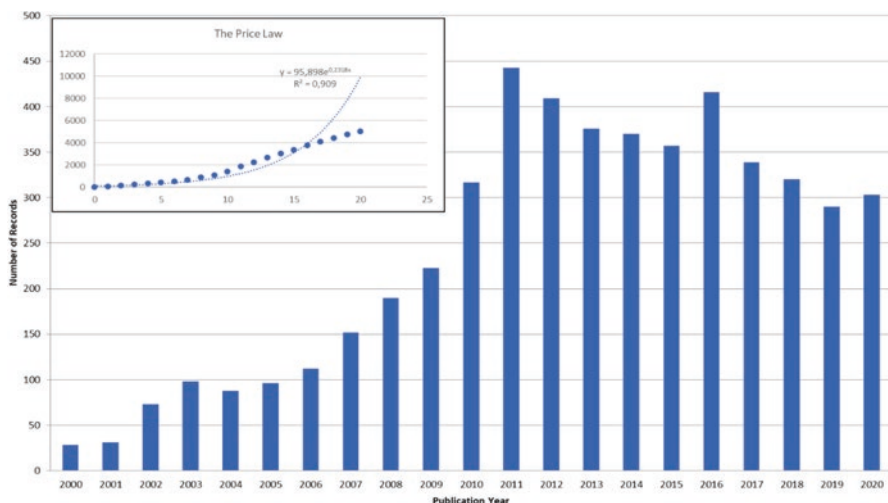


Fig. 11.1 Source: Bibliometric Unit CRAI Library, Universidad Santo Tomás (Bucaramanga). Calculations based on Scopus information (Elsevier, B.V., 2020), processed with VantagePoint software (Version 12.0, Search Technology)

On the other hand, the main scientific journals in which the research articles related to antimicrobial agents obtained from medicinal plants were published are *Journal of Ethnopharmacology* (255 records), *Pharmaceutical Biology* (110 records), *Natural Product Research* (103 records), *International Journal of Pharmacy and Pharmaceutical Sciences* (102 records), and *BMC Complementary and Alternative Medicine*, and *Phytotherapy*, each with 98 records. In addition, the top five countries with the highest number of records on this topic were India (1387 registers), Brazil (475 registers), Iran (284 registers), China (262 registers), and the United States (216 registers); at the Latin American level, in descendent order by record number, were Brazil, Mexico (236 registers), Argentina (161 registers), Colombia (29 registers), Chile (26 registers), and Cuba (18 registers).

The correlation between priority microorganisms declared by the WHO (*M. tuberculosis*, *A. baumannii*, *P. aeruginosa*, Enterobacteriaceae, *Ent. faecium*, *S. aureus*, *K. pneumoniae*, *H. pylori*, *Campylobacter* spp., *N. gonorrhoeae*, *Candida* spp. and *Aspergillus* spp.) with Latin American medicinal plants according to the data-cleaning text-mining analysis showed that the microorganisms with the highest number of records were *S. aureus* with 31 articles, *Candida* spp. with 15 articles, and *P. aeruginosa* with 14 articles. While *Bidens pilosa* (31 records), *Lippia origanoides* (13 records), *Piper regnellii* (6 records) along with *Ziziphus joazeiro*, *Amphipterygium adstringens*, and *Schinus terebinthifolia*, each with five records, stood out among the plants.

3 Some Important Statistical Data on Resistance to Antimicrobial Agents and Its Economic Impact

As reported in April 2019 by IACG (Interagency Coordination Group on Antimicrobial Resistance) for the Secretary-General of the United Nations in the document entitled “No Time to Wait: Securing the Future from Drug-Resistant Infections” and subsequently cited by the WHO in the press release entitled “New Report Calls for Urgent Action to Avert Antimicrobial Resistance Crisis,” alarms are being raised about the potential crisis by drug-resistant diseases. In this report, the IACG stated: (i) such diseases have killed at least 700,000 people every year (e.g., multidrug-resistant tuberculosis killed 230,000 people), (ii) if appropriate actions are not taken, it would cause 10 million deaths per year by 2050, according to the World Bank, (iii) as well as catastrophic damage to the economy (such as the 2008–2009 global financial crisis); AMR would push up to 24 million people into extreme poverty, by 2030 (IACG 2019; WHO 2019; Ahmed et al. 2017).

In addition, the report entitled “Estimating the Economic Costs of Antimicrobial Resistance” from the RAND corporation projected that in the next 10–44 years, the world’s population will lose 2–99 million and 11–444 million lives, respectively, due to AMR. A relevant fact included in this report is that ca. 3.2 million clinical patients contracted in-hospital (nosocomial) infections (HAI), with an incidence rate of ~623 HAI/100,000 people for OECD (Organization for Economic Cooperation and Development)/EU/EEA countries, and the most recurrent pathogenic microorganisms were *E. coli* (~16%), *S. aureus* (~12%), and *K. pneumoniae* (~9%) (Taylor et al. 2014). Likewise, common infections (respiratory, urinary, and sexually transmitted infections) are more difficult to treat. For instance, in some OECD countries, 35% of common infections are resistant to current medicines; while in some low- and middle-income countries, resistances are between 80 and 90% for some drug–bacterium combinations. The data are alarming: 6 million people die each year due to lack of or inadequate access to available antimicrobial therapies (IACG 2019).

An overwhelming statement in the IACG report (2019) is that the economic impact of non-controlled AMR could be tragic; that is, as drug-resistant pathogens spread, healthcare expenditures could increase dramatically and sustainable food/feed production (global trade in food/feed/livestock) could be increasingly threatened. For these reasons, the “One Health” initiative (designing and implementing programs/policies/legislation/research in a way that multiple sectors and stakeholders participate together to achieve better public health outcomes) is proposed as a useful tool to cover multiple scenarios.

In 2015, the Global Plan of Action on AMR was activated, and ~100 countries have developed its National Action Plans on AMR, with normative guidance from the tripartite agencies [FAO, OIE (World Organization for Animal Health) and WHO] to support the implementation. However, efforts to implement National Action Plans are currently slow and should be accelerated. Based on the “global database for the tripartite AMR” and “2019–2020 country self-assessment survey

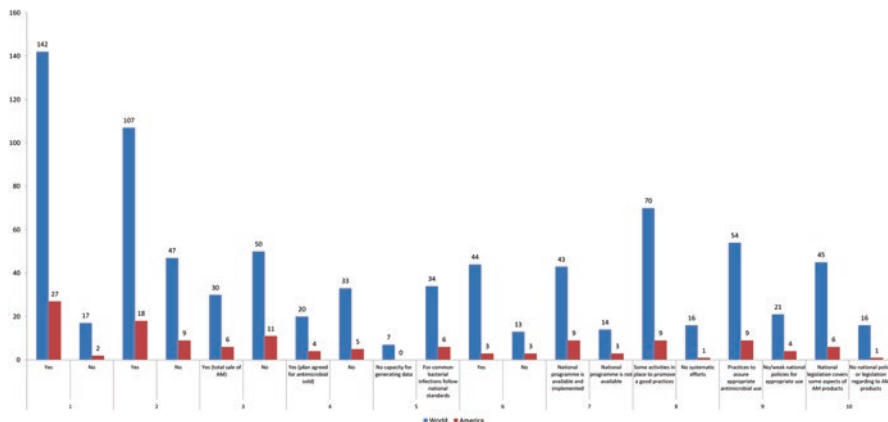


Fig. 11.2 Data related to 10 questions (WHO 2020b) considered to observe the dynamic and interest that the world (WC) and American countries (AC) have about resistance in humans and animals

(TrACSS)”, 10 questions (WHO 2020b) were considered to observe the dynamic and interest that the world (WC) and American countries (AC) have about resistance in humans and animals (Fig. 11.2).

From of this graph, it could be observed that 89% and 93% of WC and AC, respectively, have policies (P)/regulation (R) on prescription (Pr)/sale (S) of AM for human use, respectively; likewise, 67% and 62% of WC and AC have a P/R on Pr/S of AM for animal use, respectively. However, only 19% and 21% of WC and AC have a national monitoring system for consumption and rational use of AM in human health, respectively; as well only 13% and 14% of WC and AC have a national monitoring system for AM intended to be used in animals. On the other hand, 21% for both WC and AC have a national surveillance system for common bacterial infections (AMR) in humans, but only 4% of WC do not have the capacity for generating data. In the same survey, 27 % and 31 % WC and AC have a national program available and implemented for infection prevention and control in human health care, respectively; while 9–10% of WC and AC do not have any national program.

Finally, in the questions about the optimizing of the antimicrobial uses in (i) human and (ii) animal health, 34% and 31% WC and AC have practices to assure the appropriate uses for human (13–14% of WC and AC have either weak or no national policies for appropriate uses), while 28% and 21% WC and AC have national legislation that covers some aspects of AM products.

As it is well known, AMR generates multifaceted troubles which could be defined with social, economic, and anthropogenic perspectives, and its impact reaches to patients, researchers, pharmaceutical organizations, healthcare providers and businesses, and international policy-makers. For some authors, from the economic perspective, AMR could be considered as a negative externality because it has negative effects on people who do not use antibiotics; this negative effect is a

cost paid by others than the consumer itself, and the literature classifies the costs of AMR into three categories: patient level (morbi-/mortality), healthcare level, and economic level (cost across the globe). In this sense, the World Bank estimated some scenarios of the AMR impact over gross domestic products (GDP); that is, GDP is expected to fall by ~1% and ~4% in the low- and high-impact scenarios, correspondingly, by 2050. When country differences were taken into account, it was found that low-income countries could lose more than 5% of GDP in 2050 in the high-impact scenario. Moreover, the OECD/WHO/FAO/OIE predicted that patients will need more health care and resources to be able to pay for it; incidentally, the US CDC estimated that AMR generates an economic burden in the United States of around USD 55 billion (20 billion in health services and 35 billion in productivity losses per year). Furthermore, the AMR causes people to pay for extra medical expenses that set the medical poverty trap; this trap is exasperated in low-income countries with higher morbidity and mortality, *f.i.*, in Latin America, AMR explains a significant part of morbidity and mortality. The World Bank research shows that AMR would increase poverty in low-income countries in comparison to the rest of the world. (Ahmad and Khan 2019; Phyo and Nosten 2018; Shrestha et al. 2018; Jonas et al. 2017; OECD/WHO/FAO/OIE 2017; CDC 2013; ReAct 2012; NNIS System 2004).

There is a consensus on the urgency of new effective antibiotics, but incentives need to be improved to push pharmaceutical companies to reconsider antibiotic development. These incentives require government intervention that impacts on reducing R&D costs, with instruments such as research grants and tax credits. In addition, as pharmaceutical companies have shifted their focus to chronic diseases, and researchers are more interested in antiviral compounds than antibiotics, traditional medicine could be an important opportunity to take advantage of plant extracts and phytochemicals for therapeutic treatments (Roope et al. 2019; Gupta and Birdi 2017).

4 Latin American Countries as Natural Antimicrobial Hubs

In this part of chapter, some Latin American medicinal plants from different countries (Bolivia, Brazil, Colombia, Mexico and Peru) will be analyzed under the criterion of their antimicrobial potential, taking into account traditional/ethnomedicinal uses. The selected plants were *Acalypha monostachya* Cav., *Amphipterygium adstringens* (Schltdl.) Standl., *Baccharis boliviensis* (Wedd.) Cabrera, *Bidens pilosa* L. Britton, *Lippia organoides* Kunth, *Minthostachys mollis* (Kunth) Griseb., *Parastrephia lepidophylla* (Wedd.) Cabrera, *Piper regnelli* (Miq.) C.DC, *Rauvolfia tetraphylla* Linn., *Salix* spp. (*Salix humboldtiana* Willd/*Salix babylonica* L.), *Schinus terebinthifolia* Raddi, *Siparuna guianensis* Aubl., and *Ziziphus joazeiro* Mart.

In the case of Bolivia, *Baccharis boliviensis* and *Parastrephia lepidophylla* (Fig. 11.3), two Bolivian medicinal plants (used by indigenous populations), were

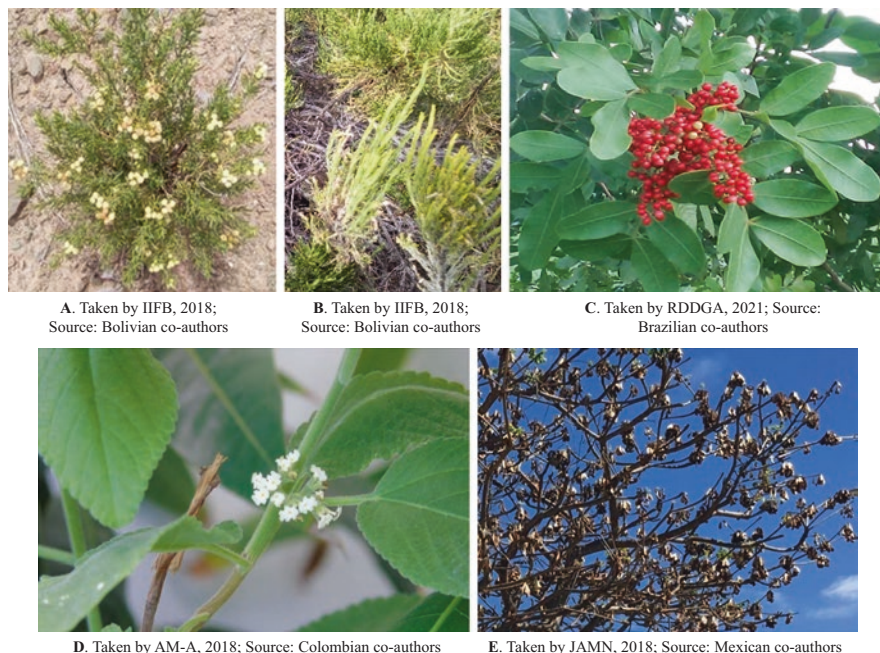


Fig. 11.3 Images of Bolivian (A. *B. boliviensis*; B. *P. lepidophylla*), Brazilian (C. *Sch. terebinthifolia*), Colombian (D. *L. organoides*), and Mexican (E. *Amp. adstringens*) plants

selected by the co-authors of the Instituto de Investigaciones Farmaco-Bioquemicas-UMSA. The two plants are perennial shrubs that grow at 3000–5000 m.a.s.l. in the altiplano region and are distributed in other countries, such as Argentina and Chile. The common names for *Bacc. boliviensis* are Jamachi thola, romero thola, tolilla (Bolivia), chijua, tola (Argentina), tola, pesco tola (Chile); while *Par. lepidophylla* is also known as t’ola, supu tola (Bolivia), tola vaca (Argentina), k’jaña tola, k’juni k’jara (Chile). Among the traditional medicinal uses described, Jamachi thola could be mentioned as a treatment for rheumatism, inflammatory processes, skin infections, and as a digestive and antiseptic, prepared in the forms of infusion/decoction/maceration/poultice. Regarding ethnobotanical uses for t’ola, the plant has been applied to treat some disorders and chronic diseases, stomach pain, to immobilize fractured limbs and as anti-inflammatory, anti-dandruff, and antiseptic (Carrizo et al. 2020; Sanchez et al. 2015; Calle et al. 2013; Benites et al. 2012; Rojo et al. 2009; Zampini et al. 2009; Freire et al. 2007; Villagran et al. 2003; Erazo et al. 2002; Girault 1987).

The most important information on antimicrobial data from the two plants is presented in Table 11.1. The evaluated Gram-positive bacteria were *S. aureus* and *Enterococcus faecalis*; the Gram-negative bacteria were *Enterobacter cloacae*, *Proteus mirabilis*, and *P. aeruginosa*; the yeast were *Candida guilliermondii*, *C. dubliniensis*, *C. albicans*, and *Saccharomyces cerevisiae*; the molds were

Table 11.1 Some promising antimicrobial results from extracts of two Bolivian plants

Plants	Antimicrobial <i>in vitro/in vivo</i> evaluation			References
<i>Bacc. boliviensis</i>	EtOH extract (aerial parts)	Antibacterial	Different <i>Staphylococcus</i> spp. strains Sensible and resistant <i>S. aureus</i> : 24-26 mm ϕ inh. zone (50 mg/mL) <i>Ent. faecalis</i> MIC/MBC 40-80/150-300 μ g/mL	Calle et al. 2013, Zampini et al. 2009
	H ₂ O:EtOH extract (aerial parts)	Antifungal	<i>C. guilliermondii</i> , <i>C. dubliniensis</i> , <i>C. albicans</i> , <i>Sac. cerevisiae</i> , <i>T. rubrum</i> , <i>T. mentagrophytes</i> , <i>M. gypseum</i> , <i>M. canis</i> , <i>Sco. brevicaulis</i> , <i>Alternaria</i> spp.	Carrizo et al. 2020
<i>Par. lepidophylla</i>	EtOH extract (aerial parts)	Antibacterial	Different <i>Staphylococcus</i> spp. strains: <i>Ent. faecalis</i> – MIC 80-150 μ g/mL <i>E. cloacae</i> F302, <i>P. mirabilis</i> F304, <i>P. aeruginosa</i> F305	Zampini et al. 2009

Trichophyton rubrum, *T. mentagrophytes*, *Microsporum gypseum*, *M. canis*, *Absidia orchidis*, *Scopulariopsis brevicaulis*, and *Alternaria* spp.

As reported by Zampini et al. (2009), the EtOH extract from *B. boliviensis* aerial parts showed MIC and MBC values of 40–80 μ g/mL and 40–300 μ g/mL, respectively, against different microbial strains [methicillin resistant and sensitive *S. aureus* (MRSA and MSSA), methicillin-resistant *Staphylococcus* coagulase negative (MRSCN) and methicillin-sensitive *Staphylococcus* coagulase negative (MSSCN)]. For EtOH extract from *P. lepidophylla* aerial parts, the MIC and MBC values were 80–150 μ g/mL and 80–2400 μ g/mL, respectively, against different microbial strains (MRSA, MSSA, MRSCN, and MSSCN). Moreover, both plants were active against *Ent. faecalis* with MIC values of 80 μ g/mL (for *B. boliviensis* and MBC values of 150–300 μ g/mL) and 80–150 μ g/mL (for *P. lepidophylla*). The extract of *P. lepidophylla* was effective on *E. cloacae*, *P. mirabilis*, and *P. aeruginosa* with MIC values of 150 μ g/mL, 300 μ g/mL, and 600 μ g/mL, respectively. In other study, Calle et al. (2013) reported that 50 mg/mL of EtOH extract from *B. boliviensis* leaves produced inhibition haloes of 24 mm and 26 mm of diameter against resistant and sensible *S. aureus*, respectively. A flavonoid/cinnamic acid derivatives-enriched fraction (339 \pm 13 mg TF/g) from EtOH extract inhibited to sensible *S. aureus* (61%, ϕ inh. zone: 23 mm). To finish, Carrizo et al. (2020) estimated the antifungal potential of hydro-alcohol extract (80%) of aerial parts from *B. boliviensis*; these authors found that fungal strains such as *C. guilliermondii* (MIC 50-800 μ g GAE/mL), *C. dubliniensis* (MIC 100 μ g GAE/mL), *C. albicans* (MIC 800 μ g GAE/mL), *S. cerevisiae* (MIC 200-800 μ g GAE/mL), *T. rubrum* (MIC 50-200 μ g GAE/mL), *T. mentagrophytes* (MIC 200 μ g GAE/mL), *M. gypseum* (MIC 200 μ g GAE/mL, and *M. canis* (MIC 100 μ g GAE/mL) showed a high sensitivity to the extract. In addition, certain non-dermatophytes strains, e.g., *A. orchidis*, *S. brevicaulis*, *Alternaria* spp., were inhibited (100%) in their radial growth by the extract (3.2 mg GAE/mL) on the

third day of the experiment. Among the constituents identified in the hydroalcoholic extract as possibly responsible for antifungal activity were terpenoids (clerodanes), phenolic acids, and flavonoids (methoxylated flavones).

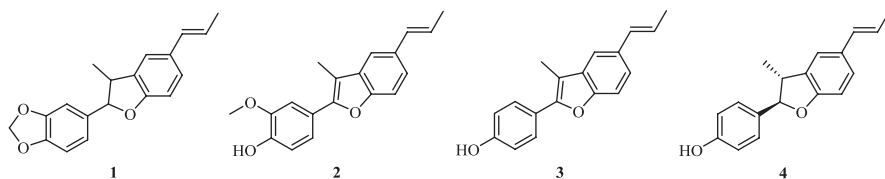
Three plants (*Piper regnelli*, *Schinus terebinthifolia*, and *Ziziphus joazeiro*) were suggested by Brazilian co-authors. First of them, *P. regnellii* (syn.: *Artanthe regnellii* Miq., *P. epunctulatum* C.DC., *P. fulvescens* C.DC) is a shrub named as pariparoba for inhabitants and it is distributed between Argentina, Paraguay, and Brazil (among the biomes of the Amazonia, Cerrado, and Mata Atlantica). The medicinal use by Brazilian natives is observed in different regions of the country; they use it in infusion or decoction (from inflorescences/leaves/roots) for the treatment of wounds, burns, anti-gastric ulcer, skin irritations, inflammation, swelling, headache, and liver disorders (Grande 2019; Guimarães et al. 2015; Ruschel 2004; de Freitas 2000; Corrêa 1984).

The antimicrobial potential of *P. regnellii* has been extensively established against bacteria, protozoa, and fungi. Thus, the essential oil (EO) from plant leaves collected in Mata Atlantica bioma showed activity against *S. aureus* and *C. albicans* (ϕ inh. zone: 15.1 ± 0.2 mm and 13 ± 3 mm, respectively) according to Constantin et al. (2001). In addition, hexane fraction of H₂O:EtOH extract along with CH₂Cl₂, EtOH, and ethyl acetate extracts from leaves presented activity against three types of *Paracoccidioides brasiliensis* with MIC values of ~ 8 $\mu\text{g/mL}$, 30 $\mu\text{g/mL}$, and 250–500 $\mu\text{g/mL}$, respectively (Johann et al. 2010). The chemical analysis by GC-MS of the most active fraction (hexane) revealed that the main components were (*E*)-anethole ($\sim 22\%$) and apiol ($\sim 21\%$).

Additionally, antifungal activity was also observed in extracts with high content of neolignans; the H₂O:EtOH extract (90%) from *P. regnellii* leaves presented a significant activity against the dermatophytes *T. mentagrophytes*, *T. rubrum*, *M. canis*, and *M. gypseum* with MIC values of ~ 62 $\mu\text{g/mL}$ for *M. gypseum* and ~ 16 $\mu\text{g/mL}$ for the other three dermatophytes; while the isolated neolignans, eupomatenoïd-3 (**1**) and eupomatenoïd-5 (**2**), were effective against *T. rubrum* with MIC values of 50 $\mu\text{g/mL}$ and ~ 6 $\mu\text{g/mL}$ (Koroishi et al. 2008). Moreover, the ethyl acetate extract from leaves was active against *C. albicans* (MIC 125 $\mu\text{g/mL}$) while for *C. krusei* as in *C. parapsilosis* it showed moderate activity (MIC 500 $\mu\text{g/mL}$ for each) (Pessini et al. 2005). Referring to the antibacterial activity, Pessini et al. (2003) found that the ethyl acetate extract from leaves was able to inhibit *S. aureus* and *Bacillus subtilis* with MIC/MBC values of $\sim 16/16$ $\mu\text{g/mL}$ whereas eupomatenoïd-6 (**3**) and **2** resulted most active than the extract on *S. aureus* with MIC values of ~ 2 $\mu\text{g/mL}$ and ~ 3 $\mu\text{g/mL}$, respectively. Also, both compounds presented MIC of ~ 3 $\mu\text{g/mL}$ against *B. subtilis* and conocarpan (**4**) was quite active against this strain (MIC ~ 6 $\mu\text{g/mL}$). The ethyl acetate extract (EAE) and its hexane fraction (HF) were also effective on MRSA and MSSA: EAE - MIC/MBC of 16/16 $\mu\text{g/mL}$ and 16/8 $\mu\text{g/mL}$, HF - MIC of 4 $\mu\text{g/mL}$ for two strains. Neolignan **2** had a MIC value of 4 $\mu\text{g/mL}$ for MRSA/MSSA (Marçal et al. 2010). Interestingly, the supercritical CO₂ extracts from *P. regnellii* leaves/stems showed activity against *M. tuberculosis* (MIC ~ 16 $\mu\text{g/mL}$ for both parts) whereas **2** and **4** exhibited good effectiveness with MIC values of ~ 2 $\mu\text{g/mL}$ and ~ 16 $\mu\text{g/mL}$, one to one (Scodro et al. 2013).

Regarding the antiprotozoal activity, **2** revealed antileishmanial activity against *Leishmania amazonensis*, acting on promastigote (IC₅₀ 13 µg/mL), axenic amastigote (IC₅₀ 9 µg/mL), and intracellular amastigote (IC₅₀ 5 µg/mL) forms of the parasite, whereas there was no toxic activity on macrophages (Vendrametto et al. 2010). Other interesting antiprotozoal activity of isolated neolignans was observed on *Trypanosoma cruzi*, etiologic agent of Chagas disease. **2–4** showed considerable activity against epimastigote forms of *T. cruzi*, with IC₅₀ values between ~7 µg/mL and 8 µg/mL (Luize et al. 2006).

Following the studies mentioned above, some researches were developed in order to improve the biological potential of *P. regnellii* extracts, either through of micro-formulations or by improving extraction processes. In this way, Brambilla et al. (2018) produced biopolymer-based microparticles (MP) containing CH₂Cl₂ extract from *P. regnellii* leaves. Extract-loaded gelatin microparticles and sodium alginate and chitosan-based MP improved the stability of the extracts and maintained the antifungal activity against *T. rubrum*. Furthermore, Lemos et al. (2013) evaluated the antifungal capability from extracts obtained by SFE-CO₂ against yeast and filamentous fungi. The most active extract was obtained from *P. regnellii* leaves at 40°C and 25 MPa, featuring a MIC value of ~4 µg/mL against *T. mentagrophytes*.



P. regnellii has been constituted by neolignans (e.g., eupomatenoids and conocarpan), phenylpropanoids and terpenoids as the most abundant components. In the leaves, the monoterpenoids myrcene and linalool were the main compounds identified in the EO from species collected in Mata Atlantica bioma, whereas myrcene, (*E*)-anethole, and bicyclogermacrene were the constituents of the species from Cerrado vegetation (dos Santos et al. 2015a, Constantin et al. 2001). On the other hand, apiol, dillapiol, and myristicin are most common phenylpropanoids in the roots, which in turn also present 4',7'-epoxy-8,3'- and 8',9'-dinor-4',7'-epoxy-8,3'-neolignans as active substances (Benevides et al. 1999). Furthermore, (*E*)-anethole and dillapiole were the main molecules found in the stems EO, whereas myrcene and bicyclogermacrene were the principal components identified in the flowers (dos Santos et al. 2015a).

The second Brazilian plant is *Schinus terebinthifolia* (*Schinus terebinthifolius*) commonly known as aroeira-da-praia, aroeira-de-remédio, aroeira-mansa, aroeira-pimenteira, cambuí and fruta-de-sabiá, among others. This fruity shrub (2–7 m height) has a strong aroma, as well as its globose drupes. *Sch. terebinthifolia* is native to Brazil and it is distributed in the biomes of the Mata Atlantica and Caatinga (from the Rio Grande do Norte to the Rio Grande do Sul). The gum-resin from plant is externally applied as a treatment for cornea diseases, tumors, leprosy; bark is

depurative, antifebrile, antineuralgic, used for hemoptysis and afflictions of the uterus (SIBBR 2020; Azevedo et al. 2002; Lorenzi 2000; Mors et al. 2000; Corrêa 1984).

The antibacterial and antifungal capabilities against some strains by *Sch. terebinthifolia* have been included in different scientific reports. For example, *Sch. terebinthifolia* EO presented certain activity against *Streptococcus mutans*, *Str. oralis*, and *Str. salivarius*, each with an MIC value of 72 mg/mL (Alves et al. 2019). It is worth noting that ripe fruit EO evidenced a good inhibitory effect against Gram-positive bacteria as *Corynebacterium* sp. (MIC 3.6 ± 0.4 $\mu\text{g/mL}$), *Bacillus* sp. (MIC 7.1 ± 0.8 $\mu\text{g/mL}$), *Nocardia* sp. (MIC 7.1 ± 0.6 $\mu\text{g/mL}$), *Streptococcus* group D (MIC 14.2 ± 0.6 $\mu\text{g/mL}$), and *S. aureus* (MIC 14 ± 0.6 $\mu\text{g/mL}$), as well against Gram-negative bacteria as *Pseudomonas* sp. (MIC 7.1 ± 0.6 $\mu\text{g/mL}$), *E. coli* (MIC 28.4 ± 0.4 $\mu\text{g/mL}$), *Klebsiella oxytoca* (MIC 28.4 ± 0.4 $\mu\text{g/mL}$), *Ent. agglomerans* (MIC 28.4 ± 0.5 $\mu\text{g/mL}$), and *Enterobacter* sp. (MIC 56.9 ± 0.8 $\mu\text{g/mL}$). All these microorganisms were of intra-hospital origin (Cole et al. 2014). Furthermore, the ethyl acetate-methanol extract fraction from *Sch. terebinthifolia* leaves showed activity *in vitro* against biofilm formation of *Str. mutans* (Minimal inhibitory concentration of adherence-MICA 7 $\mu\text{g/mL}$) and *C. albicans* (MICA 7 $\mu\text{g/mL}$). The methanol fraction of the *Sch. terebinthifolia* extract in a hydroalcoholic solvent demonstrated the best non-stick potential on *in vitro* biofilm formation for *Str. mutans* (MICA 3.5 $\mu\text{g/mL}$) and *C. albicans* (MICA 7 $\mu\text{g/mL}$) in evaluated conditions (Barbieri et al. 2014).

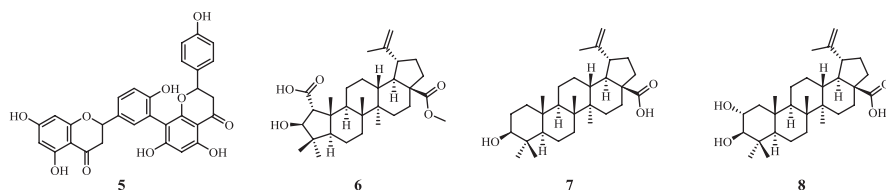
A study *in vitro* demonstrated that the H₂O:EtOH extract from *Sch. terebinthifolia* stem bark had antidermatophytic activity on *T. rubrum* and *T. mentagrophytes* with MIC/MF values of 62.5 $\mu\text{g/mL}$ (Biasi-Garbin et al. 2016). Furthermore, hexane fraction of H₂O:EtOH extract together with EtOH and CH₂Cl₂ extracts from leaves were active on three types of *Par. brasiliensis* with MIC values of 15–125 $\mu\text{g/mL}$ and 30 $\mu\text{g/mL}$, respectively (Johann et al. 2010).

Among the flavonoids and bioflavonoids [agathisflavone, amenthoflavone, and tetrahydroamentoflavone (THAF)] found in the aroeira fruits, THAF (5) has been identified as a promising antibacterial constituent. Investigations demonstrated that the antibacterial effects of bioflavonoids are strongly dependent on their specific structural properties; that is, THAF inhibited the growth of planktonic cells and biofilm formation of selected bacteria (Linden et al. 2020).

The last Brazilian plant considered is *Ziziphus joazeiro* (syn.: *Z. guaranitica*, *Z. gardneri*). This fruity tree (up to 16 m and with drupe fruits) is commonly named as joazeiro, juá-babão, juá-de-boi, joá-mirim, juá, etc., and it is native from Brazil and distributed in the Caatinga and Mata Atlantica biomes. The barks and leaves are traditionally used by northeastern natives from Brazil in the form of aqueous extract, used orally for relief gastric problems, and externally, for cleaning hair and teeth, face whitening, gingivitis, wound healing, skin diseases, and seborrhea, being even referred as hair tonic anti-dandruff (do Rego 2019; Lima 2015; Carvalho 2007).

The ethnomedicinal uses of *Z. joazeiro* can be partially explained by its diversity in antimicrobial activities. As well, some particular constituents are present in significant quantities in it as phenolic compounds, mainly flavonoids and phenolic acids as quercetin and caffeic acid, as well as saponins and ceanothane and lupine

triterpenes like methyl ceanothate (**6**), betulinic (**7**), and alphitolic (**8**) acids (Brito et al. 2015; Ribeiro et al. 2013; Leal et al. 2010). The two triterpenes **6** and **8** showed antibacterial activity against MRSA and MSSA isolated clinically, with MIC values ranged between 16 $\mu\text{g/mL}$ and 32 $\mu\text{g/mL}$ (Leal et al. 2010). The antifungal activity of saponins from this species was also observed against *C. albicans* and *A. niger* with MIC values of 156 $\mu\text{g/mL}$ and 312 $\mu\text{g/mL}$ (Ribeiro et al. 2013).



Other interesting study on antibacterial activity was conducted with aqueous extracts from leaves and stem bark. Andrade et al. (2019a) verified that both extracts did not present any action against *Str. mutans*, *P. aeruginosa*, and *E. coli* when used in isolated form, but when used in association with antibiotics such as gentamicin and norfloxacin showed a synergistic action, decreasing MIC values compared to the antibiotics alone. In other antibacterial study with leaf and bark extracts, Silva et al. (2011) demonstrated the effect of the EtOH extracts on *Micrococcus luteus* and *Mycobacterium smegmatis*. The leaf extract showed MIC values of 250–500 $\mu\text{g/mL}$ against *M. luteus* and of 125–250 $\mu\text{g/mL}$ against *M. smegmatis*, while the bark extract showed MIC of 500–1000 $\mu\text{g/mL}$ for *M. smegmatis*. The aqueous extract from inner bark still presented activity against clinical strains of five oral bacteria, which corroborated the extensive ethnomedicinal use. In this study, the extract was active on *Prevotella intermedia* (MIC 7 ± 1 mg/mL), *Porphyromonas gingivalis* (MIC 1 ± 0 mg/mL), *Fusobacterium nucleatum* (MIC 7 ± 1 mg/mL), *Str. mutans* (MIC 16 ± 0 mg/mL), and *Lactobacillus casei* (MIC 13 ± 3 mg/mL) (Alviano et al. 2008). The aqueous extract from *Z. joazeiro* stem presented antifungal activity against several clinical strains, among them *T. rubrum* (MIC 6.25 $\mu\text{g/mL}$), *C. guilliermondii* (MIC 6.25 $\mu\text{g/mL}$), *C. albicans* (MIC 25 $\mu\text{g/mL}$), *Cryptococcus neoformans* (MIC 100 $\mu\text{g/mL}$), and *Fonsecaea pedrosoi* (MIC 400 $\mu\text{g/mL}$) (Cruz et al. 2007). Other interesting property of aqueous extract from *Z. joazeiro* leaves was the inhibition of bacterial and fungal biofilm. Strong activity was observed against *S. epidermidis*, *C. albicans*, and *C. tropicalis* by optical density quantification (Andrade et al. 2019b).

As seen in *P. regnellii*, the development of formulations with antimicrobial actives from *Z. joazeiro* can improve the stability of its compounds. In this way, Guimarães et al. (2020) produced silver-nanoparticles containing leaf extract, which in turn demonstrated the enhancing in the antibacterial activity of extract on *S. aureus* and *E. coli*, in neutral pH. Lastly, the antiparasitic activity of crude extract from stem bark presented relevant activity against *L. amazonensis* and *L. infantum* promastigotes with ~82% and ~68% inhibition, respectively, evaluating a concentration of 500 $\mu\text{g/mL}$ (Andrade et al. 2019c).

From Colombia, coauthors have recommended some plants such as *Bidens pilosa*, *Lippia origanoides*, *Mystostachis mollis*, and *Salix humboldtiana*, two of which (*B. pilosa* and *S. humboldtiana*) are included in the Colombian vademecum of medicinal plants (Ministerio de la Protección Social 2008). The first of them, *B. pilosa* [syn.: *B. alba* (L.) DC., *B. chilensis* DC., *B. odorata* Cav., *Coreopsis leucantha* L., *Kerneria pilosa* (L.)] is a cosmopolitan annual shrub/herb (or weed) native to South America, but it is widely distributed throughout the world; the common names of this plant are cadillo, romerillo, carapico, chipaca, papunga, pecunia, papunca, pega-pega, amor seco, acahual, cuamba, saytilla, among others. Although all parts of this plant have wide-ranging medicinal uses for treatment of different disorders/illness, the aerial parts, prepared as a dry powder/poultice/decoction/infusion/maceration/tincture, are used to treat dysentery, diarrhea, flu-like conditions, mouth sores, hepatitis, gastroduodenal ulcers, laryngitis/pharyngitis/gingivitis, bronchitis, topical ulcers, as an antihelmintic and protozoacide agent, etc.; topically, they are used as antifungal and antiseptic, as a treatment for cuts, burns, infected wounds, and skin problems (Pérez García 2017; Bartolome et al. 2013; Arthur et al. 2012; Duke 2008; Ministerio de la Protección Social 2008; Rojas et al. 2006; Lastra Valdés and Ponce de León Rego 2001; Gupta 1995).

In scientific validation studies, the EtOH extract of leaves evaluated against three pathogenic strains (*S. aureus*, *E. coli*, and *P. aeruginosa*) showed that the most susceptible strain to the extract (200 μ L) was *S. aureus* with ϕ inhibition zones of 18 ± 2 mm (diffusion test on disk) and 30.7 ± 0.6 mm (well diffusion tests) according to Cruz-Carrillo et al. (2010). Besides, Rojas et al. (2006) validated the antimicrobial potential of different extracts (hexane and EtOH) from *B. pilosa* aerial parts against *S. aureus*, *B. cereus*, and *E. coli*. The authors reported that EtOH extract (25 μ g/mL) inhibited to *S. aureus* ($66.7 \pm 0.5\%$) and *B. cereus* ($79 \pm 5\%$) compared to antibiotic standard, while hexane extract (25 μ g/mL) inhibited to *B. cereus* ($54.5 \pm 0.3\%$) compared to the reference antibiotic. The MIC values were 36 ± 6 μ g/mL (EtOH extract on *S. aureus*), 28 ± 6 μ g/mL (EtOH extract on *B. cereus*), 8.2 ± 0.5 μ g/mL (EtOH extract on *E. coli*), and 47 ± 4 μ g/mL (hexane extract on *E. coli*). In addition, da Silva et al. (2014) estimated the ϕ inhibition zones and MIC values for hydroalcoholic extracts from *B. pilosa* flowers and leaves on oxacillin-resistant *S. aureus* (ORSA-60 strains isolated of dental clinic) and *S. aureus* ATCC. These values were 8–20 mm (flower extract on ORSA), 8–23 mm (leaf extract on ORSA), 21–23 mm (flower extract on *S. aureus*), 25–28 mm (leaf extract on *S. aureus*), 12.5 mg/mL (flower extract on ORSA), 6.25 mg/mL (leaf extract on ORSA), 1.56 mg/mL (flower extract on *S. aureus*), and 25 mg/mL (leaf extract on *S. aureus*). As described by Krummenauer et al. (2019), the decoction from *B. pilosa* leaves produced a growth inhibition (45–100%) on *S. aureus* when 2–4 mg/mL of extract were tested on the microorganism.

Other authors have also validated the antimicrobial capability of *B. pilosa*: Rabe and van Staden (1997) found that 1 mg/mL de MeOH extract from leaves inhibited to *S. aureus* and *B. subtilis* with ratio values of the inhibition zone between extract and reference of 1.15 and 0.55, respectively. MIC values were between 2–4 mg/mL; Lawal et al. (2015) determined that the aqueous extract (1000 μ g/mL) of leaves

inhibited *Sal. typhi*, *Sal. typhimurium*, *E. coli*, *Sal. paratyphi*, *S. aureus*, and *P. aeruginosa* with diameters of inhibition zones of 27 ± 1 mm, 23 ± 1 mm, 23 ± 2 mm, 22 ± 2 mm, 21 ± 2 mm, and 18 ± 1 mm, respectively. Shandukani et al. (2018) estimated the antibacterial potential of different extracts [hexane (H), CH_2Cl_2 (D), ethyl acetate (EA), acetone (A), MeOH (M)] of leaves on diarrhea-causing waterborne bacteria; the authors documented that the extracts were effective on *Shigella boydii* (MIC 80–160 $\mu\text{g/mL}$ for H, D, A, and M extracts), *K. pneumoniae* (MIC 120–310 $\mu\text{g/mL}$ for D, EA, A, and M extracts), *Vibrio parahaemolyticus* (MIC 310–470 $\mu\text{g/mL}$ for H, D, EA, and A extracts), *E. coli* (MIC 80–630 $\mu\text{g/mL}$ for D, EA, and A extracts), and *Sal. typhimurium* (MIC 310 $\mu\text{g/mL}$ for EA extract). Linhares Neto et al. (2018) evaluated anti-*Candida* activity of the leaf essential oils (dry and fresh) from *B. pilosa*. As a result, it was reported that the EO were constituted by 1-phenylhepta-1,3,5-triene (34–64%) and produced MIC₉₀ values between 16–64 $\mu\text{g/mL}$ on *C. albicans*, *C. krusei*, *C. parapsilopsis*, and *C. glabrata*. Singh et al. (2017) reported that MeOH extract (10 mg/mL) from leaves produced ϕ inh. zones of 18.2 ± 0.4 mm, 15.7 ± 0.2 mm, 14.7 ± 0.2 mm, and 14.0 ± 0.6 mm on *E. coli*, *S. aureus*, *M. luteus*, and *P. aeruginosa*, respectively; as well, MIC values were 80.00 ± 0.05 $\mu\text{g/mL}$ (*E. coli*), 110.0 ± 0.2 $\mu\text{g/mL}$ (*S. aureus*), 220.0 ± 0.2 $\mu\text{g/mL}$ (*P. aeruginosa*), and 250.0 ± 0.2 $\mu\text{g/mL}$ (*M. luteus*). Lastly, fraction 12 of the ethyl acetate extract from *B. pilosa* leaves presented the best *in vitro* and *in vivo* antiplasmodial effect (*Plasmodium berghei*) as stated by Noumedem Anangmo et al. (2020); a dose of 125 mg/kg produced *in vivo* a parasite suppression activity of 100% in mice. Among the main secondary metabolites responsible for the different biological activities could be mentioned flavonoids (e.g., sulfuretin, aurones, chalcones, etc.), esterols (campesterol, β -sitosterol, lupeol, amyryns, stigmaterols), phenylpropanoids (ferulic, caffeic, chlorogenic, p-coumaric, caffeoylquinic acids), and essential oils (rich in sesquiterpenes, e.g., caryophyllene) (Bartolome et al. 2013).

A perennial and fragrant shrub belonging to the genus *Lippia* is *L. origanoides* (syn.: *L. schomburgkiana*, *Lantana ciliaris*), which is native to Central (Mexico, Guatemala) and north South America, especially in the Amazon region (Guayana, Venezuela, Brazil, and Colombia). This plant is named as “orégano de cerro,” “orégano cimarrón,” “salvia de marajó,” “oregano de burro,” “Mexican oregano.” Among the traditional/medicinal uses can be mentioned as a spice/condiment in culinary, as well as treatment of colds, asthma, cough, lung infections, indigestion, diarrhea, and fever; also it is used as an expectorant and oral antiseptic. The main form of secondary metabolism is as an essential oil (~0.8–4.6% yield) which has at least three chemovarieties: carvacrol (37–52%), thymol (30–87%), and (*E*)-caryophyllene (9–16%)/*p*-cymene (11–20%) (Stashenko and Martínez 2020; Arango-Bedoya et al. 2012; Stashenko et al. 2008; Oliveira et al. 2007; Ruiz et al. 2007; dos Santos et al. 2004; Pascual et al. 2001).

As it is well known, thymol (9) and carvacrol (10) terpenoids have powerful antiseptic properties, which validate their medicinal/therapeutic uses (Kachur and Suntres 2020; Memar et al. 2017; Tisserand and Young 2014). Therefore, it would be expected that EO containing these phenols as main constituents would also show similar biological effects. Accordingly, it has been evidenced that EO from different

parts of the plant were effective in inhibiting different bacteria (*E. coli*, *S. aureus*, *S. epidermidis*, *S. enteritidis*) with MIC/MBC values between 98–750 µg/mL/0.75–1.5 mg/mL and nosocomial fungi (*C. albicans*, *C. parapsilosis*, *C. krusei*, *A. flavus*, and *A. fumigatus*) with MIC values between 31–198 µg/mL (Cáceres et al. 2020; Betancourt et al. 2019; Sarrazin et al. 2015; Betancur-Galvis et al. 2011; Tangarife-Castaño et al. 2011). In other reports from Venezuela, Velasco et al. (2007) found that *L. origanoides* EO (thymol type), evaluated against 39 multi-resistant bacterial strains of intrahospital origin, had a strong antibacterial activity on MRSA, ESBL-producing *K. pneumoniae*, and multi-resistant *Ac. baumannii* with diameters of inhibition zone among 11–35 mm and MIC values between 20–40 µg/mL.

One of the most important assessments of *L. origanoides* EO has been as an antimicrobial agent against strains isolated from animals (e.g., broilers/poultry, fishes, etc.) or applied *in situ* to these animals. Hence, Betancourt et al. (2019) studied the influence of EO (~78% – 9) supplementation on the performance of broilers and their bacterial cecal microbiome. Authors found that EO as supplement presented an improvement on body weight of animals (increasing 9%) and the ratio in feed conversion (increasing ~6%) under coccidian challenge; although results were not conclusive with respect to antiparasitic property (*Eimeria* sp.). In addition, Souza et al. (2015) isolated *E. coli* and *S. aureus* from samples of poultry feces (cloaca of 49 laying hens) and the *L. origanoides* EO showed a noticeable antimicrobial effect from 40 µL/mL dose on both microorganisms, with greater effectiveness on *E. coli*. Interestingly, Bandeira Junior et al. (2019) have reported the evaluation of the *in vitro* antibacterial effect by *L. origanoides* EO (~41% carvacrol) against such bacteria isolated from fish, like *Aeromonas hydrophila*, *Citrobacter freundii*, and *Raoultella ornithinolytica*. The authors found that this EO was able to inhibit all strains with MIC/MBC values of 200–800 µg/mL; they also conducted *in vivo* test of antibacterial activity, inducing *Aer. hydrophila* infection, in silver catfish (*Rhamdia quelen*). In this case, the EO (5 µL/L) acted as therapeutic agent with a success of ~58% survival rate, over 30 days of infection. Similarly, de Almeida et al. (2016) described the antiseptic activity of the *L. origanoides* EO (~33% – 10) against isolated bacteria from bovine milk; MIC values were 60 µL/mL for *S. aureus* and *E. coli*, along with 90 µL/mL for *Sal. choleraesuis* (MBC 120 µL/mL).

And finally, Hernandez et al. (2017), Chataing et al. (2012), Bueno-Sánchez et al. (2009), and Henao et al. (2009) estimated the antimicrobial potential of *L. origanoides* EO against some strains considered by the WHO as a public health problem and related to human health. Thus, Hernandez et al. (2017) tested a EO (containing ~26% carvacrol) from *L. origanoides* leaves which was effective on *E. coli* (MIC 1.25 µL/mL), *P. aeruginosa* (MIC 1.25 µL/mL), *S. aureus* (MIC 2.5 µL/mL), *Aspergillus brasiliensis* (MIC 0.62 µL/mL), and *C. albicans* (MIC 2.5 µL/mL). As well, Chataing et al. (2012) determined the antifungal (five strains) and antibacterial (six strains) properties of the EO [chemovariety thymol (~64%)] of *L. origanoides* leaves (Venezuela); authors reported that the strains were highly susceptible to the EO, f.i., *S. aureus* (φ inh. zone: 23±2 mm), *Ent. faecalis* (φ inh. zone: 20±2 mm), *E. coli* (φ inh. zone: 25±2 mm), *Pseudomonas* sp. (φ inh. zone:

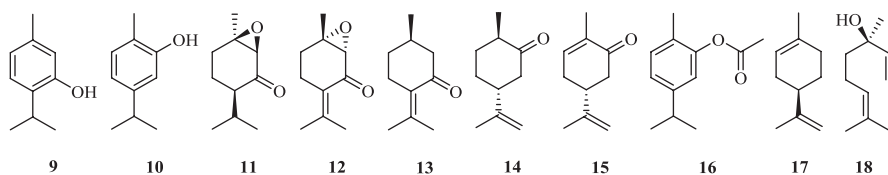
22±1 mm), *Shigella sonnei* (ϕ inh. zone: 21±2 mm), *Sal. typhimurium* (ϕ inh. zone: 20±1 mm), *A. niger* (ϕ inh. zone: 35±1 mm, MIC/MBC 250 µg/mL), *Kluyveromyces fragilis* (ϕ inh. zone: 40±2 mm), *C. albicans* (ϕ inh. zone: 34±1 mm, MIC/MBC 500 µg/mL), *Pichia pini* (ϕ inh. zone: 34±1 mm, MIC/MBC 250 µg/mL), and *Sac. cerevisiae* (ϕ inh. zone: 30±1 mm, MIC/MBC 250 µg/mL). It is worth mentioning the research by Bueno-Sánchez et al. (2009); these authors determined MIC values of 125±0 µg/mL, 160±72 µg/mL, and 400±120 µg/mL on *M. tuberculosis* from thymol (54%), carvacrol (46%), and (*E*)-caryophyllene (11%)/*p*-cymene (11%) chemovarieties, respectively, of *L. organoides* EO. Lastest, Henao et al. (2009) determined the microbial growth inhibitory capabilities of CH₂Cl₂, hexane and EtOH extracts from *L. organoides* leaves. From these extracts, the one with the highest inhibition of microbial growth was CH₂Cl₂ extract with ϕ inh. zone values between 28 mm and 21 mm for *S. aureus*, *C. albicans*, *Enterococcus gallinarum*, *Pr. mirabilis*, *P. aeruginosa*, *E. coli*, and *Ent. cloacae*.

A species of woody perennial fragrant shrub known as muña, guarmpi poleo, chancas del muerto, chancua, peperina, or poleo de Quito is *Minthostachys mollis* (syn.: *Bystropogon mollis*, *Mentha mollis*), which is the third plant of interest; it is native to Colombia, Ecuador, Peru, and Venezuela, but is also distributed in Bolivia and Argentina, and it has been used in folklore medicine as a treatment for stomach cramps and some flu disorders (Pérez García 2017; Torrenegra-Alarcón et al. 2016; Quattrocchi 2012; Carhuapoma et al. 2009; Schmidt-Lebuhn 2008). Some reports of scientific literature have mentioned their antifungal, antibacterial, and antiviral properties; thus, EO of *M. mollis* leaves [constituted by carvacrol (~21%) and thymol (~13%)] resulted active against *S. aureus* (MIC 500 µg/mL), *S. epidermidis* (MIC 600 µg/mL), and *E. coli* (MIC 500 µg/mL) (Torrenegra-Alarcón et al. 2016); in addition, the leaf EO composed by *cis*-piperitone oxide (**11** – ~30%) and piperitenone oxide (**12** – ~26%) was effective on four strains of fungi: *C. krusei* (MIC 250 µg/mL), *A. flavus* (MIC 315 µg/mL), *A. fumigatus* (MIC 315 µg/mL), and *C. parapsilosis* (MIC 375 µg/mL) (Zapata et al. 2009). As well, the leaf EO from the Venezuelan plant was able to significantly inhibit *Sal. typhi* (MIC/MBC 4/16 µg/mL), *B. subtilis* (MIC/MBC 4/32 µg/mL), *K. pneumoniae* (MIC/MBC 8/64 µg/mL), *S. aureus* (MIC/MBC 16/64 µg/mL), *E. coli* (MIC/MBC 16/64 µg/mL), and *Ent. faecalis* (MIC/MBC 64/512 µg/mL); this EO was composed by pulegone (**13** – ~55%) and *trans*-menthone (~32%) (Mora et al. 2009).

The leaf EO from the Peruvian species showed extensive antimicrobial properties: Carhuapoma et al. (2009) reported that the EO from entire plant inhibited to *H. pylori* (MIC 2 µg/mL), *Shigella dysenteriae* (MIC 4 µg/mL), *Sal. typhi* (MIC 4 µg/mL), and *P. aeruginosa* (MIC 9 µg/mL); Peña and Gutiérrez (2017) demonstrated the antimicrobial effect of muña EO on microorganisms frequently found in the lower respiratory tract; the EO constituted by pulegone (~9%) and menthone (~6%) showed ϕ inh. zones of 30 mm and 18 mm on *S. aureus* and *K. pneumoniae* with EO concentrations of 250 µg/mL and 100 µg/mL, respectively. Alcalá-Marcos et al. (2011) estimated that pure muña EO and a 50% solution of same EO had anti-*Candida* activity (*C. albicans*) with values of ϕ inh. zones ranged between 46–48mm and 39–41mm, successively, with values greater than or equal to the positive control

(fluconazole). A biological evaluation on bacteria belonging to the oral flora (e.g., *Str. mutans*, *Lactobacillus* sp., *Fusobacterium nucleatum*, *Actinobacillus actinomycetemcomitans*, now *Aggregatibacter actinomycetemcomitans*, and *Actinomyces* sp.) using the EO from *M. mollis* leaves was determined by Diaz and Moromi (2005); the authors demonstrated the *in vitro* antibacterial potential of EO against standardized oral bacteria with ϕ inh. zones between ~11–20 mm, with *F. nucleatum* being the most susceptible strain. The particular report, related to the EtOH extract (by maceration) of *M. mollis*, and carried out by Bussmann et al. (2010), showed that extract was active on *E. coli* with a MIC value of 16 mg/mL.

Other interesting antimicrobial activity of *M. mollis* EO was antiviral against human herpes virus 2 (HHV-2) with a value of 100 $\mu\text{g/mL}$. The pre-infection treatment with this EO on HHV-2 produced an EC_{50} (50% antiviral effective concentration) value of 68 and SI (antiviral selective index) value of ca. 4. The EO was constituted by pulegone (19%), (E)-caryophyllene (~18%), menthone (~12%), and bicyclogermacrene/germacrene D (~11%) (Brand et al. 2016; Olivero-Verbel et al. 2010). Finally, the variability in the chemical composition of peperina is wide; it has already been mentioned that EO from different countries contained pulegone/*trans*-menthone, pulegone/(E)-caryophyllene, *cis*-piperitone oxide/piperitenone oxide, carvacrol/timol; however, the Argentinean EO contained dihydrocarvone (**14** – ~38–57%)/carvone (**15** – ~28–46%), pulegone (~57–76%), carvacryl acetate (**16** – ~24–51 %)/carvacrol (~19–33%), limonene (**17** – ~36–43%) and linalool (**18** – ~56–84%) (van Baren et al. 2014).



And the fourth Colombian plant belongs to the genus *Salix*, which includes ca. 450 species among trees and shrubs (Argus 1997). In South America, there are two species of *Salix* (trees): *S. humboldtiana* (syn.: *S. chilensis*, *S. magellanica*) and *S. babylonica* (syn.: *S. japonica*, *S. lasiogyne*, *S. matsudana*), of which *S. humboldtiana* is the only native and it is distributed from Mexico to Argentina. Its common known names are sauce (willow), sauce de Humboldt (Humboldt willow), sauce colorado/amargo/blanco/criollo, sauco, sauz, chorão, salseiro, salso, sarã, mimbre, pajarábobo, etc.; while the common names of *S. babylonica* (distributed in Europe, Asia, as well as America) are Babylon weeping willow, sauce de Babilonia, sauce, sauce llorón. The traditional preparations and uses of the *S. humboldtiana* are as infusions/decoctions of leaves/stem/bark for the treatment of influenza, dermatosis, diarrhea, fungi infections. As for *S. babylonica*, it has been reported for its antiseptic and bactericide/fungicide properties and its effectiveness against gonorrhea, dermatosis, flu, malaria, etc. The bark contains 3–4% of salicin, which was the base for aspirin preparation (Álvarez 2019; Wahab et al. 2018; Galvis Rueda and Torres

Torres 2017; Grandtner and Chevrette 2014; Waizel-Bucay 2010; Duke 2008; Ministerio de la Protección Social 2008; Hauenstein et al. 2005).

The potential veterinary use of the hydroalcoholic extract from *S. babylonica* leaves was validated by González-Alamilla et al. (2019) against three strains (*S. aureus*, *L. monocytogenes*, and *E. coli*) that cause diseases in animals (e.g., birds, fishes, crustaceans, bovine, sheep, goat, pig, and poultry). Thus, total extract presented MIC values of 25 mg/mL, 50 mg/mL, and 100 mg/mL on *S. aureus*, *L. monocytogenes*, and *E. coli*, respectively. In addition, the extract partition in two separated phases (aqueous and organic) had the following MIC values: aqueous part – 3.1 mg/mL (*L. monocytogenes*), 12.5 mg/mL (*E. coli*), and 25 mg/mL (*S. aureus*); organic part – 1.6 mg/mL (*L. monocytogenes*), 6.25 mg/mL (*E. coli*), and 6.25 mg/mL (*S. aureus*); and subsequent fractionation produced three active fractions: F7AC (MIC values 0.78–6.25 mg/mL), F8AC (MIC values 0.39–25 mg/mL), and F10AC (MIC values 6.25–12.5 mg/mL). In turn, González-Alamilla et al. (2020) established the antibacterial capacity of the MeOH extract from *S. babylonica* aerial parts against some important bacteria in public health. The authors determined the MIC values on *B. subtilis* (12.5 mg/mL), *L. monocytogenes* (25 mg/mL), *S. aureus* (25 mg/mL), *E. coli* (12.5 mg/mL), *P. aeruginosa* (100 mg/mL), *Sal. typhi* (100 mg/mL), and *Salmonella choleraesuis* (100 mg/mL).

Likewise, Popova and Kaleva (2015) reported that 20% aqueous extracts of dried twigs/leaves from *S. babylonica* showed different degrees of growth inhibition (ϕ zones and MIC values) against *E. coli* (10±1 mm-17±7 mm, MIC 56±14 mg/mL-256±0 mg/mL), *Sal. enterica* (12±2 mm-14.2±0.4 mm, MIC 62±30 mg/mL-256±0 mg/mL), *S. aureus* (9.2±0.4 mm-11.0±0.7 mm, MIC 60±11 mg/mL-256±0 mg/mL), *Paenibacillus alvei* (isolated of European foulbrood from honeycombs – 9.2±0.4 mm- 11±1 mm, MIC 104±31 mg/mL-256±0 mg/mL) and *C. albicans* (14±6 mm-15±7 mm, MIC 70±17 mg/mL-234±38 mg/mL) strains. For its part, Wahab et al. (2018) described the antimicrobial capabilities on *E. coli*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, and *C. albicans* of 100 µg of the different fractions (petroleum ether, CH₂Cl₂, and ethyl acetate) and extract (MeOH) from *S. babylonica* bark/leaves. The values of the diameters of inhibition zone were 7–10 mm for the petroleum ether fraction (leaves) and the MeOH extract from leaves/bark, 7–9 mm for the CH₂Cl₂ fraction (leaves), 7–12 mm for the ethyl acetate fraction (leaves), 10–14 mm for the petroleum ether fraction (bark) and 9–16 mm for the CH₂Cl₂ and ethyl acetate fractions (bark) on all strains evaluated. An interesting application of hydroalcoholic extract (MeOH:H₂O 30:70) from *S. babylonica* leaves was described by Rangel-López et al. (2020) when these authors established the *in vitro* antibacterial potential against bacteria (*Aer. hydrophila*, *Listonella anguillarum*, *Edwardsiella tarda*, and *Streptococcus iniae*) affecting *Oncorhynchus mykiss* (rainbow trout) and *Oreochromis* sp. (tilapia). The MIC/MBC values were 1.6/3.1 mg/mL, 3.1/25 mg/mL, 25 mg/mL, and 25/100 mg/mL for *L. anguillarum*, *E. tarda*, *Str. Iniae*, and *Aer. hydrophila*, respectively.

Similarly, Toso et al. (2006) carried out a screening of the pharmacological potential of extracts (EtOH:H₂O) from aerial parts of native and naturalized plants (including *S. humboldtiana*) from province of La Pampa (Argentina). These authors

found that *S. humboldtiana* extract inhibited the growth of *Brucella canis* and *S. aureus*. Subsequently, Toribio et al. (2009) reported that EtOH:H₂O extract (60 µL) from *S. humboldtiana* (20 g of aerial part/100 mL) produced a diameter of the inhibition zone of 16 mm against *S. aureus*.

As a last evidence of antimicrobial potential of *Salix* spp., Muñoz et al. (2000) reported that the *in vitro* assay of the aqueous extract (10 µg/mL) from *S. humboldtiana* leaves inhibited up to 99% the growth of chloroquine-resistant *Plasmodium falciparum* strain (Indo), while the *in vivo* assay on *Pl. vinckei petteri*, an extract of 1000 mg/kg administered per four days inhibited growth by 32%. In addition, Rivero-Perez et al. (2019) determined the anticoccidial capability (against *Eimeria stiedae*, *E. magna*, *E. coecicola*, *E. media*, *E. perforans*, and *E. exigua*) in rabbits of the H₂O:EtOH (30:70) extract from *S. babylonica* leaves; the oral administration of the extract during 21 days at 25–50 mg/kg b.w. decreased the average number of oocysts per gram of feces (37±5–66±2%); nonetheless, the most significant effect (56±3–97.0±0.1%) was observed at day 28 for both concentrations. As additional relevant information, which is beyond the scope of this chapter, some authors (Salem et al. 2017; Mejia Hernandez et al. 2014) have demonstrated the anthelmintic potential of *S. babylonica* extracts in small-ruminant farms.

Two plants used by indigenous natives from Mexico were included: *Acalypha monostachya* and *Amphipterygium adstringens*. The plants are perennial shrub/herb (up to 40 cm of height and it contains a latex which could cause skin irritation/stomach upset if swallowed) with uni-bisexual inflorescences and dioecious small tree (up to 5 m height) with drupal fruits (young, shiny green; ripe red), one-to-one. The first of them, *Ac. monostachya* (syn.: *Ac. agrimonioides* D. Dietr., *Ac. anemioides* Kunth, *Ac. depressa* Sessé & Moc., *Ac. hederacea* Torr., *Ac. phleoides* Cav., *Ricinocarpus anemioides* [Kunth] Kuntze, *R. monostachyus* Cav.) is distributed from south-western of North America to northern Mexico and Guatemala; and the most common names are “hierba del cancer/del pastor,” “hojas de cobre,” round copperleaf, copper leaf, etc. The Mexican natives have used the plant as a treatment to alleviate bacterial and fungal diseases, skin rashes, as a wound healing (prepared as a poultice or for baths from the whole plant cooking) and against diarrhea (coction of aerial parts); as well as an anticancer agent (beverages or infusion) for women (Sharma et al. 2017; Jacobo-Herrera et al. 2016; Seebaluck et al. 2015; García-Regalado 2014; Canales et al. 2011; Macías et al. 2009; Canales et al. 2005; González Elizondo et al. 2004; Hernández et al. 2003).

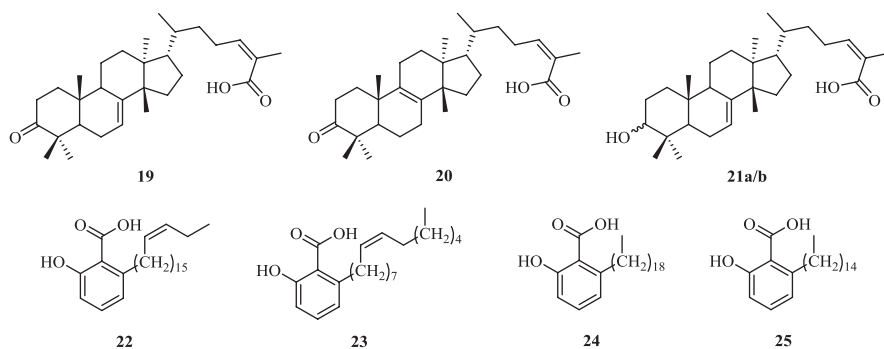
A few studies on the antimicrobial potential of *Ac. monostachya* are available in the scientific literature: (i) Canales et al. 2011 demonstrated that MeOH extract from aerial parts of copper leaf presented ϕ inh. zones on different microorganisms: 10±2 mm (*V. cholerae*), 9±2 mm (*S. aureus*), 8.6±0.6 mm (*S. epidermidis*), 8±2 mm (*Sar. lutea* and *B. subtilis*), and ≤ 7 mm (other *V. cholerae* strains); and a LC₅₀ value of 430 µg/mL on *T. mentagrophytes*. (ii) Macías et al. (2009) indicated that *S. aureus* (ϕ inh. zones: 15.0±0.01 mm and 112.0±0.02 mm) and *P. aeruginosa* (ϕ inh. zone: 11.0±0.02 mm) strains were susceptible to the aerial part extracts (aqueous and EtOH) of *Ac. monostachya*. (iii) Canales et al. (2005) found that hexane extract from aerial parts of *Ac. monostachya* produced a ϕ inh. zone of 10.0±0.5 mm; and

(iv) Hernández et al. (2003) reported that hexane extract from aerial parts of plant was able to produce the growth inhibition (ϕ inh. zone/MIC values) of *Sarcina lutea* (16.0 ± 0.5 mm/1000 $\mu\text{g/mL}$), *V. cholerae* (15.0 ± 0.5 mm/1000 $\mu\text{g/mL}$), *Ent. agglomerans* (12.7 ± 0.6 mm), *Ent. aerogenes* (12.7 ± 0.6 mm), *Sal. typhi* (12.3 ± 0.6 mm), *V. cholerae* (isolated from water) (12.0 ± 0.5 mm), *B. subtilis* (12.0 ± 0.5 mm), *Yersinia enterocolitica* (11.7 ± 0.6 mm), *V. cholerae* CDC V12Sb (11.7 ± 0.6 mm), *V. cholerae* (clinical isolate) (11.3 ± 0.6 mm), and *S. epidermidis* (11.0 ± 0.5 mm); in addition, the EtOH extract produced inhibition halos of 10 ± 1 mm and 8 ± 2 mm on *Sar. lutea* and *S. aureus*, respectively.

The second plant is *Amp. adstringens* (syn.: *Hypopterygium adstringens* Schldl., *Juliania adstringens* Schldl) is native to Central America and it is named as cuachalalá, cuachalalate, macerán, volador, palo santo, pacueco, cuachinala, matixeran, muaxalaxitli, yalaguitu, etc. In Mexico, this plant (bark) is one of the most used for the treatment of stomach ulcers and gastritis (infusion/decoction), wound healing (bath/poultice), caries/bad breath (mouthwash), and against fever, malaria, cancer (tumor/digestive tract), inflammatory conditions, and digestive/kidney disorders (Sharma et al. 2017; Quevedo-León 2015; Quattrocchi 2012; Rosas-Piñón et al. 2012; Solares-Arenas et al. 2012; Alonso-Castro et al. 2011; Ruiz-Bustos et al. 2009; Monroy-Ortiz and Monroy 2006; Canales et al. 2005; Cuevas Figueroa 2005; Grandtner 2005; Olivera-Ortega et al. 1999).

One of the most astonishing antimicrobial potentials of cuachalalate and its preparations is as an anti-*Helicobacter pylori* agent. Thus, Castillo-Juárez et al. (2009) determined that the MeOH and aqueous extracts of plant bark showed MIC values of 250 $\mu\text{g/mL}$ and 500 $\mu\text{g/mL}$ on *H. pylori* strain. In other similar report, Castillo-Juárez et al. (2007) revealed that the non-polar fraction (petroleum ether) of bark produced inhibition percentages of 90% and 100% at concentrations of 16 $\mu\text{g/mL}$ and 160 $\mu\text{g/mL}$, in turn. From this fraction, sterol compounds along with a mixture of anacardic acids [long-chain phenolic acids with side saturated chains of C_{15} (~47%), C_{16} (~7%), C_{17} (~30%) and C_{19} (~8%), and one phenol with a side mono-unsaturated chain of C_{19} (~9%)] were isolated but only the mixture of anacardic acids had a MIC value of 10 $\mu\text{g/mL}$ on *H. pylori*. Moreover, Robles-Zepeda et al. (2011) showed that MeOH extract of the plant presented MIC values of 200 $\mu\text{g/mL}$ and 200–400 $\mu\text{g/mL}$ against two strains of *H. pylori* (–25 and –43504). Alike, Rosas-Acevedo et al. (2011) demonstrated that MeOH, hexane, and ethyl acetate extracts (300 mg/kg p.o.) of bark reduced the gastric lesion induced in rats by ~85%, ~73%, and ~68%, respectively. And, Navarrete et al. (1998) revealed the gastric protective effects (%) on animal model (rat) of the MeOH, ethyl acetate and CH_2Cl_2 extracts along with four fractions from of stem bark; thus, 300 mg/kg of MeOH, ethyl acetate, and CH_2Cl_2 extracts produced a protection of ~74%, ~64%, and ~77%, in turn; and, 100 mg/kg of P1, P2, P3, and P4 fractions presented ~85%, ~96%, ~97%, and ~85%, in that order, of gastric protection. As well as, Arrieta et al. (2003) showed the gastroprotective effects (%) on *in vivo* model (rats) of the extract (MeOH), fraction (CH_2Cl_2) and isolated compounds from stem bark; thus, 30–300 mg/kg of extract produced ~44±9%–~75±6% of gastroprotection; 10–100 mg/kg of the fraction induced a gastric protection of ~30±9%–~93±6%; 10–100 mg/kg of

3 α -hydroxymasticadienonic acid (**21a**) protected between 64 \pm 8% and ~87 \pm 5%; and 10–30 mg/kg of 3-epi-oleanolic acid produced a gastroprotection between ~41 \pm 9% and ~89 \pm 5%.



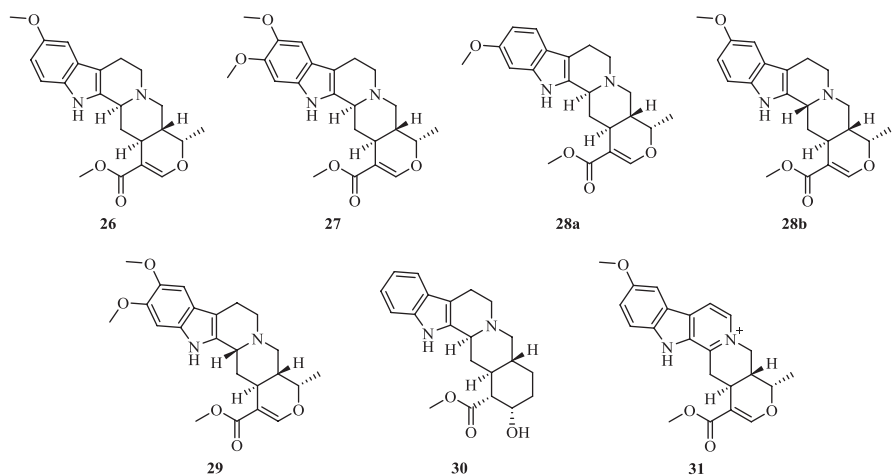
Other microbial strains in which *Amp. adstringens* and their extracts/fractions/ isolated constituents have remained active are reported by other authors; Canales et al. (2005) established that ethyl acetate extract from bark of *Amp. adstringens* caused ϕ inh. zones of 9.7 \pm 0.6 mm and 7.3 \pm 0.6 mm on *V. cholerae* CDC V12 and *S. aureus* strains, correspondingly; as well, the MeOH extract of bark was able to inhibit the growth (ϕ inh. zone/MIC values) of *Sar. lutea* (20.3 \pm 0.6 mm/125 μ g/mL), *Ent. aerogenes* (12.3 \pm 0.6 mm), *S. aureus* (11.7 \pm 0.6 mm/250 μ g/mL), *Y. enterocolitica* (11 \pm 1 mm), *S. epidermidis* (10.7 \pm 0.6 mm), *B. subtilis* (10 \pm 1 mm), *E. coli* (10.3 \pm 0.6 mm), *Ent. agglomerans* (10.0 \pm 0.5 mm), *V. cholerae* (9.7 \pm 0.6 mm), and *V. cholerae* CDC V12 (9.3 \pm 0.6 mm). Besides, Ruiz-Bustos et al. (2009) recorded that MeOH extract of this plant had MIC values of 190–250 μ g/mL and 380–500 μ g/mL on *Shigella flexneri* and *S. aureus*/*E. coli*, separately. Rosas-Piñón et al. (2012) studied the capability of extracts of Mexican plants to treat oral cavity infections; the authors found that aqueous and EtOH extracts exhibited MIC values of ~68 μ g/mL and 250 μ g/mL on *Str. mutans* and 500 μ g/mL and 250 μ g/mL on *Por. gingivalis*. Furthermore, Castillo-Juárez et al. (2013) evaluated the antiquorum-sensing potential on *Chromobacterium violaceum* and *P. aeruginosa* by hexane extract and a mixture of anacardic acids from *Amp. adstringens*; they reported that hexane extract (55 μ g/mL) and mixture of anacardic acids (166 μ g/mL) inhibited the violacin production of *Chr. violaceum* in ~92% and 94%, in that order. Whilst, mixture of anacardic acids decreased pyocyanin (86%, 200 μ g/mL) and rhamnolipid (90%, 500 μ g/mL) productions and elastase activity (75%, 500 μ g/mL) on *P. aeruginosa*. Moreover, Rivero-Cruz et al. (2011) obtained a MeOH extract and sterols and anacardic acids from *Amp. adstringens* bark; both extract and some isolated compounds were active against *Str. mutans* and *Por. gingivalis*. Thus, MeOH extract had MIC values of 69 μ g/mL (on *Str. mutans*) and 82 μ g/mL (on *Por. gingivalis*); MIC values of **19**, **22**, **23**, **24**, and **25** were 150–175 μ g/mL, 7–12 μ g/mL, 20–18 μ g/mL, 75–70 μ g/mL, and 104–126 μ g/mL, one-to-one. Finally, Gómez-Cansino et al. (2015) disclosed a remarkable antimycobacterial activity of

CH₂Cl₂:MeOH extracts of *Amp. adstringens* stem bark and leaves; these extracts (50 µg/mL) produce inhibition percentages of 88.2±0.1% (from stem bark) and 90.2±0.7% (from leaves). Additionally, Rivero-Cruz et al. (2005) reported an inhibition of 95% of *M. tuberculosis* H37Rv strain by CH₂Cl₂:MeOH extract (at 50 µg/mL) of stem bark; in addition, isolated compounds (**19** and **21**) from extract had MIC values of 64 µg/mL and 32 µg/mL, respectively.

From Peru, two plants [*Rauvolfia* (or *Rauwolfia*) *tetraphylla* and *Siparuna guianensis*] used by their ethnic inhabitants were considered; both plants are shrubs or small trees with fruits in the drupe forms (young shiny green or yellow; ripe red to black). Furthermore, *R. tetraphylla* (syn.: *R. canescens* L., *R. hirsuta* Jacq., *R. heterophylla* Willd., *R. mollissima* Markgr., *R. subpubescens* L., *R. tomentosa* Jacq.) contains a white latex and its fruit juice can be used as a substitute for ink. The plant is native to Central and northern South America, nonetheless it has also been distributed in southeast Eurasia; among the most common names could be mentioned choloquillo, chalchupa, misho runto, pelillo(a), sanango(co), tøk-ta-men, bitter bush, devil-pepper, be-syill-tree, snake root, nagboi, curarina, boboro, viborilla, amatillo, leche de sapo, etc. The Peruvian indigenous communities traditionally have used the different parts (leaves/stems/fruits/root/latex) prepared in decoction/infusion/poultice forms as a treatment for malaria, dysentery, chronic cough, fever, ulcers, syphilis, and skin diseases (e.g., ringworm and scabies) (Grandtner and Chevrette 2014; Quattrocchi 2012; Mostacero León et al. 2011; Mostacero León et al. 2002; Rengifo 2010; Alonso 2004; Cáceres 1996; Brako and Zarucchi 1993).

The antimicrobial potential of the extracts of different parts from *R. tetraphylla* has been reported by some authors; for example, Shariff et al. (2006) proved that EtOH and chloroform extracts of leaves were the most active against *Pseudomonas solanacearum* (EtOH and CHCl₃ extracts), *Xanthomonas axonopodis* pv. *malvacearum* (EtOH extract) and *E. coli* (EtOH extract), with MIC values of 1000 µg/mL for each. Likewise, Suresh et al. (2008) determined the antimicrobial susceptibility (by way of the disk diffusion method) of the EtOH extract of leaves; thus, the extract produced significant inhibition diameters between ~10–30 mm (dose-dependent manner) on *E. coli*, *Ent. Aerogenes*, and *Alcaligenes faecalis*, while *A. niger* and *Penicillium* sp. were the strains most susceptible to the extract, with a decrease in the average diameters of colonies between 48% and 50%. Alagesaboopathi (2009) found that the ethyl acetate extract (150 µL) from dried fruit was able to significantly inhibit to eight human pathogenic bacteria, e.g., *Sal. paratyphi* A (ϕ inh. - 26 mm), *Sal. typhi* (ϕ inh. - 23 mm), *P. aeruginosa* (ϕ inh. - 20 mm), *Sal. paratyphi* B (ϕ inh. - 16 mm), *Proteus vulgaris* (ϕ inh. - 16 mm), *E. coli* (ϕ inh. - 15 mm), *S. aureus* (ϕ inh. - 14 mm), and *Klebsiella mobilis* (ϕ inh. - 13 mm). In Ganga Rao et al. (2012) evaluated the antibacterial capability of ethyl acetate, MeOH, and H₂O:EtOH extracts (150 µg/cup for each) from *R. tetraphylla* root bark against Gram-negative and Gram-positive bacteria; namely, 16–18 mm of ϕ inh. zones for *Str. pneumoniae*, *B. cereus*, *S. aureus*, and *Streptomyces marienensis* with all extracts; 16–17 mm of ϕ inh. zones for *B. pumilis*; and 15–17 mm of ϕ inh. zones for *E. coli*, *Ent. aerogens*, and *P. aeruginosa*, with the three extracts.

In an interesting report by Dwivedi et al. (2015), the authors isolated six indole alkaloids [10-methoxytetrahydroalstonine (26), isoreserpiline (27), 10-demethoxyreserpiline/11-demethoxyreserpiline (28a/b), reserpiline (29), α -yohimbine (30), and serpentine (31)] from leaves (26-30) and root (31) of *R. tetraphylla*. These molecules inhibited the growth of two strains of *E. coli* (nalidixic acid-sensitive and nalidixic acid-resistant) with MIC values among 125–500 $\mu\text{g/mL}$ and being the most active, 26, 30, and 31 (each with MIC value of 125 $\mu\text{g/mL}$). Into the bargain, mixtures of each alkaloid with nalidixic acid (NAL, 10 $\mu\text{g/mL}$) increased the susceptibility of the two strains; i.e., the MIC values of mixtures were ~ 0.8 – 3.1 $\mu\text{g/mL}$ and 12.5 – 25 $\mu\text{g/mL}$ on nalidixic acid-sensitive and nalidixic acid-resistant strains, respectively. Then, the most active mixtures were 26+NAL (MIC 0.78 $\mu\text{g/mL}$) and 27+NAL, 29+NAL-31+NAL (each with MIC value of 1.56 $\mu\text{g/mL}$). Finally, Rohela et al. (2016) studied the antimicrobial effects of petroleum ether/MeOH/ CHCl_3 extracts (10%, g plant/mL ste) from *R. tetraphylla* leaves/stem/root. As relevant results were reported: (i) the MeOH extracts of leaves, stem, and root were the most active with ϕ inh. zones of ~ 8 – 25 mm, ~ 4 – 16 mm, and ~ 7 – 15 mm on the four bacterial strains (*S. aureus*, *P. aeruginosa*, *E. coli*, and *K. pneumoniae*); (ii) the MeOH extracts of leaves/stem/root were able to inhibit the growth of the four fungi strains (*A. niger*, *Penicillium* sp., *C. albicans*, and *Fusarium oxysporum*) with ϕ inh. zones of ~ 14 – 19 mm, ~ 10 – 14 mm, and ~ 10 – 22 mm; and (iii) the most susceptible strains to the MeOH extracts of leaves and root were *S. aureus* and *F. oxysporum* with ϕ inh. zones of 25 ± 2 mm and 22 ± 2 mm, respectively. The colored chemical tests on the polar extracts indicated that the main constituents were alkaloid type.

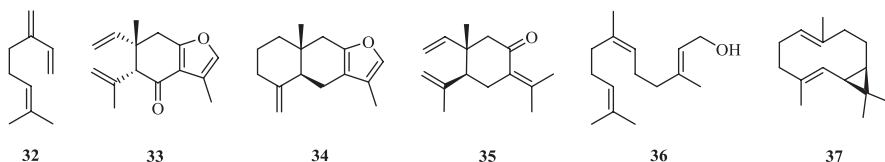


The other plant, *S. guianensis* (syn.: *Citrosma campora* Tul., *C. discolor* Poepp. & Endl., *S. archeri* A.C. Sm., *S. argyochrysea* Perkins, *S. cuspidata* A. DC., *S. duckeana* Jangoux) is native to Central and South America and its main common names are Guianan bogwood, siparuna des Guyanes, muniri dan, fever bush, árbol

del danto, culebra, cumanagoto, hierba de pasmo/bachaco, palo de bachaco/japutamo, paripari, asna/isula/picho huayo, isula-micunan, curuinsi-sacha, kapasawi, shishohuitsa, limão-bravo, muniridan, negamina, negra-mina, oema-jarakopi, päshapäsha, urcugalabili, uatakurán, yakantayuru, etc. The Latin American native populations have used the different parts (leaves/bark/flowers) prepared in decoction/infusion/bath forms to treat dyspepsia, painful spasms, cold, colics, dermatosis, fever, mange, wounds, and as antimycotic, antipyretic, and carminative (Rengifo and Nuñez Pérez 2015; Grandtner and Chevrette 2014; Quattrocchi 2012; Rengifo 2010; Brako and Zarucchi 1993).

The reviewed scientific literature mentioned that this plant has significant antimicrobial potential mainly due to its essential oils. In Moura et al. (2020) reported that EO of leaves was highly active against *E. coli*, *P. aeruginosa*, *Streptococcus pyogenes*, and *S. aureus*, by increasing the permeability of the cell wall as part of the possible mechanism of action. The MIC values were between 0.87–1.30 µg/mL and the EO was constituted by myrcene (**32**, ~40%), epicurzerenone (**33**, ~18%), and germacrene D (~14%). In the same year, de Oliveira et al. (2020) established that EO (yield 1.4%) of this plant produced ϕ inh. halos/MIC values of 13±1 mm/125 µg/mL, 12.0±0.6 mm/250 µg/mL, 11.0±0.13 mm/500 µg/mL, 11.0±0.1 mm/125 µg/mL on *C. albicans*, *Ent. faecalis*, *E. coli*, and *Str. mutans*, in that order. The chemical composition of this EO differed from that described by Moura et al. (2020); i.e., atractylone (**34**, ~19%) and (E)- β -elemenone (**35**, ~12%) were the main components.

Other study was divulged by de Melo et al. (2017) on the anticariogenic (*Str. mutans*, *Str. mitis*, *Str. sanguinis*, *Str. sobrinus*, *Str. salivarius*, *L. casei*) and antimycobacterial (*M. avium*, *M. tuberculosis*, and *M. kansasii*) capacities of EO from plant leaves. The authors found that the EO (yield 0.8%) constituted by (E,E)-farnesol (**36**, ~18%), β -myrcene (~16%), siparunone (~15%) and germacrene D (~10%) inhibited the growth of cariogenic and mycobacterial strains with MIC values between 50–400 µg/mL and 250–500 µg/mL, respectively. The most sensible microorganisms were *Str. mutans*, *Str. mitis*, and *M. avium* with MIC values of 50 µg/mL, 100 µg/mL, and 250 µg/mL. In addition, Andrade et al. (2015) evaluated the antimicrobial activities (five bacterial and four fungi strains) of EO from plant leaves. The MIC values determined for microbes were ~8 µg/mL (*A. flavus*), ~31 µg/mL (*A. niger* and *Penicillium comune*), 125 µg/mL (*Aspergillus carbonarius* and *S. aureus*), 250 µg/mL (*L. monocytogenes*), and 500 µg/mL (*E. coli*) with the chemical composition of EO represented by bicyclogermacrene (**37**, ~17%), β -myrcene (~13%), and germacrene D (~9%). Similarly, de Bessa et al. (2015) reported that leaves EO [yield: 1.2%; and composed by β -myrcene (~29%), 2-undecanone (~8%) and bicyclogermacrene (~6%)] formed inhibition halos between 10 mm and 12 mm when concentrations of EO among 95–380 µg/mL were evaluated.



Finally, Castro et al. (2008) evaluated the antibacterial potential of extracts from Brazilian plants against three pathogenic bacterial strains of fish. The authors found that the MeOH extract from *S. guianensis* steam bark inhibited the growth of *Flavobacterium columnare* with ϕ inh. zone/MIC values of ~ 12 mm/ ~ 94 μ g/mL. Besides, Lopez et al. (2001) found that MeOH extract from negramina leaves was able to inhibit to *Str. faecalis*, *Mycobacterium phlei*, *B. subtilis*, and *S. aureus*. It is worth noting that other antimicrobial activity reported for *S. guianensis* has been against protozoan parasites; thus, the leaves EO produced an IC₅₀ value (24 h) of 49 ± 4 μ g/mL on *L. amazonensis* (promastigotes), according to Andrade et al. (2016). On the other hand, the extracts or alkaloid-enriched fractions were also active against *Pl. falciparum* and *T. cruzi*; thus, EtOH extract/alkaloid fraction of leaves were active on chloroquine-resistant and chloroquine-sensitive strains of *Pl. falciparum*, with IC₅₀/IC₉₀ values of 7/34 μ g/mL and 15/49 μ g/mL (for extract), and 15/69 μ g/mL and 58/182 μ g/mL (for alkaloid fraction) (Fischer et al. 2004). Additionally, the alkaloid-enriched fraction (100 μ g/mL) from EtOH extract of leaves was able to kill up to 100% of *T. cruzi* trypomastigotes (Tempone et al. 2005).

5 Conclusions

The Latin America plants (and their parts) mentioned in this chapter have been shown to be capable of efficiently inhibiting (high ϕ of inh. zones, low MIC/MBC values) pathogenic/resistant microorganisms (Gram+/Gram- bacteria and fungi/yeast) producers of diseases in humans (of environmental or intrahospital origin) and animals (broilers/poultry, crustaceans/fishes, bovine/sheep/goat/rabbits). Furthermore, the same plants have shown potential as a treatment for transmissible vectors (protozoan parasites) of endemic tropical diseases of Latin America, such as Chagas, leishmaniasis, malaria. This topic on antimicrobial plants of Latin America becomes an important research focus due to the following: (i) in some cases, only total extracts (H₂O, MeOH, EtOH, hexane, CH₂Cl₂) or fractions (alkaloids, flavonoids) have been studied but bioactive molecules have not been isolated and chemically characterized; (ii) some extracts/fractions have been evaluated in *in vitro* models and not in *in vivo* models, and in addition to not having pharmaceutical formulations; (iii) certain extracts/fractions have some safety data of (toxicity) and not the majority; and (iv) the extracts/fractions have not been evaluated against other groups of bacteria/fungi/parasites/viruses.

Acknowledgment The authors thank: Universidad Santo Tomás de Aquino (sede Bucaramanga) by using of the Bibliometric Unit; Indigenous communities of the Peruvian Amazon (e.g., Tikunas, Kichwas, Chayahuitas); and Colciencias-SGR ["Formación de Capital Humano (O.J.C.C, Convocatoria No. 1 Becas de Excelencia Bicentenario, 2019 - Departamentos de Sucre; C.P.G.G., Convocatoria No. 810, 2018 - Departamento de La Guajira)].

References

- Abreu AC, McBain AJ, Simões M (2012) Plants as sources of new antimicrobials and resistance-modifying agents. *Nat Prod Rep* 29:1007–1021
- Ahmad M, Khan AU (2019) Global economic impact of antibiotic resistance: a review. *J Glob Antimicrob Resist* 19:313–316
- Ahmed SA, Barış E, Go DS, Lofgren H, Osorio-Rodarte I, Thierfelder K (2017) Assessing the global economic and poverty effects of antimicrobial resistance. Policy Research Working Paper No. 8133. World Bank, Washington. URL: <https://openknowledge.worldbank.org/handle/10986/27636>
- Alagesaboopathi C (2009) An investigation on the antibacterial activity of *Rauvolfia tetraphylla* dry fruit extracts. *Ethnobot Leaflets* 13:644–650
- Alcalá-Marcos KM, Alvarado-Gamarra AG, Alejandro-Paredes LA, Huayané-Linares E (2011) Actividad antimicrobica del aceite esencial de las hojas de *Minthostachys mollis* (Muña) comparado con el fluconazol en cultivo de *Candida albicans*. *CIMEL* 16:83–86
- Alonso J (2004) Tratado de fitofarmacos y nutraceuticos. Ed. Corpus Libros, Rosario, p 1350
- Alonso-Castro AJ, Villarreal ML, Salazar-Olivo LA, Gomez-Sanchez M, Dominguez F, Garcia-Carranca A (2011) Mexican medicinal plants used for cancer treatment: pharmacological, phytochemical and ethnobotanical studies. *J Ethnopharmacol* 133:945–972
- Álvarez MA (2019) Pharmacological properties of native plants from Argentina. Springer Ltd., Gewerbestrasse, p 280
- Alves VF, Figueiredo RD, Cavalcanti YW, Padilha WVN (2019) Atividade antimicrobiana de plantas medicinais indicadas para uso no Sistema Único de Saúde. *Rev Cuba Estomatol* 56:1–16
- Alviano WS, Alviano DS, Diniz CG, Antonioli AR, Alviano CS, Farias LM, Carvalho MAR, Souza MMG, Bolognese AM (2008) *In vitro* antioxidant potential of medicinal plant extracts and their activities against oral bacteria based on Brazilian folk medicine. *Arch Oral Biol* 53:545–552
- Andrade JC, da Silva ARP, Freitas MA, Ramos BA, Freitas TS, dos Santos FAG, Leite-Andrade MC, Nunes M, Tintino SR, da Silva MV, Correia MTS, de Lima-Neto RG, Neves RP, Coutinho HDM (2019b) Control of bacterial and fungal biofilms by natural products of *Ziziphus joazeiro* Mart. (Rhamnaceae). *Comp Immunol Microb* 65:226–233
- Andrade JC, Silva ARP, Santos ATL, Freitas MA, Carneiro JNP, Gonçalves MIP, de Souza A, Freitas TS, Ribeiro PRV, Brito ES, Morais-Braga MFB, Coutinho HDM (2019a) UPLC-MS-ESI-QTOF characterization and evaluation of the antibacterial and modulatory antibiotic activity of *Ziziphus joazeiro* Mart. aqueous extracts. *S Afr J Bot* 123:105–112
- Andrade JC, da Silva ARP, dos Santos ATL, Freitas MA, de Matos YMLS, Braga MFBM, Bezerra CF, Pereira Gonçalo MI, Gomez Vega MC, Rolóm M, Coronel C, Vasconcelos Ribeiro PR, de Brito ES, Melo Coutinho HD (2019c) Chemical composition, antiparasitic and cytotoxic activities of aqueous extracts of *Ziziphus joazeiro* Mart. *Asian Pac J Trop Biomed* 9:222–226
- Andrade MA, Azevedo CA, Motta FN, dos Santos ML, Silva CL, de Santana JM, Bastos IMD (2016) Essential oils: *in vitro* activity against *Leishmania amazonensis*, cytotoxicity and chemical composition. *BMC Complement Altern Med* 16:444
- Andrade MA, Cardoso MG, Gomes MS, de Azeredo CMO, Batista LR, Soares MJ, Rodrigues LMA, Figueiredo ACS (2015) Biological activity of the essential oils from *Cinnamodendron dinisii* and *Siparuna guianensis*. *Braz J Microbiol* 46:189–194
- Arango-Bedoya O, Hurtado-Benavides AM, Toro-Suárez I (2012) Efecto del origen, la época de recolección y la edad de las hojas en el rendimiento y el contenido de timol de aceites esenciales de *Lippia origanoides* H.B.K. *Acta Agron* 61:207–213
- Argus GW (1997) Infrageneric classification of *Salix* (Salicaceae) in the new world. *Syst Bot Monogr* - JSTOR 52:1–121
- Arrieta J, Benitez J, Flores E, Castillo C, Navarrete A (2003) Purification of gastroprotective triterpenoids from the stem bark of *Amphipterygium adstringens*; role of prostaglandins, sulfhydryls, nitric oxide and capsaicin-sensitive neurons. *Planta Med* 69:905–909

- Arthur GA, Naidoo KK, Cooposamy RM (2012) *Bidens pilosa* L.: agricultural and pharmaceutical importance. *J Med Plant Res* 6:3282–3287
- Azevedo CF, Quirino ZGM, Bruno RLA (2002) Estudo farmacobotânico de partes aéreas vegetativas de aroeira-vermelha (*Schinus terebinthifolius* Raddi, Anacardiaceae). *Rev Bras Plantas Med* 17:26–35
- Bandeira Junior G, Souza CF, Baldissera MD, Descovi SN, da Silveira BP, Tasca C, Mourão RHV, de Vargas APC, Baldisserotto B (2019) Plant essential oils against bacteria isolated from fish: an *in vitro* screening and *in vivo* efficacy of *Lippia organoides*. *Ciênc Rural* 49:e20190064
- Barbieri DSV, Tonial F, Lopez PVA, Sales Maia BHLN, Santos GD, Ribas MO, Glienke C, Vicente VA (2014) Antiadherent activity of *Schinus terebinthifolius* and *Croton urucurana* extracts on *in vitro* biofilm formation of *Candida albicans* and *Streptococcus mutans*. *Arch Oral Biol* 59:887–896
- Bartolome AP, Villaseñor IM, Yang W-C (2013) *Bidens pilosa* L. (Asteraceae): botanical properties, traditional uses, phytochemistry, and pharmacology. *Evid-Based Complement Alternat Med* 2013:340215
- Benevides PJC, Sartorelli P, Kato MJ (1999) Phenylpropanoids and neolignans from *Piper regnellii*. *Phytochemistry* 52:339–343
- Benites J, Gutierrez E, Lopez J, Rojas M, Rojo L, Costa MC, Vinardell MP, Calderon PB (2012) Evaluation of analgesic activities of tremetone derivatives isolated from the Chilean altiplano medicine *Parastrephia lepidophylla*. *Nat Prod Commun* 7:611–614
- Betancourt L, Hume M, Rodríguez F, Nisbet D, Sohail MU, Afanador-Tellez G (2019) Effects of Colombian oregano essential oil (*Lippia organoides* Kunth) and *Eimeria* species on broiler production and cecal microbiota. *Poult Sci* 98:4777–4786
- Betancur-Galvis L, Zapata B, Baena A, Bueno J, Ruíz-Nova CA, Stashenko E, Mesa-Arango AC (2011) Antifungal, cytotoxic and chemical analyses of essential oils of *Lippia organoides* H.B.K grown in Colombia. *Rev Univ Ind Santander Salud* 43:141–148
- Biasi-Garbin RP, Demitto FO, do Amaral RCR, Ferreira MRA, Soares LAL, Svidzinski TIE, Baeza LC, Yamada-Ogatta SF (2016) Antifungal potential of plant species from Brazilian caatinga against dermatophytes. *Rev Inst Med Trop São Paulo* 58:18
- Brako L, Zarucchi JL (1993) Catalogue of the flowering plants and gymnosperms of Peru. Missouri Botanical Garden, Saint Louis, p 1286
- Brambilla LZS, Endo EH, Cortez DAG, Lima MMS, Dias-Filho BP (2018) *Piper regnellii* extract biopolymer-based microparticles: production, characterization and antifungal activity. *J Appl Microbiol* 124:75–84
- Brand YM, Roa-Linares VC, Betancur-Galvis LA, Durán-García DC, Stashenko E (2016) Antiviral activity of Colombian Labiatae and Verbenaceae family essential oils and monoterpenes on Human Herpes viruses. *J Essent Oil Res* 28:130–137
- Brito SMO, Coutinho HDM, Talvani A, Coronel C, Barbosa AGR, Vega C, Figueredo FG, Tintino SR, Lima LF, Boligon AA, Athayde ML, Menezes IRA (2015) Analysis of bioactivities and chemical composition of *Ziziphus joazeiro* Mart. using HPLC-DAD. *Food Chem* 186:185–191
- Buckland D (2017) Analysis - Antimicrobial resistance and the race to find new antibiotics. *Prescriber* January:12–15
- Bueno-Sánchez JG, Martínez-Morales JR, Stashenko EE, Ribón W (2009) Anti-tubercular activity of eleven aromatic and medicinal plants occurring in Colombia. *Biomedica* 29:51–60
- Bussmann RW, Malca-García G, Glenn A, Sharon D, Chait G, Díaz D, Pourmand K, Jonat B, Somogy S, Guardado G, Aguirre C, Chan R, Meyer K, Kuhlman A, Townesmith A, Effio-Carbajal J, Frías-Fernandez F, Benito M (2010) Minimum inhibitory concentrations of medicinal plants used in Northern Peru as antibacterial remedies. *J Ethnopharmacol* 132:101–108
- Butaye P, Argudín MA, Threlfall J (2015) Chapter 1. Introduction to antimicrobial-resistant food-borne pathogens. In: Chen CY, Yan X, Jackson CR (eds) *Antimicrobial resistance and food safety: methods and techniques*. Academic Press, Oxford, pp 1–17
- Cáceres A (1996) Plantas de uso medicinal en Guatemala. Editorial Universitaria, Universidad San Carlos de Guatemala, p 402

- Cáceres M, Hidalgo W, Stashenko E, Torres R, Ortiz C (2020) Essential oils of aromatic plants with antibacterial, anti-biofilm and anti-*quorum* sensing activities against pathogenic bacteria. *Antibiotics* 9:147
- Calle M, Valdivieso G, Vicente JJ (2013) Propagación *in vitro* de T'ola (*Parastrephya lepidophylla* Wedd). *Info INIAF* 1:27–30
- Canales M, Hernández T, Caballero J, Romo de Vivar A, Avila G, Duran A, Lira R (2005) Informant consensus factor and antibacterial activity of the medicinal plants used by the people of San Rafael Coxcatlan, Puebla. México *J Ethnopharmacol* 97:429–439
- Canales M, Hernández T, Rodríguez-Monroy MA, Flores CM, Jiménez-Estrada M, Hernández LB, Durán A, Hernández-Moreno M, Trejo NA, Hernández AB., Ramírez JJ, Orozco J, Eleno MG and Martínez KE (2011) Evaluation of the antimicrobial activity of *Acalypha monostachya* Cav. (Euphorbiales: Euphorbiaceae). *Afr J Pharm Pharmacol* 5:640–647
- Carhuapoma M, López S, Roque M, Velapatiño B, Bell C, Whu D (2009) Actividad antibacteriana del aceite esencial de *Minthostachys mollis* Griseb “Ruyaq Muña”. *Ciencia Inv* 12:83–89
- Carrizo SL, Zampini IC, Sayago JE, Simirgiotis MJ, Bórquez J, Cuello AS, Isla MI (2020) Antifungal activity of phytotherapeutic preparation of *Baccharis* species from Argentine Puna against clinically relevant fungi. *J Ethnopharmacol* 251:112553
- Carvalho PER (2007) Juazeiro - *Ziziphus joazeiro*. Circular Técnica 139. Embrapa Florestas, Brasília, p 8. URL: <http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/313897>
- Castillo-Juárez I, García-Contreras R, Velázquez-Guadarrama N, Soto-Hernández M, Martínez-Vázquez M (2013) *Amphipterygium adstringens* anacardic acid mixture inhibits *quorum* sensing-controlled virulence factors of *Chromobacterium violaceum* and *Pseudomonas aeruginosa*. *Arch Med Res* 44:488–494
- Castillo-Juárez I, González V, Jaime-Aguilar H, Martínez G, Linares E, Bye R, Romero I (2009) Anti-*Helicobacter pylori* activity of plants used in Mexican traditional medicine for gastrointestinal disorders. *J Ethnopharmacol* 122:402–405
- Castillo-Juárez I, Rivero-Cruz F, Celis H, Romero I (2007) Anti-*Helicobacter pylori* activity of anacardic acids from *Amphipterygium adstringens*. *J Ethnopharmacol* 114:72–77
- Castro SBR, Leal CAG, Freire FR, Carvalho DA, Oliveira DF, Figueiredo HCP (2008) Antibacterial activity of plant extracts from Brazil against fish pathogenic bacteria. *Braz J Microbiol* 39:756–760
- CDC – US Centers for Disease Control and Prevention (2013) Antibiotic resistance threats in the United States, 2013. U.S. Department of Health and Human Services, CDC. URL, Atlanta, GA. <https://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf>
- Chassagne F, Samarakoon T, Porras G, Lyles JT, Dettweiler M, Marquez L, Salam AM, Shabih S, Farrokhi DR, Quave CL (2021) A systematic review of plants with antibacterial activities: a taxonomic and phylogenetic perspective. *Front Microbiol* 11:586548
- Chataing B, Rojas L, Usbillaga A, Mora D (2012) Chemical composition and bioactivity on bacteria and fungi of the essential oil from *Lippia origanoides* H.B.K. *J Essent Oil Bear PI* 15:454–460
- Chouhan G, Islamuddin M, Want MY, Ozbak HA, Hemeg HA, Sahal D, Afrin F (2015) Leishmanicidal activity of *Piper nigrum* bioactive fractions is interceded via apoptosis *in vitro* and substantiated by th1 immunostimulatory potential *in vivo*. *Front Microbiol* 6:1368
- Cole ER, dos Santos RB, Lacerda Júnior V, Martins JDL, Greco SJ, Cunha Neto A (2014) Chemical composition of essential oil from ripe fruit of *Schinus terebinthifolius* Raddi and evaluation of its activity against wild strains of hospital origin. *Braz J Microbiol* 45:821–828
- Constantin MB, Sartorelli P, Limberger R, Henriques AT, Steppe M, Ferreira MJP, Ohara MT, Emerenciano VP, Kato MJ (2001) Essential oils from *Piper cernuum* and *Piper regnellii*: antimicrobial activities, analysis by GC/MS and ¹³C-NMR. *Planta Med* 67:771–773
- Corrêa MP (1984) Dicionário das plantas úteis do Brasil e das exóticas cultivadas Vol. 1. Imprensa nacional, Rio de Janeiro, p 5, 687
- Cowan MM (1999) Plant products as antimicrobial agents. *Clin Microbiol Rev* 12:564–582

- Cruz MCS, Santos PO, Barbosa AM Jr, de Mélo DLFM, Alviano CS, Antonioli AR, Alviano DS, Trindade RC (2007) Antifungal activity of Brazilian medicinal plants involved in popular treatment of mycoses. *J Ethnopharmacol* 111:409–412
- Cruz-Carrillo A, Rodríguez N, Rodríguez CE (2010) Evaluación *in vitro* del efecto antibacteriano de los extractos de *Bidens pilosa*, *Lantana camara*, *Schinus molle* y *Silybum marianum*. *Rev UDCA Actual Divulg Cient* 13:117–124
- Cuevas Figueroa XM (2005) A revision of the genus *Amphipterygium* (Julianaceae). *Ibugana Boletín del Instituto de Botánica (CUCBA, Universidad de Guadalajara)* 13:27–47
- da Silva JJ, Cerdeira CD, Chavasco JM, Cintra ABP, da Silva CBP, de Mendonça AN, Ishikawa T, Boriollo MFG, Chavasco JK (2014) *In vitro* screening antibacterial activity of *Bidens pilosa* Linné and *Annona crassiflora* Mart. against oxacillin resistant *Staphylococcus aureus* (ORSA) from the aerial environment at the dental clinic. *Rev Inst Med Trop São Paulo* 56:333–340
- de Almeida AC, Morão RP, Martins ER, da Fonseca FSA, de Souza CN, Prates JPB, de Oliveira FD, da Silva LMV (2016) Atividade antisséptica do óleo essencial de *Lippia origanoides* Cham. (Alecim-pimenta) na presença de leite bovino. *Pesqui Vet Bras* 36:905–911
- de Bessa NGF, Pereira MAB, Ferraz V, Poletto KQ, Chagas Júnior AF, Alves A (2015) Antimicrobial activity and medicinal biomass of *Siparuna guianensis* in Brazilian Cerrado forest, a global hotspot. *J Med Plant Res* 9:968–980
- de Freitas PCD (2000) Atividade antioxidante de espécies medicinais da família Piperaceae: *Pothomorphe umbellata* (L.) Miq. e *Piper regnellii* (Miq.) C. DC. Tese de doutorado. Universidade de São Paulo, São Paulo, p 115
- de Melo DC, Miranda MLD, Ferreira Júnior WG, de Andrade PM, Alcoba AET, Silva TS, Cazal CM, Martins CHG (2017) Antocariogenic and antimycobacterial activities of the essential oil of *Siparuna guianensis* Aublet (Siparunaceae). *Orbital - Electron J Chem* 9:55–60
- de Oliveira MS, da Cruz JN, da Costa WA, Silva SG, Brito MP, de Menezes SAF, Neto AMJC, Andrade EHA, de Carvalho Junior RN (2020) Chemical composition, antimicrobial properties of *Siparuna guianensis* essential oil and a molecular docking and dynamics molecular study of its major chemical constituent. *Molecules* 25:3852
- De Solla Price DJ (1976) A general theory of bibliometric and other cumulative advantage processes. *J Assoc Inf Sci Technol* 27:292–306
- Díaz K, Moromi H (2005) Determinación antibacteriana *in vitro* de *Mentostachys mollis* (Muña) frente a bacterias orales de importancia estomatológica. *Odontol Sanmarquina* 8:3–5
- Do Rego EL (2019) Ethnobotanic study on the use of júa *Ziziphus joazeiro* Mart. (Rhamnaceae) in the Serra Verde - PE community. *Braz J Hea Rev* 2:5801–5810
- Dorman HJD, Deans SG (2000) Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J Appl Microbiol* 88:308–316
- dos Santos AL, Polidoro AS, Schneider JK, da Cunha ME, Saucier C, Jacques RA, Cardoso CAL, Mota JS, Caramão EB (2015a) Comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC/TOFMS) for the analysis of volatile compounds in *Piper regnellii* (Miq.) C. DC. essential oils. *Microchem J* 118:242–251
- dos Santos ATB, Araújo TFS, da Silva LCN, da Silva CB, de Oliveira AFM, Araújo JM, Correia MTS, Lima VLM (2015b) Organic extracts from *Indigofera suffruticosa* leaves have antimicrobial and synergic actions with erythromycin against *Staphylococcus aureus*. *Front Microbiol* 6:13
- dos Santos BS, da Silva LCN, da Silva TD, Rodrigues JFS, Grisotto MAG, Correia MTS, Napoleão TH, da Silva MV, Paiva PMG (2016) Application of omics technologies for evaluation of antibacterial mechanisms of action of plant-derived products. *Front Microbiol* 7:1466
- dos Santos FJB, Lopes JAD, Cito AMGL, de Oliveira EH, de Lima SG, Reis FAM (2004) Composition and biological activity of essential oils from *Lippia origanoides* H.B.K. *J Essent Oil Res* 16:504–506
- Duke JA (2008) *Duke's handbook of medicinal plants of Latin America*. CRC Press, Boca Raton, p 962

- Dwivedi GR, Gupta S, Maurya A, Tripathi S, Sharma A, Darokar MP, Srivastava SK (2015) Synergy potential of indole alkaloids and its derivative against drug-resistant *Escherichia coli*. *Chem Biol Drug Des* 86:1471–1481
- Erazo S, Negrete R, Zaldívar M, Backhouse N, Delporte C, Silva I, Belmonte E, López-Pérez JL, San Feliciano A (2002) Methyl psilate: a new antimicrobial metabolite from *Psila bolivien-sis*. *Planta Med* 68:66–67
- Fischer DCH, Gualda NCA, Bachiega D, Carvalho CS, Lupo FN, Bonotto SV, Alves MO, Yogi A, Di Santi SM, Avila PE, Kirchgatter K, Moreno PRH (2004) *In vitro* screening for antiplas-modial activity of isoquinoline alkaloids from Brazilian plant species. *Acta Trop* 92:261–266
- Freire SE, Urtubey E, Giuliano DA (2007) Epidermal characters of *Baccharis* (Asteraceae) species used in tradicional medicine. *Caldasia* 29:23–38
- Galvis Rueda M, Torres Torres M (2017) Etnobotánica y usos de las plantas de la comunidad rural de Sogamoso, Boyacá. *Colombia Rev Inv Agraria Amb* 8:187–206
- Ganga Rao G, Umamaheswara Rao P, Sambasiva Rao E, Mallikarjuna Rao T, Praneeth VS (2012) Evaluation of *in-vitro* antibacterial activity and anti-inflammatory activity for different extracts of *Rauwolfia tetraphylla* L. root bark. *Asian Pac. J Trop Biomed* 2:818–821
- García-Regalado G (2014) Plantas medicinales de Aguascalientes. Editorial de Universidad Autónoma de Aguascalientes, Aguascalientes, p 498
- Girault L (1987) Kallawayas, curanderos itinerantes de los Andes. Investigación sobre plantas medicinales y mágicas. Bolivia. UNICEF, Quipus, La Paz - Bolivia. 670
- Gómez-Cansino R, Espitia-Pinzón CI, Campos-Lara MG, Guzmán-Gutiérrez SL, Segura-Salinas E, Echeverría-Valencia G, Torras-Clavería L, Cuevas-Figueroa XM, Reyes-Chilpa R (2015) Antimycobacterial and HIV-1 reverse transcriptase activity of Julianaceae and Clusiaceae plant species from Mexico. *Evid-Based Complement Alternat Med* 2015:183036
- González-Alamilla EN, Gonzalez-Cortazar M, Valladares-Carranza B, Rivas-Jacobo MA, Herrera-Corredor CA, Ojeda-Ramírez D, Zaragoza-Bastida A, Rivero-Perez N (2019) Chemical constituents of *Salix babylonica* L and their antibacterial activity against gram-positive and gram-negative animal bacteria. *Molecules* 24:2992
- González-Alamilla E, Rivas-Jacobo M, Sosa-Gutiérrez C, Delgadillo-Ruiz L, Valladares-Carranza B, Rosenfeld-Miranda C, Zaragoza-Bastida A, Rivero-Pérez N (2020) Antibacterial effect of the methanol extract of *Salix babylonica* against important bacteria in public Health. *Abanico Vet* 10:e3
- González Elizondo M, López Enriquez IL, González Elizondo MS, Tena Flóres JA (2004) Plantas medicinales del estado de Durango y zonas aledañas. CIIDIR, Durango, Instituto Politécnico Nacional, México, p 209
- Grande GCC (2019) Polissacarídeos das folhas de *Piper umbellatum* e *Piper regnellii* e ativi-dade anti-inflamatória de *Piper umbellatum*. Dissertação Mestrado em Ciências. Universidade Federal do Paraná, Curitiba, p 86
- Grandtner MM (2005) Elsevier's dictionary of trees, volume 1: North America. Elsevier B.V, Amsterdam, p 1530
- Grandtner MM, Chevrette J (2014) Dictionary of trees, volume 2: South America. In: Nomenclature, taxonomy and ecology (Elsevier's Dictionary of Trees). Academic Press, London, p 1159
- Guimarães EF, Carvalho-Silva M, Monteiro D, Medeiros ES, Queiroz GA (2015) Piperaceae in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. Disponible em: <http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB12835>
- Guimarães ML, da Silva FAG Jr, da Costa MM, de Oliveira HP (2020) Green synthesis of silver nanoparticles using *Ziziphus joazeiro* leaf extract for production of antibacterial agents. *Appl Nanosci* 10:1073–1081
- Gupta MP (1995) 270 plantas medicinales iberoamericanas. CYTED Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo. Subprograma de Química Fina Farmacéutica, Convenio Andrés Bello, Bogotá, p 617
- Gupta PD, Birdi TJ (2017) Development of botanicals to combat antibiotic resistance. *J Ayurveda Integr Med* 8:266–275

- Hauenstein E, Peña-Cortés F, González M, Schlatter R (2005) Nuevos límites para la distribución de *Salix humboldtiana* Willd., Salicaceae, en Chile. *Gayana Bot* 62:46–48
- Henao J, Muñoz LJ, Ríos E, Padilla L, Giraldo GA (2009) Evaluación de la actividad antimicrobiana de los extractos de la planta *Lippia organoides* H.B.K. cultivada en el departamento del Quindío. *Rev Invest Univ Quindío* 19:159–164
- Hernandes C, Pina ES, Taleb-Contini SH, Bertoni BW, Cestari IM, Espanha LG, Varanda EA, Camilo KFB, Martinez EZ, França SC, Pereira AMS (2017) *Lippia organoides* essential oil: an efficient and safe alternative to preserve food, cosmetic and pharmaceutical products. *J Appl Microbiol* 122:900–910
- Hernández T, Canales M, Avila JG, Duran A, Caballero J, Romo de Vivar A, Lira R (2003) Ethnobotany and antibacterial activity of some plants used in traditional medicine of Zapotitlán de las Salinas, Puebla (México). *J Ethnopharmacol* 88:181–188
- IACG - Interagency Coordination Group on Antimicrobial Resistance (2019) Report to the Secretary-General of the United Nations. No time to wait: securing the future from drug-resistant infections. URL: https://www.who.int/antimicrobial-resistance/interagency-coordination-group/IACG_final_report_EN.pdf?ua=1 (Consulted on line: October 2020)
- ITFAMR - Interagency Task Force on Antimicrobial Resistance (1999) A public health action plan to combat antimicrobial resistance. Part 1. Domestic issues. URL: <https://www.cdc.gov/drugresistance/pdf/aractionplan-archived.pdf>
- ITFAMR (2011) A public health action plan to combat antimicrobial resistance. URL: <https://www.cdc.gov/drugresistance/pdf/public-health-action-plan-combat-antimicrobial-resistance.pdf>
- Jacobo-Herrera NJ, Jacobo-Herrera FE, Zentella-Dehesa A, Andrade-Cetto A, Heinrich M, Pérez-Plasencia C (2016) Medicinal plants used in Mexican traditional medicine for the treatment of colorectal cancer. *J Ethnopharmacol* 179:391–402
- Johann S, Cisalpino PS, Watanabe GA, Cota BB, de Siqueira EP, Pizzolatti MG, Zani CL, de Resende MA (2010) Antifungal activity of extracts of some plants used in Brazilian traditional medicine against the pathogenic fungus *Paracoccidioides brasiliensis*. *Pharm Biol* 48:388–396
- Jonas OB, Irwin A, Berthe FCJ, Le Gall FG, Marquez PV (2017) Drug-resistant infections: a threat to our economic future (Vol. 2). Final report (English). HNP/Agriculture Global Antimicrobial Resistance Initiative. World Bank Group, Washington D.C.. <http://documents.worldbank.org/curated/en/323311493396993758/final-report>
- Kachur K, Sutures Z (2020) The antibacterial properties of phenolic isomers, carvacrol and thymol. *Crit Rev Food Sci Nutr* 60:3042–3053
- Kirst HA (2013) Developing new antibacterials through natural product research. *Expert Opin Drug Discovery* 8:479–493
- Koroishi AM, Foss SR, Cortez DAG, Ueda-Nakamura T, Nakamura CV, Dias-Filho BP (2008) *In vitro* antifungal activity of extracts and neolignans from *Piper regnellii* against dermatophytes. *J Ethnopharmacol* 117:270–277
- Krummenauer A, Ponzilacqua B, Zani JL (2019) Atividade antibacteriana de extratos naturais sobre agentes causadores de mastite bovina. *J Biol Pharm Agricult Manag* 15:436–449
- Lastra Valdés HA, Ponce de León Rego H (2001) *Bidens pilosa* Linné. *Rev Cubana Plant Med* 6:28–33
- Lawal OA, Amisu KO, Akinyemi SK, Sanni AA, Simelane MBC, Mosa RA, Opoku AR (2015) *In vitro* antibacterial activity of aqueous extracts of *Bidens pilosa* L. (Asteraceae) from Nigeria. *Brit Microbiol Res J* 8:525–531
- Leal ICR, dos Santos KRN, Itabiana IJ, Antunes OAC, Porzel A, Wessjohann L, Kuster RM (2010) Ceanothane and lupane type triterpenes from *Ziziphus joazeiro* - an anti-staphylococcal evaluation. *Planta Med* 76:47–52
- Lemos COT, Svidzinsk TIE, Baeza LC, Miranda N, Nakamura CV, Cortez DAG, Cardozo-Filho L, Cabral VF (2013) Evaluation of antifungal activity of extracts of *Piper regnellii* obtained by supercritical fluid extraction. *Nat Prod Res* 27:2355–2359

- Lima RB (2015) Rhamnaceae in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. URL: <http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB20675> (consulted on line: September 2020)
- Linhares Neto MV, da Silva RO, de Oliveira FF, Costa LCB, Conceição AO, de Oliveira RA (2018) Avaluation anti-*Candida* of essential oils from three medicinal plants species (Astereaceae). *S Afr J Bot* 115:132–137
- Linden M, Brinckmann C, Feuereisen MM, Schieber A (2020) Effects of structural differences on the antibacterial activity of biflavonoids from fruits of the Brazilian peppertree (*Schinus terebinthifolius* Raddi). *Food Res Int* 133:109134
- Lopez A, Hudson JB, Towers GHN (2001) Antiviral and antimicrobial activities of Colombian medicinal plants. *J Ethnopharmacol* 77:189–196
- Lorenzi H (2000) Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil, 3rd edn. Instituto Plantarum de Estudos da Flora, São Paulo, p 352
- Luize PS, Ueda-Nakamura T, Dias-Filho BP, Cortez DAG, Nakamura CV (2006) Activity of neolignans isolated from *Piper regnellii* (MIQ.) C. DC. var. *pallescens* (C. DC.) Yunck against *Trypanosoma cruzi*. *Biol Pharm Bull* 29:2126–2130
- Macías KL, Juárez BI, Cárdenas NC, Aguirre JR, Jasso Y (2009) Evaluación de plantas tradicionalmente utilizadas en la desinfección de heridas. *Rev Mex Cienc Farmacéuticas* 40:5–10
- Magi G, Marini E, Facinelli B (2015) Antimicrobial activity of essential oils and carvacrol, and synergy of carvacrol and erythromycin, against clinical, erythromycin-resistant Group A *Streptococci*. *Front Microbiol* 6:165
- Manandhar S, Luitel S, Dahal RK (2019) *In vitro* antimicrobial activity of some medicinal plants against human pathogenic bacteria. *J Trop Med* 2019. ID 1895340 (5 p.)
- Marçal FJB, Cortez DAG, Ueda-Nakamura T, Nakamura CV, Dias-Filho BP (2010) Activity of the extracts and neolignans from *Piper regnellii* against methicillin-resistant *Staphylococcus aureus* (MRSA). *Molecules* 15:2060–2069
- Mejia Hernandez P, Salem AZM, Elghandour MMY, Cipriano-Salazar M, Cruz-Lagunas B, Camacho LM (2014) Anthelmintic effects of *Salix babylonica* L. and *Leucaena leucocephala* Lam. extracts in growing lambs. *Trop Anim Health Prod* 46:173–178
- Memar MY, Raei P, Alizadeh N, Akbari Aghdam M, Kafil HS (2017) Carvacrol and thymol: strong antimicrobial agents against resistant isolates. *Rev Med Microbiol* 28:63–68
- Ministerio de la Protección Social (2008) Vademécum colombiano de plantas medicinales. República de Colombia, Imprenta Nacional de Colombia, Bogotá, p 311
- Monroy-Ortiz C, Monroy R (2006) Las plantas compañeras de siempre: la experiencia en Morelos. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. Universidad Autónoma del Estado de Morelos, Corredor Biológico Chichinautzin, Comisión Nacional de Áreas Naturales Protegidas, p 206
- Monte DFM, Tavares AG, Albuquerque AR, Sampaio FC, Oliveira TCRM, Franco OL, Souza EL, Magnani M (2014) Tolerance response of multidrug-resistant *Salmonella enterica* strains to habituation to *Origanum vulgare* L. essential oil. *Front Microbiol* 5:721
- Mora FD, Araque M, Rojas LB, Ramírez R, Silva B, Usubillaga A (2009) Chemical composition and *in vitro* antibacterial activity of the essential oil of *Minthostachys mollis* (Kunth) Griseb Vaught from the Venezuelan Andes. *Nat Prod Commun* 4:997–1000
- Mors WB, Rizini CT, Pereira NA (2000) In: Defilipps RA (ed) Medicinal plants of Brazil. Reference Publications Inc., Rio de Janeiro, p 501
- Mostacero León J, Castillo-Picón F, Mejia FR, Gamarra OA, Charcape JM, Ramírez RA (2011) Plantas medicinales del Perú. Taxonomía, ecogeografía, fenología y etnobotánica. Asamblea Nacional de Rectores, Instituto de Estudios Universitarios “José Antonio Encinas”, Trujillo, p 909
- Mostacero León J, Mejia Coico F, Gamarra Torres O (2002) Taxonomía de las fanegoramas útiles del Peru. Vol. I y. II. Ed. Normas Legales, Trujillo, p 1323
- Moura WS, de Souza SR, Campos FS, Cangussu ASR, Santos EMS, Andrade BS, Gomes CHB, Viana KF, Haddi K, Oliveira EE, Nascimento VL, Aguiar RWS (2020) Antibacterial activity

- of *Siparuna guianensis* essential oil mediated by impairment of membrane permeability and replication of pathogenic bacteria. *Ind Crop Prod* 146:112142
- Mukherjee PK (2019) Quality control and evaluation of herbal drugs: evaluating natural products and traditional medicine. Elsevier, Inc., Oxford, p 760
- Muñoz V, Sauvain V, Bourdy G, Callapa J, Rojas I, Vargas L, Tae A, Deharo E (2000) The search for natural bioactive compounds through a multidisciplinary approach in Bolivia. Part II. Antimalarial activity of some plants used by Mosekene Indians. *J Ethnopharmacol* 69:139–155
- Nascimento GGF, Locatelli J, Freitas PC, Silva GL (2000) Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Braz J Microbiol* 31:247–256
- Navarrete A, Martínez-Uribe LS, Reyes B (1998) Gastroprotective activity of the stem bark of *Amphipterygium adstringens* in rats. *Phytother Res* 12:1–4
- NNIS System (2004) National nosocomial infections surveillance (NNIS) system report, data summary from January 1992 through June 2004, issue October 2004. *Am J Infect Control* 32:470–485
- Noumedem Anangmo CN, Yamssi C, Simeni Njonnou SR, Ngongang Ouankou C, Mounvera AA, Gangueu Djape CD, Nkouayep VR, Yondo J, Tsila HG, Mpoame M (2020) Antimalarial activity of ethyl acetate extract and fraction of *Bidens pilosa* against *Plasmodium berghei* (ANKA). *J Parasitol Res* 2020:8832724
- OECD/WHO/FAO/OIE (2017) Tackling antimicrobial resistance ensuring sustainable R&D. Final note. URL: <http://www.oecd.org/g20/summits/hamburg/Tackling-Antimicrobial-Resistance-Ensuring-Sustainable-RD.pdf>
- Okeke IN, Nicolle LE, Shryock TR (2003) Chapter 5. Factors contributing to the emergence of resistance. In: Knobler SL, Lemon SM, Najafi M, Burroughs T (eds) *The resistance phenomenon in microbes and infectious disease vectors: implications for human health and strategies*. The National Academies Press, Washington, P., pp 130–158
- Oliveira DR, Leitão GG, Bizzo HR, Lopes D, Alviano DS, Leitão SG (2007) Chemical and antimicrobial analyses of essential oil of *Lippia origanoides* H.B.K. *Food Chem* 101:236–240
- Olivera-Ortega AG, Soto Hernández M, Martínez Vázquez M, Terrazas Salgado T, Solares Arenas F (1999) Phytochemical study of cuachalalate (*Amphipterygium adstringens*, Schiede ex Schlecht). *J Ethnopharmacol* 68:109–113
- Olivero-Verbel J, González-Cervera T, Güette-Fernandez J, Jaramillo-Colorado B, Stashenko E (2010) Chemical composition and antioxidant activity of essential oils isolated from Colombian plants. *Rev Bras Farmacogn* 20:568–574
- Pandey AK, Kumar S (2013) Perspective on plant products as antimicrobial agents: a review. *Pharmacol Ther* 4:469–480
- Parish T (2019) Steps to address anti-microbial drug resistance in today's drug discovery. *Expert Opin Drug Discovery* 14:91–94
- Pascual ME, Slowing K, Carretero E, Sánchez Mata D, Villar A (2001) *Lippia*: traditional uses, chemistry and pharmacology: a review. *J Ethnopharmacol* 76:201–214
- Patridge E, Gareiss P, Kinch MS, Hoyer D (2016) An analysis of FDA-approved drugs: natural products and their derivatives. *Drug Discov Today* 21:204–207
- Peña D, Gutiérrez M (2017) Efecto antimicrobiano del aceite esencial de *Minthostachys mollis* sobre microorganismos frecuentes en vías respiratorias bajas. *Rev Cienc Tecnol* 13:55–66
- Pérez García W (2017) Evaluación etnobotánica medicinal de la comunidad de Buenos Aires, Jaén. Universidad Nacional de Cajamarca, Perú, Cajamarca - Perú. Trabajo de grado en Ingeniería Forestal, 122 p
- Pessini GL, Dias-Filho BP, Nakamura CV, Cortez DAG (2003) Antibacterial activity of extracts and neolignans from *Piper regnellii* (Miq.) C. DC. var. *pallescens* (C. DC.) Yunck. *Mem Inst Oswaldo Cruz* 98:1115–1120

- Pessini GL, Dias-Filho BP, Nakamura CV, Ferreira AG, Cortez DAG (2005) Neolignans and the analysis of the essential oil of *Piper regnellii* (Miq.) C. DC. var. *pallescens* (C. DC.) Yunck leaves. *Rev Bras Farmacogn.* 15:199–204
- Phyo AP, Nosten F (2018) Chapter 2. The artemisinin resistance in Southeast Asia: an imminent global threat to malaria elimination. In: Manguin S, Dev V (eds) *Towards malaria elimination - a leap forward*. IntechOpen Limited, London, pp 15–39
- Popova TP, Kaleva MD (2015) Antimicrobial effect *in vitro* of aqueous extracts of leaves and branches of willow (*Salix babylonica* L.). *Int J Curr Microbiol App Sci* 4:146–152
- Prestinaci F, Pezzotti P, Pantosti A (2015) Antimicrobial resistance: a global multifaceted phenomenon. *Pathog Glob Health* 109:309–318
- Quattrocchi U (2012) *CRC World dictionary of medicinal and poisonous plants: common names, scientific names, eponyms, synonyms, and etymology* (5 volume set). CRC Press, Boca Raton, p 4038
- Quave CL (2016) Antibiotics from nature: traditional medicine as a source of new solutions for combating antimicrobial resistance. In: website of AMR control: overcoming global antimicrobial resistance. URL: <http://resistancecontrol.info/rd-innovation/antibiotics-from-nature-traditional-medicine-as-a-source-of-new-solutions-for-combating-antimicrobial-resistance/> (consulted on line: October 2020)
- Quevedo-León Y (2015) *Plantas medicinales: un estudio de campo etnobotánico en la localidad de Ocotepec, Municipio de Cuernavaca. Morelos. Trabajo de grado para Biología, UNAM, México*, 206 p
- Rabe T, van Staden J (1997) Antibacterial activity of South African plants used for medicinal purposes. *J Ethnopharmacol* 56:81–87
- Rai J, Randhawa GK, Kaur M (2013) Recent advances in antibacterial drugs. *Int J Appl Basic Med Res* 3:3–10
- Rangel-López L, Zaragoza-Bastida A, Valladares-Carranza B, Peláez-Acero A, Sosa-Gutiérrez CG, Hetta HF, Batiha GE-S, Alqahtani A, Rivero-Perez N (2020) *In vitro* antibacterial potential of *Salix babylonica* extract against bacteria that affect *Oncorhynchus mykiss* and *Oreochromis* spp. *Animals* 10:1340
- ReAct - Action on Antibiotic Resistance (2012) Burden of antibiotic resistance. URL: <https://www.reactgroup.org/uploads/publications/react-publications/ReAct-facts-burden-of-antibiotic-resistance-May-2012.pdf>
- Rengifo EL (2010) Base de datos de plantas medicinales. Instituto de Investigaciones de la Amazonía Peruana, Iquitos, p 72
- Rengifo EL, Nuñez Pérez C (2015) *Guía práctica de las especies y usos ancestrales: comunidad nativa del grupo Yagua-Primavera. Zona fronteriza Perú-Colombia-Brasil*, Instituto de Investigaciones de la Amazonía Peruana, Iquitos, p 105
- Ribeiro BD, Alviano DS, Barreto DW, Coelho MAZ (2013) Functional properties of saponins from sisal (*Agave sisalana*) and juá (*Ziziphus joazeiro*): critical micellar concentration, antioxidant and antimicrobial activities. *Colloid Surface A* 436:736–743
- Rivero-Cruz BE, Esturau N, Sánchez-Nieto S, Romero I, Castillo-Juárez I, Rivero-Cruz JF (2011) Isolation of the new anacardic acid 6-[16^Z-nonadecenyl]-salicylic acid and evaluation of its antimicrobial activity against *Streptococcus mutans* and *Porphyromonas gingivalis*. *Nat Prod Res* 25:1282–1287
- Rivero-Cruz I, Acevedo L, Guerrero JA, Martínez S, Bye R, Pereda-Miranda R, Franzblau S, Timmermann BN, Mata R (2005) Antimycobacterial agents from selected Mexican medicinal plants. *J Pharm Pharmacol* 57:1117–1126
- Rivero-Perez N, Hernández-Alvarado JL, Valladares-Carranza B, Delgadillo-Ruiz L, Ojeda-Ramírez D, Sosa-Gutiérrez CG, Morales-Ubaldo AL, Vega-Sanchez V, Zaragoza-Bastida A (2019) *Salix babylonica* L. as a natural anticoccidial alternative in growing rabbits. *Evid-Based Complement Alternat Med* 2019:2107231

- Robles-Zepeda RE, Velázquez-Contreras CA, Garibay-Escobar A, Gálvez-Ruiz JC, Ruiz-Bustos E (2011) Antimicrobial activity of Northwestern Mexican plants against *Helicobacter pylori*. *J Med Food* 14:1280–1283
- Rohela GK, Bylla P, Korra R, Reuben C (2016) Phytochemical screening and antimicrobial activity of leaf, stem, root and their callus extracts in *Rauwolfia tetraphylla*. *Int J Agric Biol* 18:521–528
- Rojas JJ, Ochoa VJ, Ocampo SA, Muñoz JF (2006) Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: a possible alternative in the treatment of non-nosocomial infections. *BMC Complement Altern Med* 6:2
- Rojo LE, Benites J, López J, Rojas M, Díaz P, Ordóñez J, Pastene E (2009) Antioxidant capacity and polyphenolic content of twelve traditionally used herbal medicinal infusions from the South American Andes. *Bol Latinoam Caribe Plant Med Aromát* 8:498–508
- Roope LSJ, Smith RD, Pouwels KB, Buchanan J, Abel L, Eibich P, Butler CC, Tan PS, Walker AS, Robotham JV, Wordsworth S (2019) Review - The challenge of antimicrobial resistance: what economics can contribute. *Science* 364:eaau4679
- Rosas-Acevedo H, Terrazas T, González-Trujano ME, Guzmán Y, Soto-Hernández M (2011) Antitumor activity of *Cyrtocarpa procera* analogous to that of *Amphipterygium adstringens*, both assayed on the experimental gastric injury in rats. *J Ethnopharmacol* 134:67–73
- Rosas-Piñón Y, Mejía A, Díaz-Ruiz G, Aguilar MI, Sánchez-Nieto S, Rivero-Cruz JF (2012) Ethnobotanical survey and antibacterial activity of plants used in the Altiplane region of Mexico for the treatment of oral cavity infections. *J Ethnopharmacol* 141:860–865
- Ruiz C, Tunarosa F, Martínez J, Stashenko E (2007) Estudio comparativo por gc-ms de metabolitos secundarios volátiles de dos quimiotipos de *Lippia organoides* H.B.K., obtenidos por diferentes técnicas de extracción. *For Sci Technol* XIII:325–328
- Ruiz-Bustos E, Velazquez C, Garibay-Escobar A, García Z, Plascencia-Jatomea M, Cortez-Rocha MO, Hernández-Martínez J, Robles-Zepeda RE (2009) Antibacterial and antifungal activities of some Mexican medicinal plants. *J Med Food* 12:1398–1402
- Ruschel D (2004) O gênero *Piper* (Piperaceae) no Rio Grande do Sul. Dissertação Mestrado em Botânica. Universidade Federal do Rio Grande do Sul, Porto Alegre, p 84
- Salem AZM, Elghandour MMY, Kholif AE, López S, Pliego AB, Cipriano-Salazar M, Chagoyán JCV, de Oca Jiménez RM, Alonso MU (2017) Tree leaves of *Salix babylonica* extract as a natural anthelmintic for small-ruminant farms in a semiarid region in Mexico. *Agrofor Syst* 91:111–122
- Sánchez ME, De Gracia JN, Quiroga Mendiola M (2015) Guía visual de plantas nativas del Parque Nacional Los Cardones. In: Valles Calchaquies, Salta-Argentina. Salta, La Caldera, 52 p
- Saritha K, Rajesh A, Manjulatha K, Setty OH, Yenugu S (2015) Mechanism of antibacterial action of the alcoholic extracts of *Hemidesmus indicus* (L.) R. Br. ex Schult, *Leucas aspera* (Wild.), *Plumbago zeylanica* L., and *Tridax procumbens* (L.) R. Br. ex Schult. *Front Microbiol* 6:577
- Sarrazin SLF, da Silva LA, de Assunção APF, Oliveira RB, Calao VYP, da Silva R, Stashenko EE, Maia JGS, Mourão RHV (2015) Antimicrobial and seasonal evaluation of the carvacrol-chemotype oil from *Lippia organoides* Kunth. *Molecules* 20:1860–1871
- Savoia D (2012) Plant-derived antimicrobial compounds: alternatives to antibiotics. *Future Microbiol* 7:979–990
- Schmidt-Lebuhn AN (2008) Ethnobotany, biochemistry and pharmacology of *Minthostachys* (Lamiaceae). *J Ethnopharmacol* 118:343–353
- Scodro RBL, Pires CTA, Carrara VS, Lemos COT, Cardoso-Filho L, Souza VA, Corrêa AG, Siqueira VLD, Lonardoní MVC, Cardoso RF, Cortez DAG (2013) Anti-tuberculosis neolignans from *Piper regnellii*. *Phytomedicine* 20:600–604
- Seebaluck R, Gurib-Fakim A, Mahomoodally F (2015) Medicinal plants from the genus *Acalypha* (Euphorbiaceae) - a review of their ethnopharmacology and phytochemistry. *J Ethnopharmacol* 159:137–157

- Shandukani PD, Tshidino SC, Masoko P, Moganedi KM (2018) Antibacterial activity and *in situ* efficacy of *Bidens pilosa* Linn and *Dichrostachys cinerea* Wight et Arn extracts against common diarrhoea-causing waterborne bacteria. *BMC Complement Altern Med* 18:171
- Shariff N, Sudarshana MS, Umeha S, Hariprasad P (2006) Antimicrobial activity of *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracts. *Afr J Biotechnol* 5:946–950
- Sharma A, Flores-Vallejo RC, Cardoso-Taketa A, Villareal ML (2017) Antibacterial activities of medicinal plants used in Mexican traditional medicine. *J Ethnopharmacol* 208:264–329
- Shrestha P, Cooper BS, Coast J, Oppong R, Thuy NDT, Phodha T, Celhay O, Guerin PJ, Wertheim H, Lubell Y (2018) Enumerating the economic cost of antimicrobial resistance per antibiotic consumed to inform the evaluation of interventions affecting their use. *Antimicrob Resist Infect Control* 7:98
- SIBBR - Sistema de Informação sobre a Biodiversidade Brasileira (2020) *Schinus terebinthifolius* in Ficha de SiBBR. URL: https://ferramentas.sibbr.gov.br/ficha/bin/view/especie/schinus_terebinthifolius (consulted on line: October 2020)
- Siddiqui S (2019) Chapter 14. Resistance in pathogenic microorganisms. In: Gupta VK, Pandey A (eds) *New and future developments in microbial biotechnology and bioengineering - microbial secondary metabolites biochemistry and applications*. Elsevier B.V., Cambridge, pp 183–192
- Silva TCL, Almeida CCBR, Veras-Filho J, Peixoto-Sobrinho TJS, Amorim ELC, Costa EP, Araújo JM (2011) Atividades antioxidante e antimicrobiana de *Ziziphus joazeiro* Mart. (Rhamnaceae): avaliação comparativa entre cascas e folhas. *Rev Ciênc Farm Básica Apl* 32:193–199
- Singh G, Passari AK, Singh P, Leo VV, Subbarayan S, Kumar B, Singh BP, Ialhlenmawia H, Kumar NS (2017) Pharmacological potential of *Bidens pilosa* L. and determination of bioactive compounds using UHPLC-QqQ_{LT}-MS/MS and GC/MS. *BMC Complement Altern Med* 17:492
- Solares-Arenas F, Vázquez Alvarado JMP, Gálvez Cortés MC (2012) Canales de comercialización de la corteza de cuachalalate (*Amphipterigium adstringens* Schiede ex Schlecht.) en Mexico. *Rev Mex Cienc Forestales* 3:29–42
- Souza DS, Almeida AC, Andrade VA, Marcelo NA, Azevedo IL, Martins ER, Figueiredo LS (2015) Atividade antimicrobiana do óleo essencial de *Lippia organoides* e *Lippia rotundifolia* frente a enterobactérias isoladas de aves. *Arq Bras Med Vet Zootec* 67:940–944
- Stashenko E, Martínez JR (2020) Chapter 8. Study of essential oils obtained from tropical plants grown in Colombia. In: El-Shemy H (ed) *Essential oils: oils of nature*. IntechOpen Limited, London, p 28
- Stashenko E, Ruiz C, Muñoz A, Castañeda M, Martínez J (2008) Composition and antioxidant activity of essential oils of *Lippia Organoides* H.B.K. grown in Colombia. *Nat Prod Commun* 3:563–566
- Suresh K, Saravana Babu S, Harisaranraj R (2008) Studies on *in vitro* antimicrobial activity of ethanol extract of *Rauvolfia tetraphylla*. *Ethnobot Leaflets* 12:586–590
- Tangarife-Castaño V, Correa-Royero J, Zapata-Londoño B, Durán C, Stashenko E, Mesa-Arango AC (2011) Anti-*Candida albicans* activity, cytotoxicity and interaction with antifungal drugs of essential oils and extracts from aromatic and medicinal plants. *Infection* 15:160–167
- Taylor J, Hafner M, Yerushalmi E, Smith R, Bellasio J, Vardavas R, Bienkowska-Gibbs T, Rubin J (2014) Estimating the economic costs of antimicrobial resistance. Model and results. The Wellcome Trust. RAND Corp., Santa Monica. URL: https://www.rand.org/pubs/research_reports/RR911.html (consulted on line: October 2020)
- Tempone AG, Borborema SET, de Andrade HF Jr, Gualda NCA, Yogi A, Carvalho CS, Bachiega D, Lupo FN, Bonotto SV, Fischer DCH (2005) Antiprotozoal activity of Brazilian plant extracts from isoquinoline alkaloid-producing families. *Phytomedicine* 12:382–390
- Tisserand R, Young R (2014) *Essential oil safety. A guide for health care professionals*. Churchill Livingstone, Elsevier, Edinburgh, p 784
- Tiwari V, Roy R, Tiwari M (2015) Antimicrobial active herbal compounds against *Acinetobacter baumannii* and other pathogens. *Front Microbiol* 6:618

- Torbio MS, Oriani DS, Toso RE, Tortone CA, Fernandez JG (2009) *Staphylococcus aureus* sensible a extractos metanólicos obtenido de plantas nativas de la provincia de La Pampa. Argentina Ciencia Vet 11:14–18
- Torrenegra-Alarcón M, Granados-Conde C, Durán-Lengua M, León-Méndez G, Yáñez-Rueda X, Martínez C, Pájaro-Castro N (2016) The chemical composition and antibacterial activity of essential oil from *Minthostachys mollis*. Orinoquia 20:69–74
- Toso RE, Steibel PE, Troiani HO, Oriani DS, Ardoino SM, Torbilio MS, Boeris MA (2006) Búsqueda sistematizada de plantas con actividad farmacológica utilizando el banco de extractos vegetales de plantas nativas y naturalizadas de la provincia de La Pampa. Ciencia Vet 8:25–31
- Van Baren CM, Di Leo Lira P, Elechosa MA, Molina AM, Juárez MA, Martínez A, Perelman S, Bandoni AL (2014) New insights into the chemical biodiversity of *Minthostachys mollis* in Argentina. Biochem Syst Ecol 57:374–383
- Velasco J, Rojas J, Salazar P, Rodríguez M, Díaz T, Morales A, Rondón M (2007) Antibacterial activity of the essential oil of *Lippia oreganoides* against multiresistant bacterial strains of nosocomial origin. Nat Prod Commun 2:85–88
- Vendrametto MC, dos Santos AO, Nakamura CV, Dias-Filho BP, Cortez DAG, Ueda-Nakamura T (2010) Evaluation of antileishmanial activity of eupomatenoid-5, a compound isolated from leaves of *Piper regnellii* var. *pallescens*. Parasitol Int 59:154–158
- Villagrán C, Romo M, Castro V (2003) Etnobotánica del sur de los andes de la región primera de Chile: un enlace entre las culturas altiplánicas y de las quebradas altas del Loa superior. Chungara (Árica) 35:73–124
- Wahab GA, Sallam A, Elgaml A, Lahloub MF, Afif MS (2018) Antioxidant and antimicrobial activities of *Salix babylonica* extracts. World J Pharm Sci 6:1–6
- Waizel-Bucay J (2010) Plantas y compuestos importantes para la medicina: los sauces, los salicilatos y la aspirina. Rev Fitoter 10:133–145
- WHO (2020a) Antimicrobial resistance. URL: <https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance> (consulted on line: September 2020)
- WHO (2020b) Global database for the tripartite antimicrobial resistance (AMR); country self-assessment survey (TrACSS). URL: <https://amrcountryprogress.org/> (consulted on line: September 2020)
- WHO (2019) New report calls for urgent action to avert antimicrobial resistance crisis. URL: <https://www.who.int/news/item/29-04-2019-new-report-calls-for-urgent-action-to-avert-antimicrobial-resistance-crisis> (consulted on line: September 2020)
- Zampini IC, Cuello S, Alberto MR, Ordoñez RM, D’Almeida R, Solorzano E, Isla MI (2009) Antimicrobial activity of selected plant species from “the Argentine Puna” against sensitive and multi-resistant bacteria. J Ethnopharmacol 124:499–505
- Zapata B, Durán C, Stashenko E, Betancur-Galvis L, Mesa-Arango AC (2009) Antifungal activity, cytotoxicity and composition of essential oils from Labiatae family plants. Rev Univ Ind Santander Salud 41:223–230

Chapter 12

Natural Antifungal Agents Isolated from Argentine Plants. A Summary of Studies Developed in the Period 2000–2020



Gisela Seimandi, Estefanía Butassi, Melina Di Liberto, Estefanía Cordisco, Alan Blanc, Maximiliano Sortino, Laura Svetaz, and Marcos Derita

Abstract Fungal species are able to carry out beneficial actions on industrial processes that directly affect human being wellness. They can also be the cause of severe pathologies both in humans or food products. Fungicidal agents currently used to treat human or plant pathogens have many drawbacks after prolonged or inappropriate use, proving to be inefficient in a short term. Therefore, academics and the pharmaceutical or agrochemical industries are constantly encouraged to search for new chemical entities with antifungal action. In this sense, taking advantage of secondary plant metabolites to find out antifungal molecules could be of high interest. During the last 20 years, we have constituted a group of scientists that broach the subject related to antifungal products obtained from vegetable sources, and we have assayed hundreds of plant species and a considerable number of natural metabolites isolated from them, against the main fungal pathogens that affect humans and crops. The aim of this chapter is to update the plants that have demonstrated the best antifungal action during this period of time and the natural compounds responsible for this. In addition, new strategies like the evaluation of photoactive species and synergism, as well as comparisons with results obtained by other authors reported in the literature, will be discussed.

Keywords Antifungals · Natural products · Human pathogens · Fruit pathogens · Photoactivity · Synergism · Argentine plants

G. Seimandi

ICiAgo Litoral, Universidad Nacional del Litoral-CONICET, Esperanza, Argentina

E. Butassi · M. Di Liberto · E. Cordisco · A. Blanc · M. Sortino · L. Svetaz (✉)

Farmacognosia, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Argentina

M. Derita (✉)

ICiAgo Litoral, Universidad Nacional del Litoral-CONICET, Esperanza, Argentina

Farmacognosia, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Argentina

Abbreviations

1-M-3-(3',4'-DHP)-PC	1-Methyl-3-(3',4'-dihydroxyphenyl)-propyl caffeate
1-M-3-(4'-HP)-PC	1-Methyl-3-(4'-hydroxyphenyl)-propyl caffeate
3,7-DHF	3,7-Dihydroxy flavone
3-H-7,8-DMF	3-Hydroxy-7,8-dimethoxy flavone
7-H-8-MF	7-Hydroxy-8-methoxyflavanone
7-HF	7-Hydroxy flavanone
AIDS	Acquired immune deficiency syndrome
AMB	Amphotericin B
APDT	Antimicrobial photo dynamic therapy
BBT	2,2':5',2''-Terthienyl (α -T); 5-(3-buten-1-ynyl)-2,2'-bithiophene
BBTOAc	5-(4-Acetoxy-1-butynyl)-2,2'-bithiophene
BBTOH	5-(4-Hydroxy-1-butynyl)-2,2'-bithiophene
CFU	Colony forming units
DCM	DiChloroMethane
DHC	2',4'-Dihydroxychalcone
DHMC	2',4'-Dihydroxy-3'-methoxychalcone
EtOH	Ethanol
FCZ	Fluconazole
HTSS	High-throughput synergy screening
ITZ	Itraconazole
MeOH	Methanolic
MFC	Minimal fungicidal concentration
MIC	Minimal inhibitory concentration
PBT	5-(3-Penten-1-ynyl)-2,2'-bithiophene
PCM	Paracoccidioidomycosis
PDT	Photo dynamic therapy
PhytB	Phytolaccoside B (3-O- β -D-xylopiranosylphytolaccagenin)
PhytE	Phytolaccoside E (3-O- β -D-glucopyranosyl-(1,4)- β -D-xylopiranosyl-phytolaccagenin)
PhytF	Phytolaccoside F [3-O- α -L-rhamnopyranosyl-(1,2)- β -D-glucopyranosyl-(1,2)- β -D-xylopiranosyl-phytolaccagenic acid]
PhytG	Phytolaccagenin
PSs	Photosensitizer
<i>PtAqEb</i>	<i>Phytolacca tetramera</i> aqueous extract from berries
<i>PtAqEl</i>	<i>Phytolacca tetramera</i> aqueous extract from leaves
<i>PtAqEr</i>	<i>Phytolacca tetramera</i> aqueous extract from roots
<i>PtBEb</i>	<i>Phytolacca tetramera</i> butanoic extract from berries
<i>PtBEl</i>	<i>Phytolacca tetramera</i> butanoic extract from leaves
<i>PtBEr</i>	<i>Phytolacca tetramera</i> butanoic extract from roots

PtDEb	<i>Phytolacca tetramera</i>	dichloromethane	extract
	from berries		
PtDEl	<i>Phytolacca tetramera</i>	dichloromethane	extract
	from leaves		
PtDEr	<i>Phytolacca tetramera</i>	dichloromethane	extract from roots
PtMEb	<i>Phytolacca tetramera</i>	methanolic	extract from berries
PtMEl	<i>Phytolacca tetramera</i>	methanolic	extract from leaves
PtMEr	<i>Phytolacca tetramera</i>	methanolic	extract from roots
ROS	Reactive oxygen species		
TLC	Thin layer chromatography		
UHPLC-ESI-MS	Ultra-high performance liquid chromatography-electrospray ionization mass spectrometry		
UVA	Ultraviolet A radiation		

1 Introduction

Fungal diseases are life-threatening to human health and cause an important burden around the world and are associated with high numbers of morbidity and mortality. Moreover, food contaminations due to different fungal infections are frequently the cause of great economic losses in horticultural businesses and economy. However, the same fungi which may exhibit great pathogenicity could also be used by the industry for different applications:

Candida albicans and *C. glabrata* are species of yeasts which cause candidiasis, a recurrent disease that is produced in humid and warmer environments of the human body such as the oral or vaginal cavity, skin folds, oropharyngeal, and bronchial secretions (Bonifácio et al. 2019). Moreover, they could cause dangerous opportunistic fungal infections on immunocompromised hosts and candidemia in adults, mainly in patients with hematological disorders (Butassi et al. 2015). On the other hand, the production of lipids (Enshaeieh et al. 2013), butyl oleate, and ethyl oleate catalyzed by *Candida* spp. (Chen et al. 2018) is used in the industry as a source for biodiesel production. Moreover, these yeasts may be harnessed for producing biosurfactants (Nwaguma et al. 2019). *Saccharomyces cerevisiae* is another yeast that commonly colonizes mucosa and can cause superficial or invasive visceral infections associated with immunosuppressed individuals (Davicino et al. 2007). However, some studies described the biocontrol capacity of *S. cerevisiae* against other phytopathogenic fungi of fruits such as *Botrytis* spp. (Nally et al. 2012). Another study demonstrated that a strain of this fungus could synthesize the bioactive peptide pediocin PA-1 that showed its antibacterial activity against two Gram-negative strains such as *Shigella boydii* and *S. flexneri* (Nguyen et al. 2020). These systems have been regarded as safe because they are suitable for bioindustrial processes applied to food. *Cryptococcus* spp. cause important systemic mycosis globally, and their most common clinical manifestation is cryptococcal meningitis, principally in patients suffering from AIDS (Lima et al. 2016). But this fungal genus

produces useful enzymes like α -amylase, lipase, polygalacturonase, and xylanase which could be used for the treatment of polymer waste or wastewater (Basak and Das 2014; Thirunavukarasu et al. 2016).

Microsporium gypseum causes skin diseases in humans and animals such as the disseminated and recalcitrant tinea corporis eruption, particularly in immunocompromised patients (Singh 2011). In animals, it produces circular and patchy alopecia, scales, follicular papules, erythema, hyperpigmentation, and pruritus. On the other hand, some studies concluded that the application of keratinolytic microbes, such as fungi (including *M. gypseum*), could be useful for the treatment of keratinaceous wastes (Ghaffar et al. 2018). *Trichophyton* spp. are the most common dermatophytic fungi and the causal agents of fungal nail infections (onychomycosis), a very common disorder in industrialized nations, and it represents a risk for diabetics or patients with keratinization problems (Nenoff et al. 2013). These fungi affect the *stratum corneum* of the epidermis and the nail keratin, but they are not common in the hair roots. Like *M. gypseum*, the keratinases produced by *Trichophyton* spp. may actively degrade the chicken feather waste (Anbu et al. 2008). *Epidermophyton floccosum* constitutes the causal agent of many skin diseases and may lead to recurrent outbreaks of dermatophytosis in hospitals or health institutions due to its persistence in the environment (Svetaz et al. 2010). Frequently, it causes tinea cruris, tinea pedis, tinea corporis, and onychomycosis (Lacaz et al. 2002).

Paracoccidioides brasiliensis is the etiological agent of the disease paracoccidioidomycosis (PCM), the most important systemic mycosis in Latin America (do Prado et al. 2018). The isolates of this fungus are highly virulent, since it is capable of adapting to transient iron availability as strategies to survive and overcome stress conditions inside the host (do Amaral et al. 2019). *Aspergillus* spp. are airborne pathogenic fungi which may cause skin and respiratory infections which could be lethal in immunocompromised patients (Davicino et al. 2007). However, these fungi are able to produce lipases with high industrial potential (Contesini et al. 2010). Many pharmaceutical or agrochemical products have been obtained from them as well as aromatic compounds. Their capacity for effluent biodegradation and bioadsorption of the toxic metals Cd and Cr is well known (Ahmad et al. 2005).

Fusarium spp. cause pernicious infections on humans and crops but also offer some industrial applications. They could cause some opportunistic infections in humans, mostly in immunocompromised patients, such as keratitis and onychomycosis (Salah et al. 2015). Moreover, Torres et al. (2009) suggested that fumonisins (a type of toxins produced by this fungal genus) increase the incidence of esophageal cancer. Particularly, *F. verticillioides* cause root and stem rots in cereals that are cultivated in subtropical and temperate regions, and their mycotoxins are harmful to human and animal health causing leucoencephalomalacia in equines, pulmonary edema in porks, and immunosuppression in poultry (Sampietro et al. 2014). *F. oxysporum* cause accelerated wilting in banana (Forsyth et al. 2006) and tomato (Duyvesteijn et al. 2005), since it can penetrate the roots and colonize vascular tissues leading to the disruption of water translocation towards the shoots. On the other hand, lipase enzymes obtained from *F. oxysporum* have shown several

properties with significant industrial applications, such as an additive for detergents formulations (Prazeres et al. 2006). *Rhizopus* spp. are opportunist fungi which may cause different pathogenicity in humans (mucormycosis, an invasive fungal infection with high morbidity and mortality principally in immunocompromised patients) (Teal et al. 2016), animals, and vegetables (soft rot, a disease that produces great economic losses in fleshy fruits) (Pergomet et al. 2018). Species of this fungal genera are considered good bioremediators, as they can bioadsorb high concentrations of heavy metals such as Cd and Cr (Ahmad et al. 2005). Additionally, these fungi offer capacity to produce pectinolytic enzymes which have wide applications in fruit juice and wine industries (Anisa and Girish 2014). *Mucor* spp. principally cause chronic cutaneous and subcutaneous infections, but they can also produce rhinocerebral and sinopulmonary infections (frequently lethal), blood and intestinal infections, as well as septic arthritis (Morin-Sardin et al. 2017). Some *Mucor* species have been used for metabolites production or biotransformations, for biodiesel production and in the food industry as starters of cheese fermentations (Morin-Sardin et al. 2017).

During the last decades, most pathogens have developed resistance towards synthetic fungicides, and consequently, the search for new alternatives applied to the treatment of fungal diseases is urgently required (Di Liberto et al. 2017; Carrizo et al. 2020). Antifungals show different targets over the fungal cells and among them we could mention: cell wall (inhibition of β -glucan or chitin synthesis); cell membrane (binding to ergosterol or inhibition of its synthesis, e.g. phenols disrupt the cytoplasmic membrane and cause the cells leakage); inhibition of cell division (by cutting of microtubule polymerization); inhibition of RNA/DNA (by causing deficient RNA synthesis or inhibition of DNA transcription); and the inhibition of efflux pumps which function by transporting toxic substances out of the cell (inhibiting the efflux pumps, drug resistance may be reduced) (Alanís Garza 2005; Freiesleben and Jäger 2014).

Natural products are the best known reservoir of chemicals. Plants have developed different active principles for defense towards the fungal attack, and their content depends on different factors, mainly climatic conditions (Acosta de la luz 2003; Petenatti et al. 2008). The simple phenolic compounds, such as hydroxybenzoics, monophenols (e.g., cresol), diphenols (e.g., hydroquinones), and triphenols (e.g., gallic acid), are the more common secondary metabolites displaying fungicide properties (Lizcano González 2007; Martinez 2012). Moreover, compounds, such as phytoalexins, steroidal saponins, alkaloids, and some proteins, have also been depicted to have an important function in the defense systems of plants against pathogens (Montez-Belmont et al. 2000; Alanís Garza 2005; Lizcano González 2007). The secondary metabolites offer different actions against pathogens and they could be classified in three main groups according to their biosynthetic pathway (Lizcano González 2007; Freiesleben and Jäger 2014): (1) **phenolic compounds** such as coumarins and phytoalexins which are incorporated into the plant cell wall to increase rigidity and prevent the entry of pathogens; (2) **terpenoids** such as mono-, di-, or tri-terpenes, saponins, steroids, cardiac glycosides, and sterols which may act disrupting the cell membrane and directly eliminating the pathogenic

organism in order to restrain its invasion towards the rest of the plant; (3) **nitrogen-containing compounds** such as alkaloids and lectins which also eliminate the pathogenic organism and restrain its invasion through the attack to the cell membrane and cell wall. Other compounds such as phytoalexins, substances with low molecular weight and whose biosynthesis is induced by pathogens or herbivores have demonstrated a biostatic or biocidal effect at relatively low concentrations (Reichling 2018).

2 Summary of Argentinean Antifungal Plants Evaluated against Human Pathogens

Secondary metabolites are mainly obtained by distillation or extraction of aromatic or non-aromatic plant species, respectively. Plant extracts constitute a group of substances, with diverse chemical characteristics, extracted from different parts of plants such as roots, barks, seeds, buds, leaves, and fruits (Martinez 2012). On the other hand, essential oils are highly volatile substances (lipophilic molecules) synthesized and stored in glandular trichomes, which are capable to disrupt the fungal cell wall or membrane through a permeabilization process and also may inhibit the synthesis of fungal DNA, RNA, proteins, and polysaccharides (Martinez 2012; Karpiński 2020). Many researchers all over the world have investigated the properties and capacity of some plants against fungal pathogens. Table 12.1 summarizes the native or naturalized plants of Argentina which showed antifungal activities against different fungal species throughout the last 20 years (2000 to 2020).

2.1 *Zuccagnia punctata*: A Treasure Used in Traditional Medicine: Validation of its Antifungal Properties and Isolation of Bioactive Compounds

Zuccagnia punctata Cav. (Fabaceae, Caesalpinioideae) is the only representative species of this genus that is endemic of central and western arid and semi-arid regions of Argentina (Fig. 12.1). It is an aromatic shrub of 1–5 m in height which grows on hills and plains between 700–2700 m.a.s.l. It is commonly known as “jarilla macho,” “jarilla de la puna,” “jarilla pispito,” “laca,” or “pus-pus” and has a certain resemblance to the true “jarillas,” a species of the genus *Larrea* (Zygophyllaceae), with which it coexists (Vattuone et al. 2013). *Z. punctata* has a long history of use in the traditional indigenous medicine of Argentina. Infusions and decoctions in water, as well as extracts prepared by maceration in ethanol of its aerial parts, have been widely used as foot antiseptic, rubefacient, antibacterial, antifungal, anti-inflammatory, antitumor, asthma, arthritis, rheumatism, among others (Ortega et al. 2000; Vattuone et al. 2013). To date, there have been numerous

Table 12.1 Native or naturalized plants of Argentina with antifungal activities against human pathogenic fungi. Part used of each species evaluated and references are also depicted

Species	Parts used (extract types)	Reported antifungal activities	References
Aquifoliaceae			
<i>Ilex paraguariensis</i> A.St.-Hil.	Leaves (aqueous extracts)	<i>Malassezia furfur</i> (cause catheter-related systemic infections in humans and animals, dandruff, psoriasis and folliculitis)	(Filip et al. 2010)
Amaranthaceae			
<i>Amaranthus spinosus</i> L.	Aerial parts (hexane and MeOH extracts)	<i>Fusarium</i> sp.	(Thembo et al. 2010)
<i>Amaranthus viridis</i> L.	Whole plant (lectines)	<i>F. oxysporum</i>	(Kaur et al. 2006)
Anacardiaceae			
<i>Astronium urundeuva</i> Engl.	Aerial parts (saline and hydroethanolic extract)	<i>C. albicans</i> and <i>Fusarium</i> sp.	(Sa et al. 2009; Bonifácio et al. 2019)
<i>Lithraea molleoides</i> (Vell.) Engl.	Aerial parts (MeOH extracts)	Dermatophytic strains	(Muschiatti et al. 2005)
<i>Myracrodruon urundeuva</i> Allemão	Whole plant (EtOH extracts)	<i>C. albicans</i> , <i>C. neoformans</i> and <i>C. gattii</i>	(dos Santos Silva et al. 2020)
<i>Schinopsis balansae</i> Engl.	Leaves (urushiol fraction)	<i>F. graminearum</i>	(Aristimuño Ficooseco et al. 2017)
<i>Schinus areira</i> L.	Aerial parts (EtOH extracts)	<i>M. gypseum</i> , <i>T. rubrum</i> , <i>T. mentagrophytes</i> and <i>E. floccosum</i>	(Svetaz et al. 2010)
<i>Schinus molle</i> L.	Whole plant (essential oils)	<i>Fusarium</i> sp. and <i>P. brasiliensis</i>	(Sampietro et al. 2014; Prado et al. 2018)
Apiaceae			
<i>Gymnophyton polycephalum</i> Clos	Aerial parts (essential oils)	Treating dermatophyte infections	(Lima et al. 2011)
Araucariaceae			
<i>Araucaria araucana</i> (Molina) K.Koch	Wood (MeOH extracts)	<i>T. mentagrophytes</i> and <i>Fusarium</i> sp.	(Céspedes et al. 2006)
Aristolochiaceae			
<i>Aristolochia argentina</i> Griseb.	Aerial parts (EtOH extracts)	<i>F. verticillioides</i>	(Carpinella et al. 2010)
Asteraceae			
<i>Acanthostyles buniifolius</i> (hook. Ex hook. & Arn.) R.M.King & H.rob.	Aerial parts (MeOH extracts)	<i>M. gypseum</i> , <i>T. mentagrophytes</i> and <i>T. rubrum</i>	(Muschiatti et al. 2005)

(continued)

Table 12.1 (continued)

Species	Parts used (extract types)	Reported antifungal activities	References
<i>Baccharis artemisioides</i> hook. & Arn.	Aerial parts (EtOH extracts)	<i>F. verticillioides</i>	(Carpinella et al. 2010)
<i>Baccharis boliviensis</i> (Wedd.) Cabrera	Aerial parts (hydroalcoholic preparations)	Treating dermatophyte infections	(Carrizo et al. 2020)
<i>Baccharis darwinii</i> hook. & Arn.	Aerial parts (petroleum ether and MeOH extract)	<i>C. neoformans</i> , <i>M. gypseum</i> , <i>T. rubrum</i> and <i>T. mentagrophytes</i>	(Kurdelas et al. 2010)
<i>Baccharis grisebachii</i> Hieron.	Aerial parts (hexane and CH ₂ Cl ₂ extracts)	<i>Microsporum canis</i>	(Feresín et al. 2001)
<i>Baccharis inamoena</i> Gardner	Aerial parts (essential oils)	<i>T. rubrum</i>	(Sobrinho et al. 2016)
<i>Baccharis salicina</i> Torr. & A.Gray	Aerial parts (EtOH extracts)	<i>F. verticillioides</i>	(Carpinella et al. 2010)
<i>Baccharis spartioides</i> (hook. & Arn. Ex DC.) J.Rémy	Aerial parts (essential oils)	<i>C. albicans</i>	(Demo et al. 2005)
<i>Baccharis trimera</i> (less.) DC.	Aerial parts and seeds (decocción; essential oils)	<i>Saccharomyces cerevisiae</i> , <i>C. albicans</i> , <i>Trichophyton</i> sp. and <i>Microsporum</i> sp.	(Davicino et al. 2007; Caneschi et al. 2015)
<i>Chromolaena laevigata</i> (lam.) R.M.King & H.rob.	Aerial parts (essential oils)	<i>T. rubrum</i> , <i>T. mentagrophytes</i> and <i>C. albicans</i>	(Murakami et al. 2013; Valarezo et al. 2016)
<i>Flourensia oolepis</i> S.F.Blake	Aerial parts (EtOH extracts)	<i>F. verticillioides</i>	(Carpinella et al. 2010)
<i>Gaillardia megapotamica</i> (Spreng.) baker	Aerial parts (EtOH extracts)	<i>F. verticillioides</i>	(Carpinella et al. 2010)
<i>Gochnatia glutinosa</i> (D.Don) D.Don ex hook. & Arn.	Aerial parts (MeOH extracts)	Dermatophytes fungi	(Postigo et al. 2012)
<i>Heterothalamus alienus</i> (Spreng.) Kuntze	Aerial parts and roots (EtOH extracts)	<i>F. verticillioides</i> , <i>T. rubrum</i> and <i>T. mentagrophytes</i>	(Pacciaroni et al. 2008; Carpinella et al. 2010)
<i>Mikania periplocifolia</i> hook. & Arn.	Aerial parts (EtOH extracts)	<i>M. gypseum</i> , <i>T. rubrum</i> , <i>T. mentagrophytes</i> and <i>E. floccosum</i>	(Svetaz et al. 2010)
<i>Parastrephia quadrangularis</i> (Meyen) Cabrera	Aerial parts (MeOH extracts)	<i>F. verticillioides</i>	(Di Ciaccio et al. 2018)
<i>Parthenium hysterophorus</i> L.	Leaves (MeOH, EtOH and EtOAc extracts)	<i>C. albicans</i>	(Malarkodi and Manoharan 2013)

(continued)

Table 12.1 (continued)

Species	Parts used (extract types)	Reported antifungal activities	References
<i>Pluchea dodonaeifolia</i> (hook. & Arn.) H.rob. & Cuatrec.	Aerial parts (two flavanones: Naringenin and pinocembrin)	<i>C. albicans</i>	(Soberón et al. 2020)
<i>Porophyllum obscurum</i> (Spreng.) DC.	Aerial parts (hexane extracts)	<i>Candida</i> sp. (treatment of oropharyngeal candidiasis)	(Postigo et al. 2017)
<i>Pseudognaphalium gaudichaudianum</i> (DC.) Anderb.	Whole plant (EtOH/MeOH extracts and decoction)	<i>S. cerevisiae</i> , <i>Sporothrix schenckii</i> and <i>Fonsecaea pedrosoi</i>	(Davicino et al. 2007; Gaitán et al. 2011)
<i>Pterocaulon alopecuroides</i> (lam.) DC.	Aerial parts (crude MeOH/CH ₂ Cl ₂ /hexane extracts)	<i>S. cerevisiae</i> , <i>C. neoformans</i> , <i>M. gypseum</i> , <i>T. rubrum</i> and <i>T. mentagrophytes</i> (high activity); <i>C. albicans</i> , <i>C. tropicalis</i> , <i>A. flavus</i> , <i>A. niger</i> and <i>A. fumigatus</i> (moderate activity)	(Stein et al. 2005)
<i>Pterocaulon balansae</i> Chodat	Aerial parts (crude MeOH/CH ₂ Cl ₂ /hexane extracts)	<i>C. tropicalis</i> , <i>S. cerevisiae</i> , <i>C. neoformans</i> , <i>M. gypseum</i> , <i>T. rubrum</i> and <i>T. mentagrophytes</i> (high activity); <i>C. albicans</i> , <i>A. flavus</i> , <i>A. niger</i> and <i>A. fumigatus</i> (moderate activity)	(Stein et al. 2005)
<i>Pterocaulon polystachyum</i> DC.	Aerial parts (crude MeOH/CH ₂ Cl ₂ /hexane extracts)	<i>S. schenckii</i> , <i>M. gypseum</i> , <i>T. rubrum</i> and <i>T. mentagrophytes</i> (high activity); <i>C. albicans</i> , <i>C. tropicalis</i> , <i>S. cerevisiae</i> , <i>C. neoformans</i> , <i>A. flavus</i> , <i>A. niger</i> and <i>A. fumigatus</i> (moderate activity)	(Stein et al. 2005; Stopiglia et al. 2011)
<i>Senecio grisebachii</i> baker	Flowers (CH ₂ Cl ₂ /EtOH and aqueous extracts)	<i>M. gypseum</i> and <i>T. mentagrophytes</i>	(Portillo et al. 2001).
<i>Senecio nutans</i> Sch. Bip.	Aerial parts (essential oils)	<i>Fusarium</i> sp. (moderate activity)	(Galvez et al. 2020)
<i>Senecio subpanduratus</i> O.Hoffm.	Aerial parts (essential oils)	<i>C. albicans</i> , <i>C. guillermoidii</i> , <i>C. krusei</i> and <i>C. glabrata</i>	(Arancibia et al. 2010)
<i>Senecio viridis</i> Phil.	Aerial parts (essential oils)	<i>Fusarium</i> sp. (moderate activity)	(Galvez et al. 2020)
<i>Solidago chilensis</i> Meyen	Leaves (essential oils)	<i>M. gypseum</i> , <i>T. mentagrophytes</i> and <i>C. albicans</i>	(Alonso and Desmarchelier 2015)
<i>Tagetes minuta</i> L.	Roots (hexane and CH ₂ Cl ₂ extracts)	Treatment of skin mycoses and <i>Candida</i> virulence	(Giacone et al. 2020)

(continued)

Table 12.1 (continued)

Species	Parts used (extract types)	Reported antifungal activities	References
<i>Tagetes terniflora</i> Kunth	Aerial parts (essential oils)	<i>Fusarium</i> sp. (moderate activity)	(Galvez et al. 2020)
<i>Tricholine reptans</i> (Wedd.) Hieron.	Aerial parts (EtOH extracts)	<i>F. verticillioides</i>	(Carpinella et al. 2010)
<i>Vernonanthura nudiflora</i> (less.) H.rob.	Aerial parts (EtOH extracts)	<i>F. verticillioides</i>	(Carpinella et al. 2010)
Berberidaceae			
<i>Berberis microphylla</i> G.Forst.	Leaves and stems (aqueous extracts)	<i>C. albicans</i> and dermatophyte fungi	(Freile et al. 2006)
Bignoniaceae			
<i>Amphilophium cynanchoides</i> (DC.) L.G.Lohmann	Leaves and stems (EtOAc extracts)	<i>A. niger</i>	(Apud et al. 2020)
<i>Dolichandra cynanchoides</i> Cham.	Leaves and stems (CH ₂ Cl ₂ extracts)	<i>A. niger</i> and <i>A. carbonarius</i>	(Apud et al. 2020)
<i>Tecoma stans</i> (L.) Juss. Ex Kunth	Bark (EtOH extracts)	<i>F. pedrosoi</i>	(Gaitán et al. 2011)
Boraginaceae			
<i>Cordia curassavica</i> (Jacq.) Roem. & Schult.	Leaves (essential oil)	<i>C. albicans</i> and <i>C. krusei</i>	(Rodrigues et al. 2012)
Caryophyllaceae			
<i>Stellaria media</i> (L.) Vill.	Whole plant (EtOH extracts)	For psoriasis treatment	(Ríos et al. 2018)
Combretaceae			
<i>Terminalia australis</i> Cambess.	Aerial parts (EtOH extracts)	<i>M. gypseum</i> , <i>T. rubrum</i> , <i>T. mentagrophytes</i> and <i>E. floccosum</i>	(Svetaz et al. 2010)
<i>Terminalia triflora</i> (Griseb.) Lillo	Aerial parts (MeOH/EtOH extracts)	<i>M. gypseum</i> , <i>T. mentagrophytes</i> , <i>T. rubrum</i> , <i>S. schenckii</i> and <i>F. pedrosoi</i>	(Muschiatti et al. 2005; Gaitán et al. 2011)
Commelinaceae			
<i>Commelina diffusa</i> Burm.f.	Aerial parts (MeOH extracts)	<i>Trichophyton</i> sp.	(Mensah et al. 2006)
Cyperaceae			
<i>Cyperus rotundus</i> L.	Tuber (aqueous and petroleum ether extracts)	<i>Aspergillus fumigatus</i> and <i>Candida tropicalis</i>	(Biradar et al. 2010)
Euphorbiaceae			
<i>Croton urucurana</i> Baill.	Blood from bark (presence of catechins)	<i>Trichophyton</i> genus	(Gurgel et al. 2005)
<i>Sebastiania brasiliensis</i> Spreng.	Aerial parts (MeOH extracts)	<i>T. rubrum</i> , <i>T. mentagrophytes</i> , <i>E. floccosum</i> and <i>M. canis</i>	(Muschiatti et al. 2005)

(continued)

Table 12.1 (continued)

Species	Parts used (extract types)	Reported antifungal activities	References
<i>Sebastiania commersoniana</i> (Baill.) L.B.Sm. & downs	Aerial parts (MeOH extracts)	<i>T. rubrum</i> , <i>T. mentagrophytes</i> , <i>E. floccosum</i> and <i>M. canis</i>	(Muschiatti et al. 2005)
Fabaceae			
<i>Acacia caven</i> (Molina) Molina	Aerial parts (crude extracts)	<i>F. oxysporum</i>	(Quiroga et al. 2001)
<i>Albizia inundata</i> (Mart.) Barneby & J.W.Grimes	Aerial parts (MeOH extracts)	<i>C. krusei</i>	(Tempone et al. 2008)
<i>Anadenanthera colubrina</i> var. <i>cebil</i> (Griseb.) Altschul	Whole plant (EtOH extracts)	<i>C. albicans</i> , <i>C. neoformans</i> and <i>C. gattii</i>	(dos Santos Silva et al. 2020)
<i>Astragalus pehuenches</i> Niederl.	Stem (EtOH extracts)	<i>S. schenckii</i> and <i>F. pedrosoi</i>	(Gaitán et al. 2011)
<i>Dalea elegans</i> hook. & Arn.	Aerial parts (EtOH extracts)	<i>F. verticillioides</i>	(Carpinella et al. 2010)
<i>Geoffroea decorticans</i> (hook. & Arn.) Burkart	Leaves and twigs (EtOH extracts)	<i>Aspergillus</i> sp.	(Quiroga et al. 2009)
<i>Peltophorum dubium</i> (Spreng.) Taub.	Aerial parts (MeOH extracts)	<i>A. flavus</i>	(Di Ciaccio et al. 2020)
<i>Prosopis ruscifolia</i> Griseb.	Aerial parts (MeOH extracts)	<i>Aspergillus parasiticus</i> and <i>A. flavus</i> .	(Gomez et al. 2019)
<i>Pterogyne nitens</i> Tul.	Leaves (flavonoids)	<i>Epidermophyton</i> , <i>Trichophyton</i> , <i>Cryptococcus</i> , and <i>Candida</i> species	(Lima et al. 2016)
<i>Senna spectabilis</i> (DC.) H.S.Irwin & Barneby	Fruits (MeOH extracts)	<i>F. verticillioides</i>	(di Ciaccio et al. 2018)
<i>Zuccagnia punctata</i> Cav.	Aerial parts (MeOH/EtOH extracts)	<i>Candida</i> sp., <i>C. neoformans</i> , <i>S. cerevisiae</i> , <i>Aspergillus</i> sp., <i>M. gypseum</i> , <i>E. floccosum</i> , <i>S. schenckii</i> and <i>Trichophyton</i> sp.	(Svetaz et al. 2010; Gaitán et al. 2011; Butassi et al. 2015)
Gentianaceae			
<i>Gentianella multicaulis</i> (Gillies ex Griseb.) Fabris	Aerial parts	<i>M. gypseum</i> , <i>T. mentagrophytes</i> and <i>T. rubrum</i>	(Lima et al. 2012)
Iridaceae			
<i>Eleutherine bulbosa</i> (mill.) Urb.	Bulbs (EtOH/ BuOH extracts)	<i>Trichophyton</i> , <i>Trichosporon</i> , <i>Aspergillus</i> and <i>Rhizopus</i> species	(Mohanta et al. 2014)
Lamiaceae			
<i>Clinopodium gilliesii</i> (Benth.) Kuntze	Aerial parts (essential oils)	Treating dermatophyte infections	(Lima et al. 2011)

(continued)

Table 12.1 (continued)

Species	Parts used (extract types)	Reported antifungal activities	References
<i>Glechon spathulata</i> Benth.	Aerial parts (essential oils)	<i>T. rubrum</i> and <i>E. floccosum</i>	(Venturi et al. 2015)
<i>Hedeoma multiflora</i> Benth.	Leaves and stems (essential oils)	<i>A. flavus</i> and <i>A. parasiticus</i>	(Fiuza et al. 2009)
<i>Hyptis mutabilis</i> (rich.) Briq.	Leaves (essential oils)	<i>Mucor</i> spp.	(Oliva et al. 2006)
<i>Lepechinia floribunda</i> (Benth.) Epling	Aerial parts (EtOH extracts)	<i>F. verticillioides</i>	(Carpinella et al. 2010)
<i>Ocimum campechianum</i> mill.	Leaves (essential oil)	<i>C. albicans</i> and <i>S. cerevisiae</i>	(Sacchetti et al. 2004)
<i>Salvia cuspidata</i> Ruiz & Pav.	Aerial parts (EtOH extracts)	<i>F. verticillioides</i>	(Carpinella et al. 2010)
Lycopodiaceae			
<i>Lycopodiella cernua</i> (L.) pic. Serm.	Leaves (triterpenes)	<i>C. albicans</i>	(Zhang et al. 2002)
Malvaceae			
<i>Sida cordifolia</i> L.	Aerial parts (acetone extracts)	<i>Candida</i> sp. and <i>Trichosporon inkin</i>	(Ahmed et al. 2018)
Myrtaceae			
<i>Eugenia uniflora</i> L.	Leaves (essential oil)	<i>P. brasiliensis</i>	(Costa et al. 2010)
Moraceae			
<i>Maclura tinctoria</i> (L.) D.Don ex Steud.	Leaves (EtOH extracts)	<i>C. albicans</i> and <i>C. neoformans</i>	(ElSohly et al. 2001)
Oxalidaceae			
<i>Oxalis erythrorrhiza</i> Gillies ex hook. & Arn.	Aerial parts (hexane and CH ₂ Cl ₂ extracts)	<i>M. canis</i>	(Feresín et al. 2001)
Passifloraceae			
<i>Passiflora caerulea</i> L.	Leaves (MeOH extracts and nanoparticles)	<i>A. flavus</i> and dermatophytes fungi (principally <i>T. rubrum</i>)	(AL-Rubaey et al. 2019; Santhoshkumar et al. 2019)
Phytolaccaceae			
<i>Phytolacca bogotensis</i> Kunth	Leaves (CH ₂ Cl ₂ extracts)	<i>S. schenckii</i>	(Gaitán et al. 2011)
<i>Phytolacca dioica</i> L.	Berries (hydrolysis of extracts)	<i>C. albicans</i> and <i>C. neoformans</i>	(Liberto et al. 2010)
<i>Seguieria americana</i> L.	Leaves (CH ₂ Cl ₂ extracts)	<i>F. pedrosoi</i> and <i>S. schenckii</i>	(Gaitán et al. 2011)
<i>Trichostigma octandrum</i> (L.) H. Walter	Leaves (CH ₂ Cl ₂ extracts)	<i>F. pedrosoi</i>	(Gaitán et al. 2011)

(continued)

Table 12.1 (continued)

Species	Parts used (extract types)	Reported antifungal activities	References
Piperaceae			
<i>Piper</i> sp.	Aerial parts (EtOH extracts)	<i>Candida</i> sp., <i>C. neoformans</i> , <i>S. cerevisiae</i> , <i>Aspergillus</i> sp., <i>M. gypseum</i> , <i>E. floccosum</i> and <i>Trichophyton</i> sp.	(Svetaz et al. 2010)
Poaceae			
<i>Elionurus muticus</i> (Spreng.) Kuntze	Aerial parts (essential oil)	<i>Candida</i> sp.	(Sabinil et al. 2006)
Polygonaceae			
<i>Persicaria acuminata</i> (Kunth) M.Gómez	Aerial parts (CH ₂ Cl ₂ extracts)	Yeasts and dermatophytes fungi	(Derita et al. 2009)
<i>Persicaria ferruginea</i> (Wedd.) Soják	Aerial parts (MeOH and hexane extracts)	<i>E. floccosum</i> and <i>Neurospora crassa</i>	(López et al. 2011)
<i>Persicaria hydropiperoides</i> (Michx.) small	Whole plant (MeOH extracts)	<i>C. albicans</i>	(Braga et al. 2007)
<i>Persicaria maculosa</i> gray	Aerial parts (CH ₂ Cl ₂ extracts)	<i>T. mentagrophytes</i> , <i>T. rubrum</i> and <i>M. gypseum</i>	(Derita and Zacchino 2011a)
<i>Persicaria punctata</i> (Elliott) small	Aerial parts (polygodial compound)	<i>C. albicans</i> and dermatophytes fungi	(Alves et al. 2001).
Pteridaceae			
<i>Pityrogramma calomelanos</i> (L.) link	Leaves (MeOH extracts)	<i>Curvularia lunata</i>	(Guerra et al. 2020)
Solanaceae			
<i>Solanum sisymbriifolium</i> lam.	Leaves and stems (MeOH extracts)	<i>C. albicans</i> , <i>A. niger</i> , <i>A. flavus</i> , <i>A. xylinum</i>	(Vaghela et al. 2009)
Ranunculaceae			
<i>Clematis campestris</i> A.St.-Hil.	Aerial parts (EtOH extracts)	<i>M. gypseum</i> , <i>T. rubrum</i> , <i>T. mentagrophytes</i> and <i>E. floccosum</i>	(Svetaz et al. 2010)
Rutaceae			
<i>Zanthoxylum coco</i> Gillies ex Hook. f. & Arn.	Aerial parts (flavonoids and lignans)	<i>F. verticillioides</i>	(Carpinella et al. 2010)
<i>Zanthoxylum rhoifolium</i> lam.	Leaves (volatile oil)	<i>A. flavus</i>	(da Silva et al. 2006)
Smilacaceae			
<i>Smilax campestris</i> Griseb.	Aerial parts (EtOH extracts)	<i>C. krusei</i> and <i>C. gattii</i>	(Morais et al. 2014)

(continued)

Table 12.1 (continued)

Species	Parts used (extract types)	Reported antifungal activities	References
Solanaceae			
<i>Cestrum parqui</i> (lam.) L'Hér.	Leaves and flowers (EtOH/MeOH extracts and saponins)	<i>Fusarium solani</i> , <i>A. niger</i> , <i>M. gypseum</i> , <i>T. rubrum</i> , <i>T. mentagrophytes</i> , <i>E. floccosum</i> , <i>F. pedrosoi</i> and <i>S. schenckii</i>	(Svetaz et al. 2010; Gaitán et al. 2011; Ahmed et al. 2012)
Verbenaceae			
<i>Acantholippia seriphioides</i> (A.Gray) Moldenke	Aerial parts (essential oils)	Dermatophytes fungi	(Lima et al. 2011)
<i>Aloysia citriodora</i> Palau	Aerial parts (essential oils)	<i>C. albicans</i>	(Demo et al. 2005)
<i>Aloysia gratissima</i> (Gillies & Hook.) Tronc.	Aerial parts (essential oils)	<i>Fusarium</i> sp. (moderate activity)	(Galvez et al. 2020)
<i>Lippia integrifolia</i> (Griseb.) Hieron.	Aerial parts (MeOH extracts)	<i>M. canis</i> and <i>E. floccosum</i>	(Muschiatti et al. 2005)
<i>Lippia junelliana</i> (Moldenke) Tronc.	Flowers, leaves and stems (essential oils)	<i>C. albicans</i> , <i>C. parapsilosis</i> and <i>C. krusei</i>	(Córdoba et al. 2019)
Winteraceae			
<i>Drimys winteri</i> J.R.Forst. & G.Forst.	Aerial parts (EtOH extracts)	<i>Candida</i> sp., <i>C. neoformans</i> , <i>S. cerevisiae</i> , <i>aspergillus</i> sp., <i>M. gypseum</i> , <i>E. floccosum</i> and <i>Trichophyton</i> sp.	(Svetaz et al. 2010; Butassi et al. 2015)
Zygophyllaceae			
<i>Larrea cuneifolia</i> Cav.	Leaves (aqueous extracts)	<i>C. albicans</i>	(Espino et al. 2019)
<i>Larrea divaricata</i> Cav.	Aerial parts (EtOH extracts)	<i>S. cerevisiae</i> and <i>C. albicans</i>	(Davicino et al. 2007).
<i>Larrea nitida</i> Cav.	Whole plant (CH ₂ Cl ₂ extracts)	<i>C. albicans</i>	(Butassi et al. 2015)

pharmacological studies that have shown important activities that support the traditional uses of this plant (Isla et al. 2016).

Regarding its chemical composition, 13 phenolic compounds were isolated from extracts of the aerial parts of *Z. punctata*: two chalcones, 2',4'-dihydroxy-3'-methoxychalcone (DHMC) and 2',4'-dihydroxychalcone (DHC); five flavones, 3,5,7-trihydroxyflavone (galangin), 3,5-dihydroxy-7-methoxy flavone (izalpinin), 3,5,4'-trihydroxy-7-methoxy flavone (rhamnocitrin), 3-hydroxy-7,8-dimethoxy flavone (3-H-7,8-DMF); and 3,7-dihydroxy flavone (3,7-DHF); four flavanones, 7-hydroxy flavanone (7-HF), 5,7-dihydroxy flavanone (pinocembrin), 5-hydroxy-7-methoxy flavanone (pinostrobin), and 7-hydroxy-8-methoxyflavanone (7-H-8-MF); and two caffeic acid derivatives, 1-methyl-3-(4'-hydroxyphenyl)-propyl caffeate [1-M-3-(4'-HP)-PC] and 1-methyl-3-(3',4'-dihydroxyphenyl)-propyl caffeate [1-M-3-(3',4'-DHP)-PC]. Chalcones being the main components of such extracts (Ortega et al. 2000; Nuño 2015). In the essential oil of the aerial parts of *Z.*



Fig. 12.1 *Zuccagnia punctata* Cav. (Fabaceae, Caesalpinioideae). (a) specimens in their natural environment. (b) Aerial parts

punctata, 80 constituents were identified, mainly oxygenated monoterpenes. The main components were identified as linalool and (–)-5,6-dehydrocamphor (Álvarez et al. 2012).

Below we describe the results obtained from our recent studies on the antifungal activity of extracts, essential oil, and compounds obtained from *Z. punctata* against phytopathogenic and human pathogenic fungi.

The activity of the crude ethanolic extract of aerial parts of *Z. punctata* was evaluated by the agar dilution method (Zacchino et al. 1999), against strains of fungi isolated from soybean plants growing in the most important producing regions of Argentina that presented typical symptoms of disease (*Phomopsis longicolla*, *Alternaria alternata*, *Sclerotium bataticola*, *Fusarium equiseti*, *F. graminearum*, and *Colletotrichum truncatum*) (Svetaz et al. 2004). The ethanolic extract showed activity against all of the fungi tested with Minimal Inhibitory Concentration (MIC) values between 100 and 500 $\mu\text{g/mL}$. This extract was successively partitioned between *n*-hexane, chloroform, and *n*-butanol; the chloroform extract showed the lowest MIC value (62.5 $\mu\text{g/mL}$) against the most relevant pathogenic fungus on soybean seed (*P. longicolla*). Repeated bioassay-guided chromatographies of the chloroform extract led to the isolation of DHMC, DHC, 7-HF, 1-M-3-(4'-HP)-PC, and 1-M-3-(3',4'-DHP)-PC. The chalcones DHMC and DHC showed potent antifungal activities against all fungi tested with MIC values ranging from 6.25 and 3.12 to 50 $\mu\text{g/mL}$, respectively. 7-HF and 1-M-3-(4'-HP)-PC showed very interesting activities only against *P. longicolla* (MIC = 6.25 $\mu\text{g/mL}$), while 1-M-3-(3',4'-DHP)-PC did not show any activity up to 50 $\mu\text{g/mL}$. It is interesting to note that four of the five compounds isolated from the antifungal chloroform extract of *Z. punctata* displayed very good activities (MIC \leq 6.25 $\mu\text{g/mL}$) against *P. longicolla*. Additionally, DHMC and DHC showed strong activities against *C. truncatum* (MIC = 6.25 $\mu\text{g/mL}$) (Svetaz et al. 2004). Both pathogens are the cause of the most serious soybean diseases due to their high incidence and persistence, causing reduction of seed quality and yields. *P. longicolla* is a primary agent of seed decay, a severe pathogen that affects soybean seed quality and *C. truncatum* is the causal agent of soybean anthracnose, a disease acquired mainly in the last growing step that affects stems and pods diminishing the number of seeds and their weight (Pioli et al. 2000).

Concerning to human pathogenic fungi, petroleum ether and dichloromethane extracts of fruits, aerial parts, and resinous exudate (obtained by dipping the fresh aerial parts in dichloromethane) of *Z. punctata* showed moderate antifungal activities against the yeasts *C. albicans*, *S. cerevisiae*, and *C. neoformans* (MICs: 62.5–250 µg/mL) and very strong antifungal activities against the dermatophytes *M. gypseum*, *T. rubrum*, and *T. interdigitale* (MICs: 8–16 µg/mL) thus supporting the ethnopharmacological use of this plant. Antifungal activity-directed fractionation of the most active extract led to the isolation of DHMC and DHC as the compounds responsible for the antifungal activity. DHCM displayed a selective and strong activity against dermatophytes (MIC = 8 µg/mL) and DHC showed a broader spectrum of activity inhibiting the yeasts *C. albicans*, *S. cerevisiae*, and *C. neoformans* (MICs: 16–31.2 µg/mL) and dermatophytes (MIC = 4 µg/mL). Second-order studies showed that DHC, in addition to being the most active chalcone, is fungicidal rather than fungistatic and that it would act through a different mechanism of action from that of antifungal drugs in current clinical use, so it appears to be a very promising antifungal agent (Svetaz et al. 2007).

The essential oil obtained by hydrodistillation from aerial parts of *Z. punctata* was evaluated against a panel of human opportunistic and pathogenic fungi using standardized procedures (CLSI 2017). The microbroth dilution method showed that the essential oil was not active against *C. albicans*, *C. tropicalis*, *S. cerevisiae*, or *C. neoformans* (MIC >1000 µg/mL) and possessed marginal activity against *Aspergillus niger*, *A. flavus*, and *A. fumigatus* (MIC = 1000 µg/mL). In contrast, it showed an interesting antifungal activity against dermatophytes (*M. gypseum*, *T. rubrum*, and *T. interdigitale*) with MIC values between 15.6 and 125 µg/mL, being *T. rubrum* the most susceptible species. A total of 80 constituents were identified in the oil, with linalool and (–)-5,6-dehydrocamphor being the main constituents. Both compounds were also tested against the same fungal strains. Linalool showed moderate activity against dermatophytes (MICs: 125–250 µg/mL) but was inactive against yeasts and *Aspergillus* spp. The other major component was inactive up to 250 µg/mL against all the fungi tested. The main components of the essential oil would not be responsible for its antifungal activity, but the identification of thymol and carvacrol as minor components suggests that they could contribute to this activity. The results of their antifungal evaluation undoubtedly suggest that both compounds could contribute to the activity of the essential oil since they were active against all of the evaluated fungal species (MICs: 15.6–250 µg/mL). Both compounds displayed interesting activities against dermatophytes (MICs: 15.6–31.2 µg/mL), while carvacrol also showed very good activity against *A. flavus* and *A. fumigatus* (MIC = 31.2 µg/mL) (Álvarez et al. 2012).



Fig. 12.2 *Phytolacca tetramera* Hauman (Phytolaccaceae)

2.2 *Phytolacca tetramera*: Berries Extracts and Compounds with Potential Antifungal Activity

Phytolacca tetramera Hauman (Phytolaccaceae), commonly known as “ombusillo,” is an endemic species from Argentina (Fig. 12.2). It is currently considered one of the threatened plant species, being in critical danger of extinction (Delucchi et al. 2006), mainly due to anthropic causes that lead to the reduction of their habitat, such as human settlements, construction of roads, the periodic weeding of these roads, agricultural and livestock activity, industrial facilities, etc. (Hernandez et al. 2008).

Our research group has studied this plant species for several years, and the information about the chemical composition, antifungal activity, and mechanism of action allowed us to know the importance of this plant for the treatment of fungal infections, promoting thus its conservation. From our studies, an area related to research and development for the conservation *in situ* of *P. tetramera* was created (Petri et al. 2010).

After Escalante et al. (2002) studies, the antifungal activity of methanolic, dichloromethanic, butanolic, and aqueous extracts of *P. tetramera* obtained not only from berries but also from leaves and roots (*PtMEb*, *PtDEb*, *PtBEb*, *PtAqEb*, *PtMEl*, *PtDEl*, *PtBEl*, *PtAqEl*, *PtMEr*, *PtDEr*, *PtBEr*, and *PtAqEr*) was assessed with the standardized CLSI (2017) microbroth dilution method against the yeasts *C. albicans* and *C. glabrata* (Butassi et al. 2019). The use of the most recent guidelines of CLSI (2017) for yeasts assured more reproducible and more reliable results. Table 12.2 shows the results corresponding to the re-evaluation of the antifungal activity of *P. tetramera* extracts.

Results showed that *PtMEb*, *PtDEb*, and *PtBEb* were moderately active against *C. albicans* and *C. glabrata*, with *PtDEb* being the most active against the tested strains (MIC = 250 µg/ml), followed by *PtMEb* (MICs between 500 and 1000 µg/

Table 12.2 MICs ($\mu\text{g/ml}$) of the different extracts of *P. tetramera* against *C. albicans* (Ca) CCC 125–2000 and *C. glabrata* (Cg) CCC 115–2000 determined by the microbroth dilution method recommended by CLSI (2017). ITZ = itraconazole (used as standard drug)

	PtMEb	PtDEb	PtBEb	PtAqEb	PtMEI	PtDEI	PtBEI	PtAqEI	PtMEr	PtDEr	PtBEr	PtAqEr	ITZ
Ca	1000	250	1000	i	i	i	i	i	i	i	i	i	0.50
Cg	500	250	1000	i	i	i	i	i	i	i	i	i	2.00

i: inactive (MIC > 1000 $\mu\text{g/ml}$). *C. albicans* and *C. glabrata* were clinically isolates obtained from CEREMIC, Centro de Referencia en Micología, FCByF, UNR

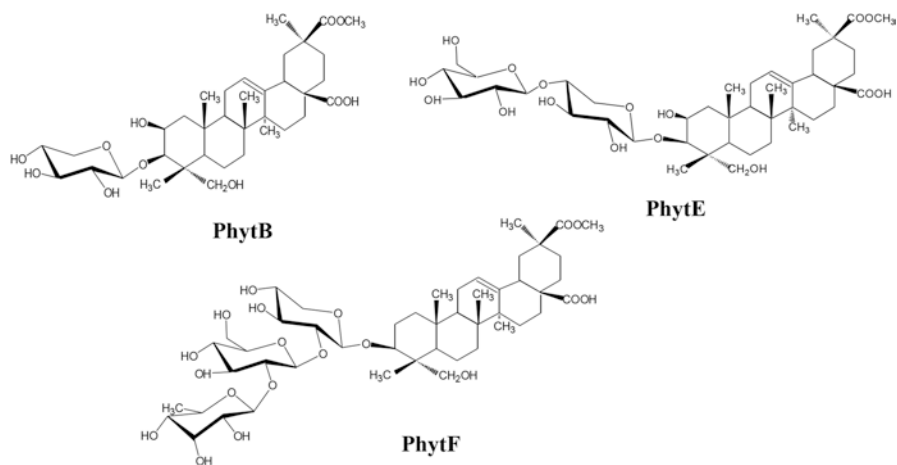


Fig. 12.3 Phytolaccoside B, phytolaccoside E and phytolaccoside F isolated from berries of *P. tetramera* butanolic extract

ml) and *PtBEb* (MIC = 1000 $\mu\text{g/ml}$). The rest of the extracts was inactive (MIC >1000 $\mu\text{g/ml}$) (Table 12.2).

By bioassay-guided fractionation (Escalante et al. 2002), three monodesmosidic triterpenoid saponins were isolated from *PtBEb*: phytolaccoside B (3-O- β -D-xylopyranosylphytolaccagenin) (PhytB), phytolaccoside E (3-O- β -D-glucopyranosyl-(1,4)- β -D-xylopyranosyl-phytolaccagenin) (PhytE), and phytolaccoside F [3-O- α -L-rhamnopyranosyl-(1,2)- β -D-glucopyranosyl-(1,2)- β -D-xylopyranosyl-phytolaccagenic acid] (PhytF) (Fig. 12.3). The three saponins belong to the olean-type triterpenoid saponins, possessing 28,30 dicarboxylic groups and an olefinic double bond on C-12. PhytB and PhytE, but not PhytF, showed antifungal activities against a panel of human pathogenic opportunistic fungi. PhytB was the most active compound and showed the broadest spectrum of action (MICs between 25 and 125 $\mu\text{g/ml}$) (Escalante et al. 2002).

The chemical composition of *P. tetramera* extracts obtained from berries, leaves, and roots was studied using Thin Layer Chromatography (TLC). These studies allowed the detection of PhytB, PhytE, and phytolaccagenin (PhytG) in all the extracts except in aqueous ones (Butassi et al. 2019) (Fig. 12.4).

According to this chemical analysis, the antifungal activity of PhytB, PhytE, and PhytG was evaluated against *C. albicans* and *C. glabrata*. PhytB and PhytG were active against both pathogens with MIC = 62.5 $\mu\text{g/ml}$. In contrast, PhytE did not show activity (MIC >250 $\mu\text{g/ml}$) (Butassi et al. 2019). Based on these results, PhytB and PhytG were selected as active markers (EMA 2010) and were quantified in all the extracts using UHPLC-ESI-MS (Table 12.3) (Butassi et al. 2019). The most potent extract of *P. tetramera* (*PtDEb*) showed the highest amount of active markers, followed by *PtMEb* and *PtBEb*. The extracts from leaves and roots contained a low level of both PhytB and PhytG. Therefore, it could be stated that these two compounds contribute strongly to the antifungal activity of the active extracts.

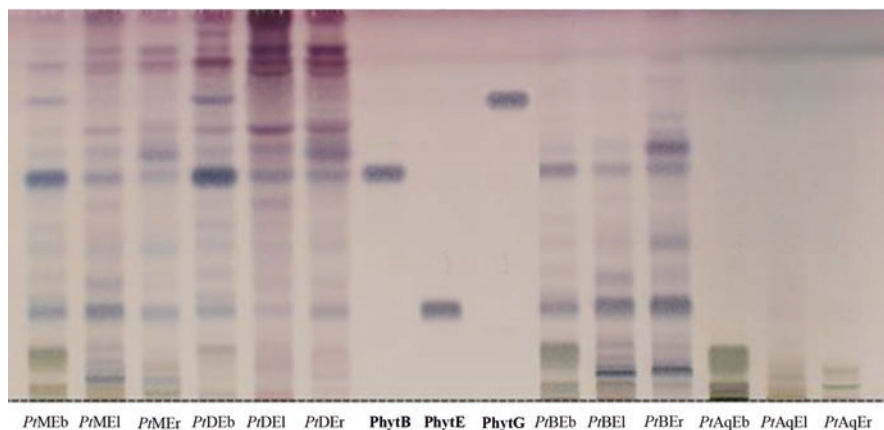


Fig. 12.4 Chemical profile of different extract types obtained from *P. tetramera* berries, using PhytB, PhytE, and PhytG as markers

Table 12.3 Content (mg of compound/g plant material) of active markers PhytB and PhytG in *P. tetramera* extracts analyzed by UHPLC-ESI-MS. Values are the mean \pm standard deviation ($n = 3$). Nd: not detected

	Extracts	PhytB (mg/g)	PhytG (mg/g)
Berries	<i>PtMEb</i>	66.12 \pm 0.33	24.92 \pm 0.09
	<i>PtDEb</i>	155.24 \pm 5.27	121.48 \pm 1.36
	<i>PtBEb</i>	36.27 \pm 0.30	0.68 \pm 0.03
Leaves	<i>PtMEl</i>	2.11 \pm 0.28	0.01 \pm 0.005
	<i>PtDEl</i>	0.61 \pm 0.11	0.06 \pm 0.0002
	<i>PtBEL</i>	3.13 \pm 0.66	Nd
Roots	<i>PtMEr</i>	0.09 \pm 0.01	0.004 \pm 0.0003
	<i>PtDEr</i>	0.56 \pm 0.10	0.017 \pm 0.005
	<i>PtBEr</i>	0.55 \pm 0.21	Nd

Additionally, the mechanism of action of the active markers PhytB and PhytG and the most active extract *PtDEb* was studied. For that, morphological studies (using scanning electron, phase contrast, and fluorescence microscopies) which target the fungal cell wall [cellular sorbitol assay and enzymatic (1,3)- β -D-glucan synthase (GS) and chitin synthase 1 (ChS) assays] and studies which target the fungal cell membrane (ergosterol-binding assay) were carried out. Table 12.4 summarizes the results obtained.

Table 12.4 Mechanism of action studies of the active markers PhytB and PhytG and the most active extract *PtDEB*

	Mechanism of antifungal action	References
PhytB	Produces shortening of <i>Neurospora crassa</i> hyphae and highly branched bulbous hyphal tips	(Escalante et al. 2008)
	Modifies the normal morphology of the yeast <i>S. cerevisiae</i> producing aggregates and swollen cells	(Escalante et al. 2008)
	Produces an increase of chitin synthase 1 activity. A high deposit of chitin would lead ultimately to the arrest of cell growth	(Escalante et al. 2008)
PhytG	Binds to ergosterol and disrupts the fungal plasma membrane causing cell wall damage and cell death	(Butassi et al. 2019)
<i>PtDEB</i>	Modifies the normal morphology of the yeast <i>Schizosaccharomyces pombe</i> producing smaller, wrinkled, brighter, deformed, and swollen cells. The wrinkled and brighter appearance of <i>S. pombe</i> cells indicates a phenotype of dead cells, which was more abundant during the process of cell separation	(Butassi et al. 2019)
	Modifies the normal morphology of the yeast <i>C. albicans</i> producing swollen or elongated and refringent cells, which suggest sick or dead cells due to an altered plasma membrane. Produces an enrichment of chained cells, indicating a defect in the final process of cell separation	(Butassi et al. 2019)
	Binds to ergosterol and disrupts the fungal plasma membrane causing cell wall damage and cell death	(Butassi et al. 2019)

2.3 The Genus *Polygonum*: An Update for its Antifungal Effects and Influence of Chemotaxonomy

Polygonum constitutes one of the plant genera most used by Argentinian traditional medicine to treat fungal conditions. This genus comprises about 250 species that have a wide geographical distribution, from Polar Regions to the tropics of all continents. Formerly, it was divided into five sections: *Echinocaulon*, *Amblygonum*, *Persicaria*, *Tiniaria* and *Polygonum* but nowadays, due to its botanical and phytochemical complexity, species are classified into two true genera: *Polygonum* and *Persicaria*. According to the plant list (www.plantlist.org) most of them have been regrouped with accepted names, but their synonyms are still used (Álvarez et al. 2020). In Argentina, about 20 species belonging to both genera grow throughout the country, but the most active in terms of their antifungal properties is *Persicaria acuminata* syn. *Polygonum acuminatum* and *Persicaria maculosa* syn. *Polygonum persicaria* (Fig. 12.5) (Derita and Zacchino 2011a).

From the bio-guided fractionation of *P. acuminata* aerial parts, five sesquiterpenes with drimane skeleton were isolated: drimenol, isopolygodial, confertifoline, polygodial, and 1- β - (p-methoxycinnamoyl) polygodial. Among them, the most active was polygodial, inhibiting the growth of *C. albicans*, *C. neoformans*, and the dermatophytes *M. gypseum*, *T. rubrum*, and *T. mentagrophytes* with MICs between 3.9 and 62.5 $\mu\text{g/mL}$ and MFCs between 7.8 and 125 $\mu\text{g/mL}$, being fungicide as well as a strong inhibitor of fungal growth (Derita et al. 2013).

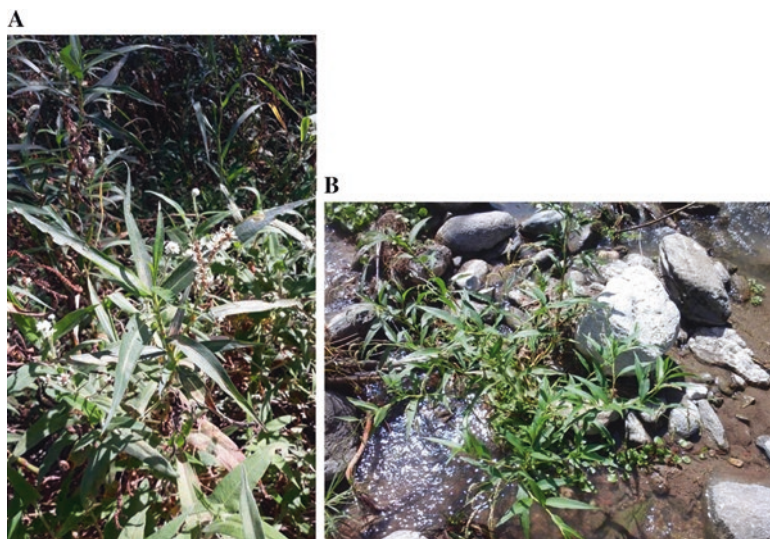


Fig. 12.5 *Persicaria acuminata* (a) and *P. maculosa* (b)

The bioguided fractionation of *P. maculosa*, in addition to the sesquiterpenes mentioned above, allowed the isolation of the flavanone pinostrobin and the chalcones flavokawin B and cardamonin. The latter compounds were active against *C. albicans*, *C. neoformans*, and the dermatophytes *M. gypseum*, *T. rubrum*, and *T. mentagrophytes* displaying MICs between 15.6 and 500 $\mu\text{g/mL}$. Unlike *P. acuminata*, the antifungal activity of *P. maculosa* was due not only to polygodial and isopolygodial but also to flavonoids, which added an interesting chemotaxonomical data (Derita and Zacchino 2011b).

Flavonoids have played an important role in the systematics of *Polygonum* species being proposed as chemotaxonomic markers of the genus. However, as stated before, the finding of polygodial within the *Persicaria* section promoted a sesquiterpene (not a flavonoid) to be proposed as a chemotaxonomic marker for the *Persicaria* section. Consequently, with the aim of finding compounds that could be suggested as new chemical markers for the delimitation of *Persicaria* section of the genus, a comparative study about the presence and quantification of sesquiterpenes and flavonoids in six species belonging to the section was carried out. It was found that *P. hydropiperoides*, *P. lapathifolia*, and *P. ferruginea*, which were the three species that did not contain polygodial, showed the presence of the three flavonoids. In turn, *P. punctata* and *P. acuminata* contained the four sesquiterpenes but no flavonoids. Surprisingly, *P. persicaria* was the only species belonging to this section that presented both types of compounds (Derita and Zacchino 2011b). All these findings contributed to the regrouping of these species into the current *Polygonum* and *Persicaria* genera (Álvarez et al. 2021).

3 Photoactivity: An Underexplored Property for Detection of Antifungal Plants

Antimicrobial photodynamic therapy (APDT) is increasingly being recognized as an alternative clinical treatment for fungal infections. Their advantages include a broad spectrum of activities (viruses, protozoa, Gram-positive and Gram-negative bacteria and fungi), the possibility of eliminating microorganisms independently of their antimicrobial resistance pattern, low probability of adverse side effects and low cost (Donnelly et al. 2008). In addition, APDT is highly selective (Dai et al. 2012), resistance has not been described (Wainwright et al. 2017), it has been implicated in changes in the expression of virulence determinants factors and showed efficacy against biofilms (Kato et al. 2013). PDT involves the administration of a non-toxic photosensitizer (PSs) and harmless visible light of the correct wavelength to generate reactive oxygen species (ROS), which can oxidatively damage surrounding biomolecules, such as lipids, proteins, and nucleic acids, thus killing pathogenic microorganisms (Liang et al. 2016; Pinto et al. 2018). ROS are produced by the reaction of the excited PSs and oxygen (O_2) through type I reactions, that involve electron-transfer reactions that generate hydroxyl radical (OH^\bullet), hydrogen peroxide (H_2O_2), and superoxide radical ($O_2^{\bullet-}$) and type II reactions that involve energy transference to produce singlet oxygen 1O_2 (Maisch et al. 2007).

PSs are present in certain plants as a chemical defense mechanism developed to protect themselves from the action of microbes and herbivorous attack. This action is triggered when these secondary metabolites are excited to higher energy levels by absorbing solar or artificial radiation at a particular wavelength range (Mamone et al. 2014). Siewert and Stuppner (2019) described 10 classes of natural PSs: thiophenes, furanocoumarins, polyacetylenes, curcumins, xanthenoids, alkaloids, anthraquinones and perylenequinones, phenalenones, and porphyrins. Thiophenes is one of the larger groups with more than 150 natural biologically active compounds with absorbance maximum for photobiological effects between 314–350 nm (Postigo et al. 2017; Siewert and Stuppner 2019), present in plants of the family Asteraceae including the genus *Porophyllum*, *Tagetes*, and *Flaveria* widely distributed in Argentina (Downum and Towers 1983; Ibrahim et al. 2016). *Porophyllum* comprises 25 species, six of them inhabit the Argentinean central-western region, they are annual or perennial plants with secretory cavities in oil-bearing leaves and bracts which emanate a strong foul odor (Johnson 1964; Loockerman et al. 2003). *Tagetes* includes approximately 56 species that have been used as a source of essential oil for flavoring in the food industries, and their flowers, which are rich in orange–yellow carotenoids, are used as food coloring (Vasudevan et al. 1997). *Flaveria* comprises 21 species widely distributed in America, only *F. bidentis* and *F. haumanii* occur in Argentina (de los A. Páez et al. 2019).

Below we describe the photodynamic antifungal activity of different extracts obtained from six species belonging to the genus *Porophyllum*, *Tagetes*, and *Flaveria* which were evaluated against *C. albicans*. The experiments were carried on following the guidelines of CLSI (2017) document that assures confident and reproducible

Table 12.5 Minimal Fungicide Concentrations (MFC expressed in $\mu\text{g/mL}$) against *C. albicans* (light/darkness)

Species Voucher specimen	Parts used	Extract type			
		Hex	DCM	EA	Met
<i>Flaveria bidentis</i> (L.) Kuntze (Del Vitto & Petenatti #9491, UNSL)	Whole plant	0.24 / NA	0.98 / NA	NA / NA	NA / NA
<i>Porophyllum lanceolatum</i> DC. (Del Vitto & Petenatti #9478, UNSL)	Whole plant	500 / NA	125 / NA	NA / NA	NA / NA
<i>Porophyllum obscurum</i> (Spreng.) DC (Del Vitto & Petenatti # 9436, UNSL)	Whole plant	0.98 / NA	7.81 / NA	NA / NA	NA / NA
<i>Porophyllum ruderale</i> (Jacq.) Cass (Del Vitto & Petenatti #9539, UNSL)	Whole plant	62.50 / NA	31.25 / NA	NA / NA	NA / NA
<i>Tagetes minuta</i> L. (Del Vitto and Petenatti #9230, UNSL)	Stems	3.91 / NA	31.25 / NA	NA / NA	NA / NA
	Leaves	7.81 / NA	7.81 / NA	NA / NA	NA / NA
	Flowers	31.25 / NA	31.25 / NA	NA / NA	NA / NA
	Roots	1.95 / NA	0.49 / NA	62.5 / NA	NA / NA
<i>Tagetes patula</i> L. (Del Vitto & Petenatti #9239, UNSL)	Aerial parts	250 / NA	500 / NA	NA / NA	NA / NA
	Roots	1.95 / NA	15.63 / NA	NA / NA	NA / NA

Hex: Hexane; DCM: dichloromethane; EA: ethyl acetate; Met: methanol. NA: not active (MFC >1000 $\mu\text{g/mL}$). In bold, photoactive extracts

results. Microplates were submitted to irradiation (light) or kept in darkness. In light experiments, UVA irradiations were performed with a homemade UVA light array composed of a set of three lamps (Alic, Buenos Aires, Argentina), emitting at 315–400 nm (100 W). Microplates were aligned perpendicular to the samples that illuminate uniformly the entire area of the microplate, placed at a distance to 12 cm from the light source and irradiated for 60 min. In darkness experiments, assays were carried out in the same conditions but microplates were wrapped with aluminum foil to avoid exposure to light. Table 12.5 shows the Minimal Fungicide Concentrations (MFC expressed in $\mu\text{g/mL}$) against *C. albicans* under light/darkness conditions.

No significant differences were observed in the number of yeasts Colony Forming Units (CFU/mL) in the growth controls between darkness and light experiments, suggesting that the action of UVA irradiation did not reduce *C. albicans* viability. None of the evaluated extracts, at a concentration up to 1000 $\mu\text{g/mL}$, showed antifungal activity in experiments performed without irradiation. All hexane and DCM

extracts were considered photoactive, because they showed antifungal activity against *C. albicans* only under UVA irradiation. Most ethyl acetate and methanolic extracts did not exhibit antifungal photosensitive activity at concentrations up to 1000 µg/mL. The observed higher activity of the apolar extracts, compared with the more polar extracts, could be attributed to the presence of apolar thiophenic compounds previously reported for the studies on related species (Gil et al. 2002; Postigo et al. 2017; Ibrahim et al. 2018; Giacone et al. 2020). The main photoactive components of these extracts were identified as 2,2':5',2''-terthienyl (α -T); 5-(3-buten-1-ynyl)-2,2'-bithiophene (BBT); 5-(4-acetoxy-1-butynyl)-2,2'-bithiophene (BBTOAc); 5-(4-hydroxy-1-butynyl)-2,2'-bithiophene (BBTOH); and 5-(3-penten-1-ynyl)-2,2'-bithiophene (PBT). When different parts of the plant were studied, the highest activity was obtained in root extracts, that is, the organ where thiophenes accumulate (Marotti et al. 2010).

4 Probing Synergistic Effects to Increase Antifungal Activity in Plants

Combination therapy has emerged as an effective strategy for antifungal treatment to fight against microbial resistance (Hemaiswarya et al. 2008). In fact, the high incidence rates of *Candida* infections are supposed to be closely related with their recalcitrant resistance to conventional antifungals and their capacity for biofilm formation (Zavrel and White 2015). Synergism, defined as a phenomenon in which the combined action of two agents is more effective than the action of a single agent, has been the main focus for combinatory therapy as it greatly reduces the effective dosages of them required to treat an infection (Yang et al. 2017). Combinations of drugs with different targets prevents the development of drug resistance, may improve the interaction with its target and can reduce toxicity, since lower concentrations of both agents can be used (Ayaz et al. 2019). Research of new antimicrobials boost the use of bioactive compounds and extracts from plants, either alone, combined, or together with antibiotics (Ríos and Recio 2005) that can potentiate the activity of antimicrobials by targeting different sites in the microbial cell (multi-target effect), by improving their solubility or bioavailability (pharmacokinetic or physicochemical effects) or by targeting the resistance mechanism (Wagner and Ulrich-Merzenich 2009).

In recent years, there has been an increased interest in using herbs along with conventional drugs rather than using them in place of drugs that raises concerns about studying herb–drug interactions (Spitzer et al. 2017). Here we detail the results of our research on the antifungal activity of plant extracts of the Argentine flora alone and in combination with currently used antifungal drugs (Cordisco et al. 2019). The antifungal activity of 253 plant extracts used in traditional Argentine medicine (obtained from 153 species of plants, belonging to 120 genera and 56 families) was evaluated alone and in combination with the antifungal drugs amphotericin B (AMB), fluconazole (FCZ), and itraconazole (ITZ), against *C. albicans*.

MICs alone and in combination were determined with the broth microdilution technique following the guidelines of CLSI (2017) and using a modification of the High-Throughput Synergy Screening (HTSS) test, respectively (Zhang et al. 2007). Only 27 extracts tested alone showed activity (MICs between 31.25–1000 $\mu\text{g/mL}$), which represents 10.67% of the total evaluated. For this reason, we decided to re-explore all extracts through combination trials with antifungal drugs, hoping to detect those with enhanced activity and thus take full advantage of nature's chemodiversity and find new structures with antifungal activity.

First, we analyzed the importance of ethnopharmacological uses in the selection of extracts by investigating whether the probability of detecting plant extracts which enhance the activity of antifungal compounds is higher when obtained from species with reports of ethnopharmacological uses related to fungal infections. To achieve this, the plant extracts were classified into three groups according to whether they have reports of ethnopharmacological uses related to fungal infections (Group I = 37 species), if they are not reputed as antifungal but belong to a genus which has species with ethnopharmacological use related to antifungal activity (Group II = 11 species) and without any ethnopharmacological use related to fungal infections (Group III = 46 species). The results obtained from the combinations between the commercial antifungals and the extracts of the different group of plants showed that 7/37 (18.92%), 1/11 (9.09%), and 1/46 (2.17%) plant species of Groups I–III, respectively, enhanced the activity of AMB (Fig. 12.6a); 7/37 (18.92%), 1/11 (9.09%), and 4/46 (8.69%) plant species of Groups I–III respectively, enhanced the activity of FCZ (Fig. 12.6b) and 10/37 (27.03%), 2/11 (18.18%), and 7/46 (15.22%) plant species of Groups I–III, respectively, enhanced the activity of ITZ (Fig. 12.6c). These results indicated that when extracts came from plants with ethnopharmacological use related to fungal pathologies, there were more possibilities of finding extracts that enhance the activity of commercial antifungal drugs, suggesting that the ethnopharmacological approach is useful in designing extract–antifungal combinations with enhanced activity. Regarding the antifungal drug, the activity of azoles, especially ITZ, has been improved to a greater extent with respect to AMB (Cordisco et al. 2019).

Additionally, we evaluated whether the probability of detecting plant extracts which enhance the activity of antifungal compounds is greater when they have antifungal activities alone (MIC \leq 1000 $\mu\text{g/mL}$) than when they do not possess it (MIC $>$ 1000 $\mu\text{g/mL}$). Our results indicated that there is a greater probability of finding an enhancement in the activity of commercial drugs when the combination is performed with extracts that have shown activity alone as compared to those previously inactive extracts. It is important to note that 42 extracts behaved as enhancers in combination with at least one of the antifungal agents evaluated and that among these, a total of 27 extracts had not shown activity alone. These extracts would have been considered inactive and discarded for further studies according to the classic strategy, however, by using this new paradigm, they remain potential candidates in the search for new antifungals (Cordisco et al. 2019).

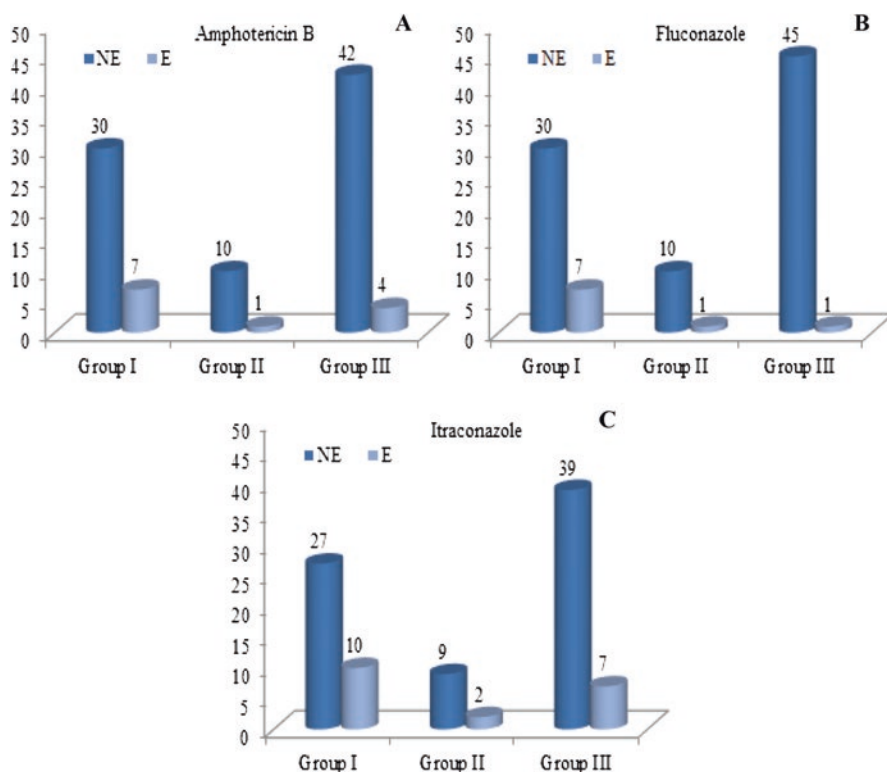


Fig. 12.6 Number of species from Groups I, II, and III which showed no enhancement (NE) and enhancement (E) of the activity of (a) amphotericin B; (b) fluconazole and (c) itraconazole

5 An Extension of Plant Antifungal Properties toward the Control of Phytopathogenic Fungi on Fruits

Phytopathogenic fungi cause pre- and post-harvest diseases in vegetable, cereal, and fruit crops and are responsible for considerable world agriculture economic losses. The most common species of phytopathogenic fungi that cause the deterioration of fruits, leaves, stems, and ground organs (roots, tubers, corms, etc.) belong to the genera *Alternaria*, *Botrytis*, *Diplodia*, *Monilinia*, *Penicillium*, *Colletotrichum*, *Phomopsis*, *Fusarium*, *Rhizopus*, and *Mucor* (Juárez-Becerra et al. 2010).

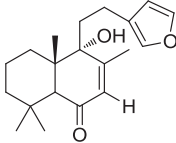
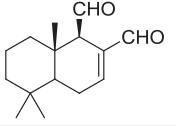
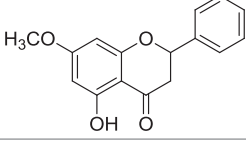
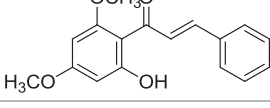
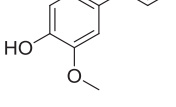
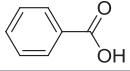
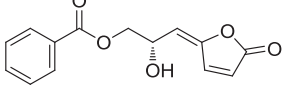
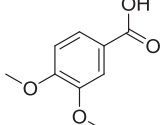
Plant extracts and their isolated active compounds have been tested for their efficacy in the control of a wide range of phytopathogenic fungi (Jiménez-Reyes et al. 2019). In this sense, an *in vitro* study revealed the antifungal activity of 18 Argentinean plants species against four phytopathogenic fungi that greatly affect the post-harvest stage of commercially important fruits, including *Penicillium digitatum*, *Botrytis cinerea*, *Monilinia fructicola*, and *Rhizopus stolonifer*. All the species studied were at least active against one fungus of the panel, while three of them (*Solidago chilensis* Meyen, *Drimys winteri* J.R.Forst, and *Polygonum stelligerum*

Cham.) displayed high antifungal properties inhibiting the growth of the selected pathogens. The antifungal activity of these plants was attributed to the presence of solidagenone in *S. chilensis*, polygodial in *D. winteri* and pinostrobin, and flavokawin B in *P. stelligerum* (Di Liberto et al. 2019). All these compounds have been identified as antimicrobial compounds (Muñoz-Concha et al. 2007; Derita and Zacchino 2011a; Ramirez et al. 2013; Carrasco et al. 2017) with the exception of solidagenone, which has been recently evaluated for its antiproliferative potential (Gomes et al. 2018). In a similar study, 17 Chinese medicinal plants were determined against eight species of plant pathogenic fungi, including *Rhizoctonia cerealis*, *F. graminearum*, *Gaeumannomyces graminis*, *F. oxysporum*, *Valsa mali*, *Colletotrichum gloeosporioides*, *F. oxysporum* sp. *Cucumebrium*, and *Colletotrichum lagenarium*. The results showed that the ethanol extracts of *Syzygium aromaticum* (L.) Merr. Et Perry (Myrtaceae) has the highest antifungal effect over the tested pathogens, isolating 2-methoxy-4-(2-propenyl) phenol (eugenol) as the active compound (Yang et al. 2019). Moreover, the antifungal activity of hexane, dichloromethane, and methanol extracts of 45 Thai plants were *in vitro* screened against plant phytopathogenic fungi (*Alternaria porri*, *C. gloeosporioides*, *F. oxysporum*, and *Phytophthora parasitica*). Seven extracts strongly inhibited the mycelial growth of the fungi. The plant extract with highest antifungal activity was *Melodorum fruticosum* Lour. (Annonaceae). Two of the eight isolated compounds (benzoic acid and melodorinol) exhibited strong activity against mycelial growth of *P. parasitica* (Mongkol et al. 2016). Extracts from the Chilean plants *Ephedra breana* Phil. (Ephedraceae) and *Nolana sedifolia* Poepp. (Solanaceae) have revealed antifungal activity against *B. cinerea* (Vio-Michaelis et al. 2012). The bioactive compounds of these plants were veratric, *p*-hydroxybenzoic, and caffeic acids in *E. breana*, and *p*-coumaric and ferulic acids in *N. sedifolia*. All these compounds have been identified as antimicrobials (Fu et al. 2010). These examples, regarding the antifungal potential of plant products, are listed in Table 12.6.

On the other hand, essential oils have gained popularity in the agricultural sector due to their great antifungal activity and mycotoxin inhibition. They are ecological, biodegradable, and safe to human health (Dwivedy et al. 2016; Stegmayer et al. 2020). Their volatility makes them suitable as fumigants in protected environments and for post-harvest diseases of horticultural crops (Shukla 2018). The antimicrobial activity of essential oils is attributed mainly due to the bioactivity of the major compound or the overall synergistic effect of all major and minor compounds (Mishra et al. 2013). Although their modes of action are still unclear, they are known to decline the biosynthesis of ergosterol and disrupt the cell membrane.

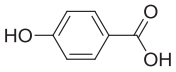
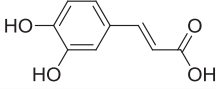
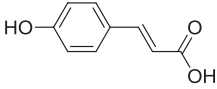
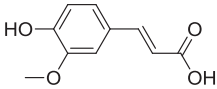
Essential oils from *Illicium verum* Hook.f (Schisandraceae) and its main component *trans*-anethole showed potent antifungal activity against several phytopathogenic fungi and could be developed as natural fungicides for disease control in fruit and vegetable preservation (Huang et al. 2010). Essential oils from the Lamiaceae plants peppermint (*Mentha piperita* L.) and sweet basil (*Ocimum basilicum* L.) proved to be effective fumigants against the two post-harvest phytopathogens *M. fructicola* and *R. stolonifer* in stored peach (*Prunus persica*) (Ziedan and Farrag 2008). A number of essential oils based on food preservatives are commercially

Table 12.6 Natural products isolated from plants which proved to be potent against different phytopathogenic fungi

Structure	Compound	Source	Controlled plant pathogen	Reference
	Solidagenone	<i>S. chilensis</i>	<i>P. digitatum</i> ; <i>B. cinerea</i> ; <i>M. fruticola</i> ; <i>R. stolonifer</i>	(Di Liberto et al. 2019)
	Polygodial	<i>D. winteri</i>	<i>P. digitatum</i> ; <i>B. cinerea</i> ; <i>M. fruticola</i> ; <i>R. stolonifer</i>	(Di Liberto et al. 2019)
	Pinostrobin	<i>P. stelligerum</i>	<i>P. digitatum</i> ; <i>B. cinerea</i> ; <i>M. fruticola</i> ; <i>R. stolonifer</i>	(Di Liberto et al. 2019)
	Flavokawin B	<i>P. stelligerum</i>	<i>P. digitatum</i> ; <i>B. cinerea</i> ; <i>M. fruticola</i> ; <i>R. stolonifer</i>	(Di Liberto et al. 2019)
	Eugenol	<i>S. aromaticum</i>	<i>R. cerealis</i> , <i>F. graminearum</i> , <i>G. graminis</i> , <i>F. oxysporum</i> f. sp. <i>vasinfectum</i> , <i>V. mali</i> , <i>C. gloeosporioids</i> , <i>F. oxysporum</i> sp. <i>cucumebrium</i> , <i>C. lagenarium</i>	(Yang et al. 2019)
	Benzoic acid	<i>M. fruticosum</i>	<i>P. parasitica</i>	(Mongkol et al. 2016)
	Melodorinol	<i>M. fruticosum</i>	<i>P. parasitica</i>	(Mongkol et al. 2016)
	Veratric acid	<i>E. breana</i>	<i>B. cinerea</i>	(Vio-Michaelis et al. 2012)

(continued)

Table 12.6 (continued)

Structure	Compound	Source	Controlled plant pathogen	Reference
	<i>p</i> -Hydroxybenzoic acid	<i>E. breana</i>	<i>B. cinerea</i>	(Vio-Michaelis et al. 2012)
	Caffeic acid	<i>E. breana</i>	<i>B. cinerea</i>	(Vio-Michaelis et al. 2012)
	<i>p</i> -Coumaric acid	<i>N. sedifolia</i>	<i>B. cinerea</i>	(Vio-Michaelis et al. 2012)
	Ferulic acid	<i>N. sedifolia</i>	<i>B. cinerea</i>	(Vio-Michaelis et al. 2012)

used and listed in “Generally Recognized as Safe” category by the Food and Drug Administration (FDA) and Environment Protection Agency (EPA) in the United States (Burt 2004), but only a few commercial biopesticides containing essential oils or artificial mixtures of terpene constituents are available. Cinnamite and Valero (from Mycotech Corporation) are commercialized as aphicide/fungicide being based on cinnamaldehyde and cinnamon oil, respectively. SPoran (a fungicide based on rosemary oil), from EcoSMART Technologies, is another example of biopesticide commercially available.

6 Conclusions

Throughout this chapter, we highlighted the most relevant results obtained during the last 20 years in our laboratory, which is internationally recognized for its quality on antifungal studies. The main human and plant fungal pathogens as well as the properties of fungi that are exploited in different industries were deeply discussed. An extensive list of Argentine plants belonging to different botanic families which have been described in the literature as antifungals was exhaustively detailed, taking into account its parts used, the fungi type against they were active, and the research group that generated the information.

The results obtained up to date give support to the ethnopharmacological use of *Z. punctata*, *P. acuminata*, *P. maculosa*, and *P. tetramera* as the most important species used as antiseptic and antifungal in the traditional medicine of Argentina, indicating the potential of their extracts, essential oils, and metabolites isolated from them for the control of a wide range of fungi affecting both crops and humans.

In view of the lack of new classes of drugs or different molecular targets against the most threatening yeast *C. albicans*, photodynamic therapy emerged as an

alternative approach to treat fungal infections. Plants containing phototoxic compounds were discovered in various botanic families, and several researchers have demonstrated that herbal extracts could be used in antimicrobial treatments, including antifungal. Aligned with this, the re-exploration of inactive extracts, by combining them with commercial antifungal agents, allowed to obtain synergistic mixtures, offering new possibilities for antifungal formulations. A greater probability of finding an enhancement in the activity of commercial drugs was observed when the combination was performed with extracts that had shown activity alone compared to the previously inactive extracts. Notably, 42 extracts behaved as enhancers in combination with at least one of the antifungal agents evaluated and 27 of them had not shown activity alone. These extracts would have been considered inactive and discarded for further studies according to the classic strategy, however, by using this new paradigm, they remain potential candidate in the search for new antifungals.

Acknowledgments Authors gratefully acknowledge Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) and Universidad Nacional de Rosario (UNR) for financial support (PIP N° 2015-0524, PICT N° 2015-2259, PICT N° 2016-1833 and BIO571-UNR). GS, EB, MDL, EC, and AB are also thankful to CONICET for their doctoral fellowships.

References

- de la Luz LA (2003) Principios agroclimáticos básicos para la producción de plantas medicinales. *Rev Cubana Plant Med* 1:1–10
- Ahmad I, Zafar S, Ahmad F (2005) Heavy metal biosorption potential of *aspergillus* and *Rhizopus* sp. isolated from wastewater treated soil. *J Appl Sci Environ Mgt* 9(1):123–126
- Ahmed DB, Chaieb I, Salah KB, Boukamcha H, Jannet HB, Mighri Z, Daami-Remadi M (2012) Antibacterial and antifungal activities of *Cestrum parqui* saponins: possible interaction with membrane sterols. *Int Res J Plant Sci* 3(1):001–007
- Ahmed H, Juraimi AS, Swamy MK, Ahmad-Hamdani MS, Omar D, Rafii MY, Sinniah UR, Akhtar MS (2018) Botany, chemistry, and pharmaceutical significance of *Sida cordifolia*: A traditional medicinal plant. In: Akhtar MS, Swamy MK (eds) *Anticancer plants: properties and application*. Springer, Singapore, pp 517–537
- Al-Rubaey NKF, Abbas FM, Hameed IH (2019) Antibacterial and anti-fungal activity of Methanolic extract of *Passiflora caerulea*. *Indian J Public Health Res Dev* 10(1):930–935
- Alanís Garza BA (2005) Rastreo de la actividad antifúngica de plantas del noreste de México, contra los principales agentes causales de micosis pulmonar en la región. In: pH thesis. Universidad Autónoma de Nuevo León
- Alonso J, Desmarchelier C (2015) *Plantas medicinales autóctonas de la Argentina: bases científicas para su aplicación en atención primaria de la salud*, 1st edn. Corpus Libros Médicos y Científicos, Buenos Aires, Argentina
- Álvarez NH, Fernández LN, Seimandi GM, Stegmayer MI, Ruiz VE, Derita MG (2021) Antifungal activity validation of wild plants used in Argentine Ethnomedicine. In: Rai M, Bhattarai S, Feitosa C (eds) *Ethnopharmacology of wild plants*. CRC Press/Taylor & Francis Group, U.S.A., pp 111–128. in press
- Álvarez SL, Cortadi A, Juárez MA, Petenatti E, Tomi F, Casanova J, van Baren CM, Zacchino S, Vila R (2012) (–)-5,6-Dehydrocamphor from the antifungal essential oil of *Zuccagnia punctata*. *Phytochem Lett* 5:194–199

- Alves TMDA, Ribeiro FL, Kloos H, Zani CL (2001) Polygodial, the fungitoxic component from the Brazilian medicinal plant *Polygonum punctatum*. Mem Inst Oswaldo Cruz 96(6):831–833
- Anbu P, Hilda A, Sur HW, Hur BK, Jayanthi S (2008) Extracellular keratinase from *Trichophyton* sp. HA-2 isolated from feather dumping soil. Int Biodeterior Biodegradation 62(3):287–292
- Anisa SK, Girish K (2014) Pectinolytic activity of *Rhizopus* sp. and *Trichoderma viride*. J Pure Appl Microbiol 4(2):28–31
- Apud GR, Aredes-Fernández PA, Kritsanida M, Grougnet R, Sampietro DA (2020) Antifungal activity of Bignoniaceae plants on *aspergillus carbonarius* and *aspergillus Niger*. Nat Prod Res 34(18):2656–2659
- Arancibia L, Naspi C, Pucci G, María ARCE (2010) Aromatic plants from Patagonia: chemical composition and antimicrobial activity of the essential oil of *Senecio mustersii* and *S. subpan-duratus*. Bol Latinoam Caribe Plantas Med Aromat 9(2):123–126
- Aristimuño Ficooseco ME, Sequin CJ, Aceñolaza PG, Vattuone MA, Catalán CAN, Sampietro DA (2017) Antifungal metabolites from *Schinopsis balansae* Engl (Anacardiaceae): isolation, identification and evidences of their mode of action on *fusarium graminearum* Schwabe. Nat Prod Res 31(12):1450–1453
- Ayaz M, Ullah F, Sadiq A, Ullah F, Ovais M, Ahmed J, Devkota HP (2019) Synergistic interactions of phytochemicals with antimicrobial agents: potential strategy to counteract drug resistance. Chem Biol Interact 308:294–303
- Basak G, Das N (2014) Characterization of sophorolipid biosurfactant produced by *Cryptococcus* sp. VITGBN2 and its application on Zn (II) removal from electroplating wastewater. J Environ Biol 35(6):1087–1094
- Biradar SS, Wadkar GH, Rasal VP, Kane SR (2010) Anti-fungal screening of tubers of *Cyperus rotundus* Linn. Res J Pharmacogn Phytochem 2(4):317–318
- Bonifácio B, Vila T, Fantacini Masiero I, Bento da Silva P, Da Silva IC, Lopes de Oliveira É, dos Santos Ramos MA, Perez de Souza L, Vilegas W, Rogério Pavan F, Chorilli M, Lopez-Ribot JL, Bauab TM (2019) Antifungal activity of a Hydroethanolic extract from *Astronium urundeuva* leaves against *Candida albicans* and *Candida glabrata*. Front Microbiol 10:1–12
- Braga FG, Bouzada MLM, Fabri RL, Matos MDO, Moreira FO, Scio E, Coimbra ES (2007) Antileishmanial and antifungal activity of plants used in traditional medicine in Brazil. J Ethnopharmacol 111(2):396–402
- Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods-a review. Int J Food Microbiol 94(3):223–253
- Butassi E, Svetaz LA, Ivancovich JJ, Feresin GE, Tapia A, Zacchino SA (2015) Synergistic mutual potentiation of antifungal activity of *Zuccagnia punctata* Cav. And *Larrea nitida* Cav. Extracts in clinical isolates of *Candida albicans* and *Candida glabrata*. Phytomedicine 22(6):666–678
- Butassi E, Svetaz LA, Zhou S, Wolfender JL, Cortes JCG, Ribas JC, Diaz C, Perez del Palacio J, Vicente F, Zacchino SA (2019) The antifungal activity and mechanisms of action of quantified extracts from berries, leaves and roots of *Phytolacca tetramera*. Phytomedicine 60:152884
- Caneschi CA, Martins FJ, Larrudé DG, Romani EC, Brandão MAF, Raposo NRB (2015) In vitro antifungal activity of *Baccharis trimera* less (DC) essential oil against dermatophytes. Trop J Pharm Res 14(11):2083–2089
- Carpinella MC, Ruiz G, Palacios SM (2010) Screening of native plants of Central Argentina for antifungal activity. Allelopathy J 25(2):423–431
- Carrasco H, Robles-Kelly C, Rubio J, Olea AF, Martínez R, Silva-Moreno E (2017) Antifungal effect of polygodial on *Botrytis cinerea*, a fungal pathogen affecting table grapes. Int J Mol Sci 18(11):2251
- Carrizo SL, Zampini IC, Sayago JE, Simirgiotis MJ, Bórquez J, Cuello AS, Isla MI (2020) Antifungal activity of phytotherapeutic preparation of *Baccharis* species from Argentine Puna against clinically relevant fungi. J Ethnopharmacol 251:112553
- Céspedes CL, Avila JG, García AM, Becerra J, Flores C, Aqueveque P, Bittner M, Hoeneisen M, Martínez M, Silva M (2006) Antifungal and antibacterial activities of *Araucaria araucana* (Mol.) K. Koch heartwood lignans. Z. Naturforsch. C. 61(1–2):35–43

- Chen C, Cai D, Qin P, Chen B, Wang Z, Tan T (2018) Bio-plasticizer production by hybrid acetone-butanol-ethanol fermentation with full cell catalysis of *Candida* sp. 99–125. *Bioresour Technol* 257:217–222
- CLSI (Clinical and Laboratory Standards Institute) (2017) Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard-document M-27, 4th edn. Wayne, Pennsylvania, USA
- Contesini FJ, Lopes DB, Macedo GA, da Graça Nascimento M, de Oliveira Carvalho P (2010) *Aspergillus* sp. lipase: potential biocatalyst for industrial use. *J Mol Catal B Enzym* 67(3–4):163–171
- Cordisco E, Sortino M, Svetaz L (2019) Antifungal activity of traditional medicinal plants from Argentina: effect of their combination with antifungal drugs. *Curr Tradit Med* 5(1):75–95
- Córdoba S, Vivot W, Szusz W, Albo G (2019) Antifungal activity of essential oils against *Candida* species isolated from clinical samples. *Mycopathologia* 184:615–623
- Costa DP, Alves Filho EG, Silva LM, Santos SC, Passos XS, Silva MRR, Seraphin JC, Ferri PH (2010) Influence of fruit biotypes on the chemical composition and antifungal activity of the essential oils of *Eugenia uniflora* leaves. *J Braz Chem Soc* 21(5):851–858
- da Silva SL, Figueredo PM, Yano T (2006) Antibacterial and antifungal activities of volatile oils from *Zanthoxylum rhoifolium*. *Leaves Pharm Biol* 44(9):657–659
- Dai T, Fuchs BB, Coleman JJ, Prates RA, Astrakas C, St Denis TG, Ribeiro MS, Mylonakis E, Hamblin MR, Tegos G (2012) Concepts and principles of photodynamic therapy as an alternative antifungal discovery platform. *Front Microbiol* 3:120
- Davicino R, Mattar MA, Casali YA, Correa SG, Pettenati EM, Micalizzi B (2007) Actividad antifúngica de extractos de plantas usadas en medicina popular en Argentina. *Rev Peru Biol* 14(2):247–252
- De Los Páez AV, Albornoz PL, Lizarraga E, Sobrero MT, Chaila S (2019) Anatomía foliar y caulinar, y caracterización fitoquímica foliar de *Flaveria bidentis* y *F. haumanii* (Asteraceae) de Santiago del Estero, Argentina. *Act. Bot. Mex*:126
- Delucchi G (2006) Las especies vegetales amenazadas de la Provincia de Buenos Aires: Una actualización. *Aprona Bol Cient* 39:19–31
- Demo M, Oliva MDL, López ML, Zunino MP, Zygodlo JA (2005) Antimicrobial activity of essential oils obtained from aromatic plants of Argentina. *Pharm Biol* 43(2):129–134
- Derita MG, Leiva ML, Zacchino SA (2009) Influence of plant part, season of collection and content of the main active constituent, on the antifungal properties of *Polygonum acuminatum* Kunth. *J Ethnopharmacol* 124(3):377–383
- Derita M, Montenegro I, Garibotto F, Enriz R, Cuellar Fritis M, Zacchino S (2013) Structural requirements for the antifungal activities of natural drimane sesquiterpenes and analogues, supported by conformational and electronic studies. *Molecules* 18(2):2029–2051
- Derita M, Zacchino S (2011a) Validation of the ethnopharmacological use of *Polygonum persicaria* for its antifungal properties. *Nat Prod Comm* 6(7):931–933
- Derita M, Zacchino S (2011b) Chemotaxonomic importance of sesquiterpenes and flavonoids in Argentinian species of *Polygonum* genus. *J Ess Oil Res* 23:11–14
- Di Ciaccio LS, Catalano AV, López PG, Rojas D, Cristos D, Fortunato RH, Salvat AE (2020) In vitro antifungal activity of *Peltophorum dubium* (Spreng.) Taub. Extracts against *aspergillus flavus*. *Plants* 9(4):438
- di Ciaccio LS, Fortunato RH, Salvat AE (2018) Antifungal activity of species of the genus *Senna* (Caesalpinioideae, Leguminosae) from northern Argentina against *fusarium verticillioides*. *Rev Investig Agropecu* 44(1):111–120
- Di Ciaccio LS, Spotorno VG, Córdoba Estévez MM, Rios DJL, Fortunato RH, Salvat AE (2018) Antifungal activity of *Parastrephia quadrangularis* (Meyen) Cabrera extracts against *fusarium verticillioides*. *Lett Appl Microbiol* 66(3):244–251
- Di Libertò MGD, Stegmayer MI, Svetaz LA, Derita MG (2019) Evaluation of Argentinean medicinal plants and isolation of their bioactive compounds as an alternative for the control of post-harvest fruits phytopathogenic fungi. *Rev Bras Farmacol* 29(5):686–688

- Di Liberto M, Svetaz L, Castelli MV, Rai M, Derita M (2017) Role of essential oils for the cure of human pathogenic fungal infections. In: Rai M, Zacchino S, Derita M (eds) Essential oils and nanotechnology for treatment of microbial diseases. CRC Press/Taylor & Francis Group, U.S.A, pp 127–142
- do Amaral CC, Fernandes GF, Rodrigues AM, Burger E, de Camargo ZP (2019) Proteomic analysis of *Paracoccidioides brasiliensis* complex isolates: correlation of the levels of differentially expressed proteins with in vivo virulence. PLoS One 14(7):e0218013
- do Prado AC, Garces HG, Bagagli E, Rall VLM, Furlanetto A, Fernandes Junior A, Furtado FB (2018) Schinus molle essential oil as a potential source of bioactive compounds: antifungal and antibacterial properties. J Appl Microbiol 126(2):516–522
- Donnelly RF, McCarron PA, Tunney MM (2008) Antifungal photodynamic therapy. Microbiol Res 163(1):1–12
- dos Santos Silva F, Landell MF, Paulino GVB, Coutinho HDM, Albuquerque UP (2020) Antifungal activity of selected plant extracts based on an ethnodirected study. Acta Bot Bras 34(2):442–448
- Downum KR, Towers GHN (1983) Analysis of thiophenes in the Tageteae (Asteraceae) by HPLC. J Nat Prod 46(1):98–103
- Duyvesteijn RG, Van Wijk R, Boer Y, Rep M, Cornelissen BJ, Haring MA (2005) Frp1 is a *fusarium oxysporum* F-box protein required for pathogenicity on tomato. Mol Microbiol 57(4):1051–1063
- Dwivedy AK, Kumar M, Upadhyay N, Prakash B, Dubey NK (2016) Plant essential oils against food borne fungi and mycotoxins. Curr Opin Food Sci 11:16–21
- ElSohly HN, Joshi AS, Nimrod AC, Walker LA, Clark AM (2001) Antifungal chalcones from *Maclura tinctoria*. Planta Med 67(1):87–89
- EMA (European Medicines Agency) (2010) Guidelines on Declaration of Herbal Substances and Herbal Preparations in Herbal Medicinal Products/Traditional Herbal Medicinal Products. EMA/HMPC/CHMP/CVMP/287539/2005 Rev 1. Available at http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003272.pdf. (Accessed 27.09.2020)
- Enshaeieh M, Abdoli A, Nahvi I (2013) Medium optimization for biotechnological production of single cell oil using *Yarrowia lipolytica* M7 and *Candida* sp. J Cell Molecular Research 5(1):17–23
- Escalante A, Gattuso M, Pérez P, Zacchino S (2008) Evidence for the mechanism of action of the antifungal phytolaccoside B isolated from *Phytolacca tetramera* Hauman. J Nat Prod 71:1720–1725
- Escalante A, Santecchia C, López S, Gattuso M, Gutiérrez Ravelo A, Delle Monache F, Zacchino S (2002) Isolation of antifungal saponins from *Phytolacca tetramera*, an Argentinean species in critic risk. J Ethnopharmacol 82(1):29–34
- Espino M, Solari M, de los Ángeles Fernández M, Boiteux J, Gómez MR, Silva MF (2019) NADES-mediated folk plant extracts as novel antifungal agents against *Candida albicans*. J Pharm Biomed Anal 167:15–20
- Feresín G, Alejandro T, Silvia NL, Susana AZ (2001) Antimicrobial activity of plants used in traditional medicine of San Juan province, argentine J. Ethnopharmacol 78(1):103–107
- Filip R, Davicino R, Anesini C (2010) Antifungal activity of the aqueous extract of *Ilex paraguayensis* against *Malassezia furfur*. Phytother Res 24:715–719
- Fiuza TDS, de Sabóia-Morais SMT, de Paula JR, Tresvenzol LM, Pimenta FC (2009) Antimicrobial activity of the crude ethanol extract from *Hyptidendron canum* leaves. Pharm Biol 47(7):640–644
- Forsyth LM, Smith LJ, Aitken EA (2006) Identification and characterization of non-pathogenic *fusarium oxysporum* capable of increasing and decreasing fusarium wilt severity. Mycol Res 110(8):929–935
- Freiesleben S, Jäger A (2014) Correlation between plant secondary metabolites and their antifungal mechanisms—a review. Medicinal Aromat Plan Theory 3(2):1–6

- Freile M, Giannini F, Sortino M, Zamora M, Juárez A, Zacchino S, Enriz D (2006) Antifungal activity of aqueous extracts and of berberine isolated from *Berberis heterophylla*. *Acta Farm Bonaer* 25(1):83–88
- Fu J, Cheng K, Zhang ZM, Fang RQ, Zhu HL (2010) Synthesis, structure and structure–activity relationship analysis of caffeic acid amides as potential antimicrobials. *Eur J Med Chem* 45(6):2638–2643
- Gaitán I, Paz AM, Zacchino SA, Tamayo G, Giménez A, Pinzón R, Cáceres A, Gupta MP (2011) Subcutaneous antifungal screening of Latin American plant extracts against *Sporothrix schenckii* and *Fonsecaea pedrosoi*. *Pharm Biol* 49(9):907–919
- Galvez CE, Jimenez CM, Gomez ADLA, Lizarraga EF, Sampietro DA (2020) Chemical composition and antifungal activity of essential oils from *Senecio nutans*, *Senecio viridis*, *Tagetes terniflora* and *Aloysia gratissima* against toxigenic *Aspergillus* and *Fusarium* species. *Nat Prod Res* 34(10):1442–1445
- Ghaffar I, Imtiaz A, Hussain A, Javid A, Jabeen F, Akmal M, Qazi JI (2018) Microbial production and industrial applications of keratinases: an overview. *Int Microbiol* 21(4):163–174
- Giacone L, Cordisco E, Garrido MC, Petenatti E, Sortino M (2020) Photodynamic activity of *Tagetes minuta* extracts against superficial fungal infections. *Med Mycol* 58(6):797–809
- Gil A, Ghersa CM, Perelman S (2002) Root thiophenes in *Tagetes minuta* L. accessions from Argentina: genetic and environmental contribution to changes in concentration and composition. *Biochem. Syst. Ecol* 30(1):1–13
- Gomes D, Zanchet B, Locateli G, Benvenuti R, Dalla Vecchia C, Schönell A, Diel K, Zilli G, Miorando D, Ernetti J, Oliveira B, Zanotelli P, Santos M, Barisson A, Banzato T, Ruiz A, Carvalho J, Cechinel Filho V, García P, San Feliciano A, Roman Junior W (2018) Antiproliferative potential of solidagenone isolated of *Solidago chilensis*. *Rev Bras Farmacogn* 28:703–709
- Gomez AA, Terán Baptista ZP, Mandova T, Barouti A, Kritsanida M, Grougnet R et al (2019) Antifungal and antimycotoxicigenic metabolites from native plants of Northwest Argentina: isolation, identification and potential for control of *aspergillus* species. *Nat Prod Res* 1–4
- Guerra F, Ansari AA, Kurup R, Subramanian G (2020) Antifungal activity of *Senna alata*, *Senna bicapsularis* and *Ptyrogramma calomelanos*. *J Complement Altern Medical Res* 10(3):11–21
- Gurgel LA, Sidrim JJC, Martins DT, Cechinel F, Rao V, V.S. (2005) In vitro antifungal activity of dragon's blood from *croton urucurana* against dermatophytes. *J Ethnopharmacol* 97(2):409–412
- Hemaiswarya S, Kruthiventi AK, Doble M (2008) Synergism between natural products and antibiotics against infectious diseases. *Phytomedicine* 15(8):639–652
- Hernández M, Rodríguez A, Gallo D, Fernández D (2008) *Phytolacca tetramera* Hauman, una especie amenazada de la Provincia de Buenos Aires. In: Resumen Jornada “Seminario Técnico en la Reserva de Biosfera Parque Costero del Sur como herramienta del conocimiento científico”. Buenos Aires, Municipalidad de Magdalena
- Huang Y, Zhao J, Zhou L, Wang J, Gong Y, Chen X, Guo Z, Wang Q, Jiang W (2010) Antifungal activity of the essential oil of *Illicium verum* fruit and its main component trans-anethole. *Molecules* 15(11):7558–7569
- Ibrahim SRM, Abdallah HM, El-Halawany AM, Esmat A, Mohamed GA (2018) Thiotagetin B and tagetannins A and B, new acetylenic thiophene and digalloyl glucose derivatives from *Tagetes minuta* and evaluation of their in vitro antioxidative and anti-inflammatory activity. *Fitoterapia* 125:78–88
- Ibrahim SRM, Abdallah HM, El-Halawany AM, Mohamed GA (2016) Naturally occurring thiophenes: isolation, purification, structural elucidation, and evaluation of bioactivities. *Phytochem Rev* 15:197–220
- Isla MI, Moreno MA, Nuño G, Rodríguez F, Carabaja A, Alberto MR, Zampini IC (2016) *Zuccagnia punctata*: a review of its traditional uses, phytochemistry, pharmacology and toxicology. *Nat Prod Commun* 11(11):1749–1755
- Jiménez-Reyes MF, Carrasco H, Olea AF, Silva-Moreno E (2019) Natural compounds: a sustainable alternative to the phytopathogens control. *J Chil Chem Soc* 64(2):4459–4465

- Johnson RR (1964) Monograph of the plant genus *Porophyllum* (Compositae, Helenieae). University of Kansas
- Juárez-Becerra GP, Sosa-Morales ME, López-Malo A (2010) Hongos fitopatógenos de alta importancia económica: descripción y métodos de control. *Temas Selectos de Ingeniería de Alimentos* 4(2):14–23
- Karpiński TM (2020) Essential oils of Lamiaceae Family plants as antifungals. *Biomol Ther* 10(1):103
- Kato IT, Prates RA, Sabino CP, Fuchs BB, Tegos GP, Mylonakis E, Hamblin MR, Ribeiro MS (2013) Antimicrobial photodynamic inactivation inhibits *Candida albicans* virulence factors and reduces in vivo pathogenicity. *Antimicrob Agents Chemother* 57(1):445–451
- Kaur N, Dhuna V, Kamboj SS, Agrewala JN, Singh J (2006) A novel antiproliferative and antifungal lectin from *Amaranthus viridis* Linn seeds. *Protein Pept Lett* 13(9):897–905
- Kurdelas RR, Lima B, Tapia A, Egly Feresin G, Gonzalez Sierra M, Rodríguez MV, Zacchino S, Enriz RD, Freile ML (2010) Antifungal activity of extracts and prenylated coumarins isolated from *Baccharis darwinii* Hook & Arn. (Asteraceae). *Molecules* 15(7):4898–4907
- Lacaz CDS, Porto E, Martins JEC, Heins-Vaccari EM, Takahashi de Melo N (2002) Tratado de micología médica. *Rev Inst Med Trop S Paulo* 44(5):297–298
- Liang YI, Lu L, Chen Y, Lin Y (2016) Photodynamic therapy as an antifungal treatment. *Exp Ther Med* 12:23–27
- Liberto MD, Svetaz L, Furlán RL, Zacchino SA, Delporte C, Novoa MA, Asencio M, Cassels BK (2010) Antifungal activity of saponin-rich extracts of *Phytolacca dioica* and of the saponinins obtained through hydrolysis. *Nat Prod Commun* 5(7):1013–1018
- Lima B, López S, Luna L, Agüero MB, Aragón L, Tapia A, Zacchino S, López ML, Zygadio J, Feresin GE (2011) Essential oils of medicinal plants from the Central Andes of Argentina: chemical composition, and antifungal, antibacterial, and insect-repellent activities. *Chem Biodivers* 8(5):924–936
- Lima CS, Polaquini CR, dos Santos MB, Gullo FP, Leite FS, Scorzoni L, da Silva Bolzani V, Mendez-Giannini MJ, Fusco-Almeida AM, Alvez Rezende A, Regasini LO (2016) Anti-*Candida* and anti-*Cryptococcus* evaluation of 15 non-alkaloidal compounds from *Pterogyne nitens*. *Asian Pac J Trop Biomed* 6(10):841–845
- Lima B, Sánchez M, Luna L, Agüero MB, Zacchino S, Filippa E, Palermo JA, Tapia A, Feresin GE (2012) Antimicrobial and antioxidant activities of *Gentianella multicaulis* collected on the Andean slopes of San Juan Province, Argentina *Z Naturforsch C* 67(1–2):29–38
- Lizcano González MC (2007) Evaluación de la actividad antifúngica del extracto de tomillo (*Thymus vulgaris*) contra *Botrytis cinerea*. Pontificia Universidad Javeriana, Bogotá, Fusarium oxysporum y Sclerotinia sclerotiorum. pH Tesis
- Loockerman DJ, Turner BL, Jansen RK (2003) Phylogenetic relationships within the Tageteae (Asteraceae) based on nuclear ribosomal ITS and chloroplast ndhF gene sequences. *Syst Bot* 28(1):191–207
- López SN, Furlan RLE, Zacchino SA (2011) Detection of antifungal compounds in *Polygonum ferrugineum* Wedd. Extracts by bioassay-guided fractionation. Some evidences of their mode of action. *J Ethnopharmacol* 138(2):633–636
- Maisch T, Baier J, Franz B, Maier M, Landthaler M, Szeimies RM, Baumler W (2007) The role of singlet oxygen and oxygen concentration in photodynamic inactivation of bacteria. *Proc Natl Acad Sci* 104(17):7223–7228
- Malarkodi E, Manoharan A (2013) Antifungal activity of *Parthenium hysterophorus* L. *J Chem Pharm Res* 5(1):137–139
- Mamone L, Di Venosa G, Gándara L, Sáenz D, Vallecorsa P, Schickinger S, Rossetti MV, Battle A, Buzzola F, Casas A (2014) Photodynamic inactivation of gram-positive bacteria employing natural resources. *J Photochem Photobiol B Biol* 133:80–89
- Marotti I, Marotti M, Piccaglia R, Nastri A, Grandi S, Dinelli G (2010) Thiophene occurrence in different *Tagetes* species: agricultural biomasses as sources of biocidal substances. *J Sci Food Agric* 90:1210–1217
- Martínez JA (2012) Natural fungicides obtained from plants. INTECH Open Access Publisher

- Mensah AY, Houghton PJ, Dickson RA, Fleischer TC, Heinrich M, Bremner P (2006) In vitro evaluation of effects of two Ghanaian plants relevant to wound healing. *Phytother Res* 20(11):941–944
- Mishra PK, Singh P, Prakash B, Kedia A, Dubey NK, Chanotiya CS (2013) Assessing essential oil components as plant-based preservatives against fungi that deteriorate herbal raw materials. *Int Biodeterior Biodegradation* 80:16–21
- Mohanta YK, Laxmipriya P, Panda SK (2014) Antifungal activity of *Eleutherine bulbosa* bulb against mycelial fungus. *Int J Agric Technol* 10(5):1165–1171
- Mongkol R, Piapukiew J, Chavasiri W (2016) Chemical constituents from *Melodorum fruticosum* Lour. Flowers against plant pathogenic fungi. *Agric Nat Resour* 50(4):270–275
- Montez-Belmont RM, Cruz VC, Martínez GM, García GS, Licona RG, Domínguez SZ, Bravo-Luna L, Bermúdez-Torres K, Flores-Moctezuma HE, Caravajal-Moreno MC (2000) Propiedades antifúngicas en plantas superiores. Análisis retrospectivo de investigaciones. *Rev Mex Fitopatol* 18(2):125–131
- Morais M, Amaral Pinto M, Araújo S, Fonseca Castro A, Duarte Almeida J, Rosa L, Rosa C, Johann S, Rodríguez dos Santos Lima, L. (2014) Antioxidant and antifungal activities of *Smilax campestris* Griseb. (Smilacaceae). *Nat Prod Res* 28(16):1275–1279
- Morin-Sardin S, Nodet P, Coton E, Jany JL (2017) *Mucor*: a Janus-faced fungal genus with human health impact and industrial applications. *Fungal Biol Rev* 31(1):12–32
- Muñoz-Concha D, Vogel H, Yunes R, Razmilic I, Bresciani L, Malheiros A (2007) Presence of polygodial and drimenol in *Drimys* populations from Chile. *Biochem Sys Ecol* 35(7):434–438
- Murakami C, Lago JH, Perazzo FF, Ferreira KS, Lima ME, Moreno PR, Young MC (2013) Chemical composition and antimicrobial activity of essential oils from *Chromolaena laevigata* during flowering and fruiting stages. *Chem Biodivers* 10(4):621–627
- Muschietti L, Derita M, Sülsen V, de Dios Muñoz J, Ferraro G, Zacchino S, Martino V (2005) In vitro antifungal assay of traditional argentine medicinal plants. *J Ethnopharmacol* 102(2):233–238
- Nally MC, Pesce VM, Maturano YP, Muñoz CJ, Combina M, Toro ME, Castellanos de Figueroa LI, Vazquez F (2012) Biocontrol of *Botrytis cinerea* in table grapes by non-pathogenic indigenous *Saccharomyces cerevisiae* yeasts isolated from viticultural environments in Argentina. *Postharvest Biol Technol* 64(1):40–48
- Nenoff P, Krüger C, Ginter-Hanselmayer G, Tietz HJ (2013) Mycology—an update. Part 1: dermatomycoses: causative agents, epidemiology and pathogenesis. *J Dtsch Dermatol Ges* 12(3):188–210
- Nguyen TPA, Nguyen TTM, Nguyen NH, Nguyen TN, Dang TTP (2020) Application of yeast surface display system in expression of recombinant pediocin PA-1 in *Saccharomyces cerevisiae*. *Folia Microbiol* 2020
- Niño G (2015) Aislamiento y caracterización de biomoléculas producidas por especies vegetales que crecen en ecosistemas semiáridos. pH Tesis. Universidad Nacional de Tucumán
- Nwaguma IV, Chikere CB, Okpokwasili GC (2019) Effect of cultural conditions on biosurfactant production by *Candida* sp. isolated from the sap of *Elaeis guineensis*. *Biotechnol J Int* 23(3):1–14
- Oliva MM, Demo MS, López AG, López ML, Zygodlo JA (2006) Antimicrobial activity and composition of *Hyptis mutabilis* essential oil. *Int J Geogr Inf Syst* 11(4):57–63
- Ortega CA, María AOM, Gianello JC (2000) Chemical components and biological activity of *Bidens subalternans*, *B. aurea* (Asteraceae) and *Zuccagnia punctata* (Fabaceae). *Molecules* 5(3):465–470
- Pacciaroni ADV, de los Angeles Gette M, Derita M, Ariza-Espinar L, Gil RR, Zacchino SA, Silva GL (2008) Antifungal activity of *Heterothalamus alienus* metabolites. *Phytother Res* 22(4):524–528
- Pergomet J, Di Liberto M, Derita M, Bracca A, Kaufman T (2018) Activity of the pterophyllins 2 and 4 against postharvest fruit pathogenic fungi. Comparison with a synthetic analog and related intermediates *Fitoterapia* 125:98–105

- Petenatti E, Gette M, Derita M, Petenatti M, Solís C, Zuljan F, Del Vitto L.A, Zacchino, S. (2008) Importance of the ethnomedical information for the detection of antifungal properties in plant extracts from the Argentine flora. South American medicinal plants as potential source of bioactive compounds. Transworld Research Network, Kerala, pp 15–38
- Petri I, Gallo D, Ollier F (2010) First protected area in the partido de Magdalena for the preservation of ombusillo (*Phytolacca tetramera* Hauman), in situ. Agreement between the Department of Transportation of the province of Buenos Aires and the Faculty of Agricultural Sciences and Forestry of the National University of La Plata. Argentina Rev Colomb Biotecnol 12(2):259–261
- Pinto AP, Rosseti IB, Carvalho ML, da Silva BGM, Alberto-Silva C, Costa MS (2018) Photodynamic antimicrobial chemotherapy (PACT), using toluidine blue O inhibits the viability of biofilm produced by *Candida albicans* at different stages of development. Photodiagn Photodyn Ther 21:182–189
- Pioli RN, Benavídez R, Morandi EN, Bodrero M (2000) Epidemiological study of diseases associated to soybean carpels and seeds, in Santa Fe, Argentina. Fitopatología 35(2):111–118
- Portillo A, Vila R, Freixa B, Adzet T, Cañigual S (2001) Antifungal activity of Paraguayan plants used in traditional medicine. J Ethnopharmacol 76(1):93–98
- Postigo A, Funes M, Petenatti E, Bottai H, Pacciaroni A, Sortino M (2017) Antifungal photosensitive activity of *Porophyllum obscurum* (Spreng.) DC.: correlation of the chemical composition of the hexane extract with the bioactivity. Photodiagnosis Photodyn Ther 20:263–272
- Postigo A, Svetaz LA, Derita MG, Gette MA, Petenatti M, Del Vitto L, Petenatti E, Zacchino S (2012) Discovery of antifungal plants in Argentinean San Luis Province: ethnomedical information or random selection? Revista Eletrônica de Farmácia 9(1):22–22
- Prazeres JND, Cruz JAB, Pastore GM (2006) Characterization of alkaline lipase from *fusarium oxysporum* and the effect of different surfactants and detergents on the enzyme activity. Braz J Microbiol 37(4):505–509
- Quiroga EN, Sampietro DA, Sgariglia MA, Soberón JR, Vattuone MA (2009) Antimycotic activity of 5'-prenylisoflavanones of the plant *Geoffroea decorticans*, against *aspergillus* species. Int J Food Microbiol 132(1):42–46
- Quiroga EN, Sampietro AR, Vattuone MA (2001) Screening antifungal activities of selected medicinal plants. J Ethnopharmacol 74(1):89–96
- Ramirez J, Cartuche L, Morocho V, Aguilar S, Malagon O (2013) Antifungal activity of raw extract and flavanons isolated from *Piper ecuadorensis* from Ecuador. Rev Bras Farmacogn 23(2):370–373
- Reichling J (2018) Plant-microbe interactions and secondary metabolites with antibacterial, antifungal and antiviral properties. Annu Plant Rev 39:214–347
- Ríos JL, Recio MC (2005) Medicinal plants and antimicrobial activity. J Ethnopharmacol 100(1–2):80–84
- Ríos JL, Schinella GR, Andújar I (2018) Antipsoriatic medicinal plants, from traditional use to clinic. In: Martínez JL, Muñoz-Acevedo A, Rai M (eds) Ethnobotany: local knowledge and traditions. CRC Press, Taylor and Francis Group, pp 158–186
- Rodrigues FF, Oliveira LG, Rodrigues FF, Saraiva ME, Almeida SC, Cabral ME, Campos AR, Costa JGM (2012) Chemical composition, antibacterial and antifungal activities of essential oil from *Cordia verbenacea* DC leaves. Pharm Res 4(3):161–165
- Sa RA, Argolo AC, Napoleao TH, Gomes FS, Santos ND, Melo CM, Albuquerque AC, Xavier HS, Coelho LCB, Bieber LW, Paiva PM (2009) Antioxidant, *fusarium* growth inhibition and *Nasutitermes corniger* repellent activities of secondary metabolites from *Myracrodruon urundeuva* heartwood. Int Biodeterior Biodegradation 63(4):470–477
- Sabinil LI, Gabrielli PC, Torres CV, Escobar FM, Cacciabue M, Rovera M, Kolb N (2006) Study of the cytotoxic and antifungal activity of the essential oil of *Elyonurus muticus* against *Candida* spp. Strain Mol Med Chem 11(2):31–33
- Sacchetti G, Medici A, Maietti S, Radice M, Muzzoli M, Manfredini S, Braccioli E, Bruni R (2004) Composition and functional properties of the essential oil of Amazonian basil, *Ocimum*

- micranthum* Willd., Labiatae in comparison with commercial essential oils. *J Agric Food Chem* 52(11):3486–3491
- Salah H, Al-Hatmi AM, Theelen B, Abukamar M, Hashim S, van Diepeningen AD, Lass-Flörl C, Boekhout T, Almaslamani M, Taj-Aldeen SJ (2015) Phylogenetic diversity of human pathogenic *fusarium* and emergence of uncommon virulent species. *J Infect* 71(6):658–666
- Sampietro DA, Belizan MME, Baptista ZPT, Vattuone MA, Catalán CA (2014) Essential oils from *Schinus* species of Northwest Argentina: composition and antifungal activity. *Nat Prod Commun* 9(7):1019–1022
- Santhoshkumar J, Sowmya B, Kumar SV, Rajeshkumar S (2019) Toxicology evaluation and anti-dermatophytic activity of silver nanoparticles synthesized using leaf extract of *Passiflora caerulea*. *S Afr J Chem Eng* 29:17–23
- Shukla AC (2018) Essential oils as green pesticides for postharvest disease management. *Acta Hort* 1210:199–206
- Siewert B, Stuppner H (2019) The photoactivity of natural products—An overlooked potential of phytomedicines? *Phytomedicine* 60:152985
- Singh CJ (2011) Extracellular protease expression in *Microsporium gypseum* complex, its regulation and keratinolytic potential. *Mycoses* 54(4):e183–e188
- Soberón JR, Sgariglia MA, Torrez JAC, Aguilar FA, Pero EJ, Sampietro DA, de Luco JF, Labadie GR (2020) Antifungal activity and toxicity studies of flavanones isolated from *Tessaria dodeifolia* aerial parts. *Heliyon* 6(10):e05174
- Sobrinho ACN, de Souza EB, Rocha MFG, Albuquerque MRJ, Bandeira PN, dos Santos HS, de Paula Cavalacante CS, Souza Oliveira S, Rodrigues Aragão P, de Moraes, S.M. dos Santos Fontenelle, R.O. (2016) Chemical composition, antioxidant, antifungal and hemolytic activities of essential oil from *Baccharis trinervis* (lam.) Pers. (Asteraceae). *Ind. Crops Prod* 84:108–115
- Spitzer M, Robbins N, Wright GD (2017) Combinatorial strategies for combating invasive fungal infections. *Virulence* 8(2):169–185
- Stegmayer MI, Fernandez LN, Olivella L, Gutierrez HF, Favaro MA, Derita MG (2020) Aceites esenciales provenientes de plantas nativas para el control de hongos fitopatógenos que afectan a frutales. *Revista FAVE* 20. in press
- Stein AC, Sortino M, Avancini C, Zacchino S, von Poser G (2005) Ethnoveterinary medicine in the search for antimicrobial agents: antifungal activity of some species of *Pterocaulon* (Asteraceae). *J Ethnopharmacol* 99(2):211–214
- Stopiglia CDO, da Rocha Vianna D, de Carvalho Meirelles G, Teixeira H, Von Poser GL, Scroferneker ML (2011) Antifungal activity of *Pterocaulon* species (Asteraceae) against *Sporothrix schenckii*. *J Mycolm Méd* 21(3):169–172
- Svetaz L, Agüero MB, Alvarez S, Luna L, Feresin G, Derita M, Tapia A, Zacchino S (2007) Antifungal activity of chalcones from *Zuccagnia punctata* Cav. Acting against clinically important fungi and studies of mechanism of action. *Planta Med* 73:1074–1080
- Svetaz L, Tapia A, López S, Furlán R, Petenatti E, Pioli R, Schmeda-Hirschmann G, Zacchino S (2004) Antifungal chalcones and new caffeic acid esters from *Zuccagnia punctata* acting against soybean infecting fungi. *J Agric Food Chem* 52(11):3297–3300
- Svetaz L, Zuljan F, Derita M, Petenatti E, Tamayo G, Cáceres A, Filho VC, Giménez A, Pinzón R, Zacchino S, Gupta M (2010) Value of the ethnomedical information for the discovery of plants with antifungal properties. A survey among seven Latin American countries. *J Ethnopharmacol* 127(1):137–158
- Teal LJ, Schultz KM, Weber DJ, Gergen MF, Miller MB, DiBiase LM, Sickbert-Bennett E, Rutala WA (2016) Invasive cutaneous *Rhizopus* infections in an immunocompromised patient population associated with hospital laundry carts. *Infect Control Hosp Epidemiol* 37(10):1251–1253
- Tempone AG, Sartorelli P, Teixeira D, Prado FO, Calixto IA, Lorenzi H, Melhem MS (2008) Brazilian flora extracts as source of novel antileishmanial and antifungal compounds. *Mem Inst Oswaldo Cruz* 103(5):443–449

- Thembo KM, Vismar HF, Nyazema NZ, Gelderblom WCA, Katerere DR (2010) Antifungal activity of four weedy plant extracts against selected mycotoxigenic fungi. *J Appl Microbiol* 109(4):1479–1486
- Thirunavukarasu K, Purushothaman S, Sridevi J, Aarthi M, Gowthaman MK, Nakajima-Kambe T, Kamini NR (2016) Degradation of poly (butylene succinate) and poly (butylene succinate-co-butylene adipate) by a lipase from yeast *Cryptococcus* sp. grown on agro-industrial residues. *Int. Biodeterior. Biodegradation* 110:99–107
- Torres AM, Ramirez ML, Chulze SN (2009) *Fusarium* and fumonisins in maize in South America. In: Rai M, Varma A (eds) *Mycotoxins in food, Feed and Bioweapons*. Springer, Berlin, Heidelberg, pp 179–200
- Vaghela J, Rana M, Savalia V, Sheth NR (2009) Evaluation of antifungal activity of methanolic extract of leaves and stems of *Solanum sisymbriifolium* LAM. *Pharmacologyonline* 3:1–5
- Valarezo E, Arias A, Cartuche L, Meneses M, Ojeda-Riascos S, Morocho V (2016) Biological activity and chemical composition of the essential oil from *Chromolaena laevigata* (lam.) RM King & H. rob. (Asteraceae) from Loja, Ecuador. *J Essent Oil-Bear Plan Theory* 19(2):384–390
- Vasudevan P, Kashyap S, Sharma S (1997) Tagetes: a multipurpose plant. *Bioresour Technol* 62(1–2):29–35
- Vattuone MA, Soberón JR, Sgariglia MA, Quiroga EN, Sampietro DA (2013) *Zuccagnia punctata* Cav.: Phytochemistry, traditional uses and pharmacology. In: Céspedes CL, Sampietro DA, Seigler DS, Rai M (eds) *Natural antioxidants and biocides from wild medicinal plants*. CABI, pp 178–186
- Venturi CR, Danielli LJ, Klein F, Apel MA, Montanha JA, Bordignon SA, Roehe PM, Fuentesfria AM, Henriques AT (2015) Chemical analysis and in vitro antiviral and antifungal activities of essential oils from *Glechon spathulata* and *Glechon marifolia*. *Pharm Biol* 53(5):682–688
- Vio-Michaelis S, Apablaza-Hidalgo G, Gómez M, Peña-Vera R, Montenegro G (2012) Antifungal activity of three Chilean plant extracts on *Botrytis cinerea*. *Bot Sci* 90(2):179–183
- Wagner H, Ulrich-Merzenich G (2009) Synergy research: approaching a new generation of phyto-pharmaceuticals. *Phytomedicine* 16(2–3):97–110
- Wainwright M, Maisch T, Nonell S, Plaetzer K, Almeida A, Tegos GP, Hamblin MR (2017) Photoantimicrobials-are we afraid of the light? *Lancet Infect Dis* 17(2):49–55
- Yang CJ, Gao Y, Du KY, Luo XY (2019) Screening of 17 Chinese medicine plants against phytopathogenic fungi and active component in *Syzygium aromaticum*. *J Plant Dis Prot* 127:237–244
- Yang SK, Yusoff K, Mai CW, Lim WM, Yap WS, Lim SH, Lai KS (2017) Additivity vs synergism: investigation of the additive interaction of cinnamon bark oil and meropenem in combinatory therapy. *Molecules* 22(11):1733
- Zacchino SA, López SN, Pezzenati G, Furlán RL, Santecchia CB, Muñoz L, Giannini FA, Rodríguez AM, Enriz RD (1999) In vitro evaluation of antifungal properties of phenylpropanoids and related compounds acting against dermatophytes. *J Nat Prod* 62(10):1353–1357
- Zavrel M, White TC (2015) Medically important fungi respond to azole drugs: an update. *Future Microbiol* 10(8):1355–1373
- Zhang Z, ElSohly HN, Jacob MR, Pasco DS, Walker LA, Clark AM (2002) Natural products inhibiting *Candida albicans* secreted aspartic proteases from *Lycopodium cernuum*. *J Nat Prod* 65(7):979–985
- Zhang L, Yan K, Zhang Y, Huang R, Bian JM, Zheng C, Sun H, Chen Z, Sun N, An R, Min F, Zhao W, Zhuo Y, You J, Song Y, Yu Z, Liu Z, Yang K, Gao H, Dai H, Zhang X, Wang J, Fu C, Pei G, Liu J, Zhang S, Goodfellow M, Jiang Y, Kuai J, Zhou G, Chen X (2007) High-throughput synergy screening identifies microbial metabolites as combination agents for the treatment of fungal infections. *Proc Natl Acad Sci* 104(11):4606–4611
- Ziedan EHE, Farrag ESH (2008) Fumigation of peach fruits with essential oils to control postharvest decay. *Res J Agric Biol Sci* 4(5):512–519

Part III
Mushrooms/Beehive as Antimicrobials and
Delivery Systems

Chapter 13

Natural Antimicrobials from Basidiomycota Mushrooms



Vladimir Elisashvili, Mikheil D. Asatiani, Tamar Khardziani,
and Mahendra Rai

Abstract Basidiomycota mushrooms are one of the best and rich sources of natural bioactive compounds, including antitumor, antioxidant, antimicrobial, and many others. The search for natural sources of chemically new, safe, and effective antibiotics for biological control of invasive organisms and the development of effective and competitive technologies for their production has become a scientific and technological challenge. This chapter mostly summarizes recently published reports and own data on the occurrence of antibacterial and antifungal activities among Basidiomycota species collected from different geographical regions and ecological niches. Antibiotic activities of extracts from fruiting bodies and mycelial cultures against different groups of microorganisms are compared, focusing on the diversity, common characteristics, and unique properties of individual mushrooms, as well as on several physiological approaches and strategies that enhance the biosynthetic potential of mushrooms and their antimicrobial activity to provide a sustainable source of a safe, useful, and cheap medicine for the treatment of infectious diseases.

Keywords Basidiomycetes · Medicinal mushrooms · Antimicrobial activity · Antimicrobial compounds · Cultivation conditions

Abbreviations

ABA	Antibacterial activity
AFA	Antifungal activity
CFU	Colony-forming units
CL	Culture liquid
EPS	Exopolysaccharides
FB	Fruiting bodies

V. Elisashvili (✉) · M. D. Asatiani · T. Khardziani
Institute of Microbial Biotechnology, Agricultural University of Georgia, Tbilisi, Georgia
e-mail: v.elisashvili@agruni.edu.ge

M. Rai
Department of Biotechnology, SGB Amravati University, Amravati, Maharashtra, India

GI	Growth inhibition
G-	Gram-negative
G+	Gram-positive
IC ₅₀	Concentration inhibiting 50% of the growth
IZD	Internal zone diameter
MIC	Minimal inhibitory concentration
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
PDA	Potato dextrose agar

1 Introduction

Bacterial and fungal infections that cause significant damage to people, farm animals, crops, and other organisms lead to health problems and huge economic losses. Over the past decades, antibiotics have been effectively used to combat infections while synthetic fungicides have been used in the fight against phytopathogenic fungi. However, their use has led to the emergence and spread of multidrug resistance of pathogenic microorganisms, environmental pollution with poorly biodegradable, toxic, and carcinogenic chemicals, as well as the penetration of these compounds into food products. Nowadays, one of the main challenges for the scientific community and pharmaceutical companies is the lack of structurally new classes of antibiotics effective against pathogens (Genilloud 2012; Shen et al. 2017). Therefore, the search for new natural sources of chemically novel, safe, and effective antimicrobial compounds for biological control of invasive organisms and the development of efficient and competitive technologies for their production has become a scientific and technological task.

Basidiomycota fungi represent taxonomically, ecologically, physiologically, and genetically extremely diverse group of eukaryotic organisms. Among them, medicinal mushrooms have a proven history of use in many countries around the world. Mushrooms are mainly used in large quantities for food because of their high nutritional value and beneficial properties for human health (Wasser 2014; Gargano et al. 2017; Badalyan et al. 2019). During the last decades, medicinal mushrooms have become an attractive source of low-calorie, low fat-containing functional food, and therapeutic products mainly because of their chemical composition and ability to synthesize many highly beneficial bioactive compounds, primary and secondary metabolites, such as proteins, essential amino acids, fatty acids, dietary fiber, polysaccharides (mainly β -glucans), vitamins, macro- and microelements, lectins, terpenoids, steroids, statins, phenols, alkaloids, and antibiotics (Lindequist et al. 2005; Cohen et al. 2014; Gargano et al. 2017; Xue et al. 2020).

The literature data provides clear evidence that many species of higher basidiomycetes from different genera, such as *Agaricus*, *Coprinus*, *Pleurotus*, *Lentinula*, *Ganoderma*, *Trametes*, *Schizophyllum*, *Hericium*, *Grifola*, and many others produce bioactive substances with diverse pharmaceutical properties useful for the treatment and prevention of various diseases. Medicinal mushrooms are believed to have over

130 medicinal functions including antitumor, immunomodulating, anti-inflammatory, antioxidant, free radical scavenging, cholesterol-lowering, antidiabetic, antiviral, antibacterial, antifungal, and many other effects (Asatiani et al. 2007, 2018; Gargano et al. 2017; Wasser 2017; Chaturvedi et al. 2018; Hetland et al. 2020).

Among different bioactive compounds, polysaccharides and phenolic compounds represent one of the major classes of chemical substances found in mushrooms. Numerous bioactive polysaccharides or polysaccharide–protein complexes from medicinal mushrooms seem to enhance innate and cell-mediated immune responses, and they exhibit antitumor and immune-protective activities in animals and humans (Wasser 2017, Venturella et al. 2019; Hetland et al. 2020). Several of the mushroom polysaccharide compounds already have proceeded through Phase I, II, and III clinical trials and are used extensively and successfully as drugs in Asian countries to treat various cancers and other diseases. It is worth noting that the effect of polysaccharides and other substances derived from mushrooms are especially beneficial when used in conjunction with chemotherapy or radiotherapy. Pharmaceutical products derived from mushrooms, such as lentinan, schizophyllan, krestin, ganoderic acid, hericenone, grifolan have been developed as adjuvant anti-cancer drugs for immunotherapy in oncological clinical settings (Piotrowski et al., 2015, Wasser 2017; Wasser 2017; Gargano et al. 2017).

It has been reported that different types of phenolic compounds are effective antioxidants in biological systems, acting as free radical inhibitors, peroxide decomposers, or oxygen scavengers (Sanchez 2017). It is a very important and timely finding since in recent years it became known that synthetic antioxidants have a toxic effect and could be responsible for different types of tumor and liver damage (Panico et al. 2019). Therefore, the search for new and effective natural compounds with high antioxidant properties is a serious challenge. Both polysaccharides and phenolic compounds produced by mushrooms have been documented as antioxidants. At the same time, the phenolic extracts and polysaccharides from many mushroom exhibit antimicrobial activity against a wide range of pathogens and therefore can serve as a potential source of both antimicrobial and antioxidant compounds (Adebayo et al. 2018; Angelini et al. 2019, 2020; Özdal et al. 2019; Bach et al. 2019; Badalyan et al. 2019; Adongbede et al. 2020).

In recent years, the use of basidiomycetes with potential therapeutic properties has attracted global interest for several reasons (Lindequist et al. 2005; Wasser 2011; Alves et al. 2013; Badalyan et al. 2019; Fukushima-Sakuno 2020). First, so-called medicinal mushrooms already demonstrated their nutritional and pharmacological properties as well as efficiency against numerous diseases and metabolic disorders. Second, they are largely unexplored alternative sources of new natural myco-pharmaceuticals with unique structure and chemical composition that differ from those isolated from traditional sources of antibiotics. Third, bioactive metabolites can be easily obtained not only from wild mushrooms but also from industrially cultivated FB and mycelial biomass and supernatant of submerged cultures. Finally, medicinal mushrooms are not expensive, the majority of them are non-toxic and contain many other compounds beneficial to human health.

ABA and AFA have been identified in many mushroom extracts. In this chapter, we summarize recent advances in the study of the antimicrobial potential of *Basidiomycota* mushrooms against bacteria and fungi, focusing on the diversity, common characteristics, and unique properties of individual mushrooms, as well as on several approaches and strategies that provide enhanced production of antimicrobial metabolites.

2 The Occurrence of Antimicrobial Activity among *Basidiomycota* Mushrooms

Mushrooms exhibiting antimicrobial properties are widespread in different regions and ecosystems of the world with diverse environmental and biological conditions. It is believed that mushrooms produce antimicrobial compounds to defend themselves against various pathogenic microbes and survive in their natural environment (Lindequist et al. 2005; Rai et al. 2015). The ability to show antimicrobial activity is widespread among *Basidiomycota* species belonging to different taxonomic groups. Among them are saprotrophic, mycorrhizal (several species are capable of saprotrophic nutrition), and parasites (biotrophic and necrotrophic). Most of the mushrooms tested are wild and/or edible. Barseghyan et al. (2015) reported that the highest ABA occurred among members of the *Ganodermatales*, *Poriales*, *Agaricales*, and *Stereales* that constitute a good source for developing new antibiotics. Shen et al. (2017) summarized information on the antimicrobial properties of 158 mushroom species belonging to 88 genera, collected from various ecosystems in different regions of the world. In screening studies of hundreds of wild and edible mushrooms, important differences were observed between different strains of the same species and genera, confirming the influence of habitat and geographic location on the production of antimicrobial metabolites (Barros et al. 2007; Aqueveque et al. 2010; Bala et al. 2012; Owaid et al. 2017b; Spremo et al. 2017; Khardziani et al. 2020).

As already noted (Alves et al. 2013), the comparison of the results reported by different authors is not easy due to the diverse methodologies used to evaluate the antimicrobial activity of mushrooms. Antimicrobial activity depends on the mushroom species and even strain, mushroom form, method of extraction, and many other circumstances. Therefore, in some cases, the literature data on the antimicrobial effects of mushroom extracts are contradictory. To effectively extract the antibiotic compounds from mushrooms, a wide range of solvents with different polarity were tested while to assess the antimicrobial activity of mushrooms, mainly microdilution, disk diffusion, and agar streak dilution method have been used. Accordingly, antimicrobial activity was expressed through the determination of MIC, IC₅₀, IZD values. In some studies, a method with the incorporation of the extract in the culture medium and further determination of CFU was used.

2.1 Antimicrobial Activity of Mushroom FB

Antibacterial and antifungal activities of extracts from wild and commercially cultivated mushroom FB were the most studied. Bala et al. (2012) evaluated the antimicrobial activity of a range of Australian mushrooms from five different orders and nine different families against two Gram+ bacteria, two Gram- bacteria, and two fungi. Mushrooms belonging to the genera *Fomitopsis*, *Hohenbuehelia*, *Psathyrella*, and *Ramaria* showed promising antimicrobial activity. Alves et al. (2013) reviewed 52 mushroom species as having AFA. Among them, 44 species are edible mushrooms, 21 species are saprotrophic, 16 species are mycorrhizal, 5 species are saprotrophic but also mycorrhizal, 6 are biotrophic parasites, and 4 species are necrotrophic parasite fungi. Shen et al. (2017) summarized that among 88 mushroom genera with antimicrobial properties, 45 genera exhibited antibacterial activities and 42 genera demonstrated both antibacterial and antifungal properties. The most common mushrooms genera with antimicrobial properties include *Lentinula*, *Pleurotus*, *Dictyophora*, *Cordyceps*, *Ganoderma*, and *Tremella*. Similar to Alves et al. (2012), the authors emphasized that the number of mushrooms with activities against Gram-positive bacteria is much greater than that with activity against Gram-negative bacteria.

Barros et al. (2007) found different selectivity of methanolic extracts from Portuguese wild edible mushrooms *Lactarius deliciosus*, *Sarcodon imbricatus*, and *Tricholoma portentosum*. Among them, *L. deliciosus* distinguished with the higher content of phenols and flavonoids and great antimicrobial activity. The extracts from the entire mushroom and the cap inhibited *Bacillus cereus*, *B. subtilis*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Cryptococcus neoformans*, while the mushroom stipe extract inhibited only *B. cereus*, *P. aeruginosa*, and *C. neoformans*. The *T. portentosum* extract was effective only against Gram+ bacteria (*B. cereus*, *B. subtilis*) and *C. neoformans*, while *S. imbricatus* showed activity only against *B. cereus* and *C. neoformans* with the lowest MIC. It is worth noting that the cap extract of *S. imbricatus* was selective for *B. cereus*, while the stipe extract was not effective against the tested microorganisms. Venturini et al. (2008) evaluated the antimicrobial activity of aqueous, methanol, hexane, and ethyl acetate extracts from 48 edible wild and cultivated mushroom species against nine foodborne pathogenic bacterial strains. Extracts from 19 mushroom species did not express ABA. Of the 97 active extracts, 72.2% were active against Gram-positive bacteria and 27.8% were active against Gram-negative bacteria. *Clitocybe geotropa* appeared to be active against the majority of bacterial strains and gave inhibition zones with the largest diameters. Likewise, *L. edodes* had antimicrobial activity against all the Gram-positive bacteria and *Vibrio parahaemolyticus* and *Yersinia enterocolitica*. *Hygrophorus limacinus* was the only carpophore whose hexane extract resulted in an inhibition zone of more than 20 mm in diameter against *Clostridium*.

Tunisian mushrooms *Phellinus torulosus*, *Fomes fomentarius*, *Trametes versicolor*, *Pisolithus albus*, and *Fomitopsis pinicola* were extracted using ethanol and antimicrobial activities were assessed against eight bacterial species (Khadhri et al.

2017). The mushroom extracts exhibited a broad spectrum of ABA against all the tested bacterial species although with a different degree; the IZD varied from 9 mm (*E. coli*, *P. aeruginosa*, *A. hydrophila*, and *B. subtilis*) to 17 mm (*E. faecalis*). In particular, the IZD of *F. pinicola* extracts ranged from 10 mm (*S. typhimurium*, *L. monocytogenes*) to 16–17 mm (*P. aeruginosa*, *E. coli*, *E. faecalis*, *B. subtilis*). MIC values of *F. pinicola* extracts against the Gram+ and Gram- bacteria were 6.25–25 mg/mL and 12.5–25 mg/mL, respectively.

Of 75 mushroom samples collected in the vicinity of Oxford, Ohio (USA), the 60 °C water extracts of 25 samples had antibiotic activity against at least 1 bacterial strain tested, while water extracts of *Polyporus squamosus*, *Ganoderma applanatum*, *Lentinellus subaustralis*, *Laetiporus sulphureus*, *Ganoderma lucidum*, and *T. versicolor* exhibited strong antibiotic activity against all tested bacteria (*P. aeruginosa*, *P. fluorescens*, *B. subtilis*, *Staphylococcus epidermidis*, and *Micrococcus luteus*) (Hassan et al. 2019). The *G. lucidum* and *L. sulphureus* extracts displayed the strongest inhibition, with a MIC of 0.1 mg/mL. It is worth noting that several samples of the same mushroom species, collected from different places, showed different levels of ABA. Thus, unlike *T. versicolor* isolate 3, the extract of *T. versicolor* isolate 2 did not inhibit the growth of *P. aeruginosa*. Due to observed intra-species differences, the researchers concluded that the local ecosystem or microenvironment can have a large impact on the antibiotic compounds that the fungus produces in the wild.

Ganoderma spp. have been considered as the best source of various secondary metabolites with antimicrobial activity. Several mushrooms exhibited exceptionally high AFA. Thus, methanolic extract from *G. lucidum* showed activity against *Trichoderma viride* and *Penicillium funiculosum* with MIC 0.005 mg/mL and 0.09 mg/mL, respectively, higher than the tested standard, bifonazole (MIC 0.15 and 0.20 mg/mL, respectively) and ketoconazole (MIC 1.0 and 0.2 mg/mL, respectively) (Heleno et al. 2013). In another study, *G. lucidum*, *G. applanatum*, and *G. australe* extracts expressed differently their antimicrobial activity depending on the solvent used for extraction (Jonathan and Awotona 2010). Compared with methanol and ethanol, water appeared to be a poor solvent while ethanol was better than methanol. The best ABA showed the crude methanol extract of *G. lucidum* against *P. mirabilis* with an IZD of 20.3 mm while the highest AFA (24.3 mm) exhibited the crude ethanol extract of *G. lucidum* against *Aspergillus niger*. For the comparison, the aqueous extract of *G. australe* showed IZD of 2.3 mm against *Escherichia coli*. The MIC values for the ethanol extract ranged between 1.7 and 5.0 mg/mL for bacteria and between 2.0 and 6.0 mg/mL for fungi. Likewise, the FB of *Ganoderma boninense* effectively suppressed the growth of tested pathogens but a degree of inhibition depended on the type of used solvent (Chan and Chong 2020). Ethyl acetate extract appeared to be the most suitable for extraction purposes exhibiting antimicrobial activity against a wide range of both Gram+ and Gram- bacteria (*Enterobacter* sp., *P. aeruginosa*, *P. mirabilis*, *Acinetobacter* sp., *K. pneumoniae*, *S. marcescens*, *S. pyogenes*, coagulase negative staphylococci, and MRSA). In this study, the effectiveness of other solvents was as follows: hot water > acetone > methanol > ethanol and chloroform extract exhibited the least antimicrobial activity.

Karnwal and Kaur (2020) assessed the *Agaricus bisporus* S-II extracts as a bio-controlling agent against human pathogens. The methanol extract of FB showed the maximum level of GI of 21.8%, 15%, and 26.5% at 100% extract strength against *P. aeruginosa*, *B. cereus*, and *S. aureus*, respectively, while 100% ethanolic extract gave GI of 14%, 13.82%, and 17% against the same bacteria, respectively. The lowest MBC values (10 mg/ml) were recorded for *P. aeruginosa* and *S. aureus*, whereas no bactericidal effect of the extract was noticed on *B. cereus*.

Pleurotus spp. are one of the best-studied mushrooms for antimicrobial activity. Some species of this genus exhibit both antifungal and antibacterial activities. Evaluation of the AFA of FB of four oyster mushrooms species revealed the best inhibition zone (16 mm) against *Trichoderma harzianum* by the dried aqueous extract (2 mg/disc) from *P. ostreatus* var. *florida* grown on the substrate containing 70% wheat straw, 20% hardwood sawdust, and 10% date palm fibers (Owaid et al. 2017a). However, as compared with *T. harzianum*, *Pythium* sp. and *Verticillium* sp. appeared to be less sensitive against mushroom extracts. Moreover, these authors (Owaid et al. 2017b) obtained liquid cultures of four *Pleurotus* spp. and revealed that the culture filtrate of *P. ostreatus* caused 55%, 43.94%, and 33.33% inhibition of growth in liquid medium of *T. harzianum*, *Verticillium* sp., and *Pythium* sp., respectively. Lesser inhibitions (13.64% and 15%) were recorded in *P. cornucopiae* filtrate against *Verticillium* sp. and *T. harzianum*, respectively. Adebayo et al. (2018) compared antibacterial properties of standardized hydro-alcoholic extracts of four *Pleurotus* species against eight clinically relevant species. Among them, *P. tuber-regium* expressed remarkable activity toward both Gram+ and Gram- bacteria with MIC of 0.006–0.048 mg/mL. Younis et al. (2015) showed that the water extracts from FB of *P. ostreatus* had the widest spectrum and the highest growth inhibitory effect against tested fungi, especially toward *C. albicans*, *Cryptococcus humicola*, and *Trichosporon cutaneum* (IZD = 30 mm). Hexane and chloroform extracts from carpophore and sclerotium of *P. tuber-regium* contained compounds that inhibited the growth of 11 Gram+ and Gram- bacteria with MIC values 6.25–12.5 mg/mL (Metsebing et al. 2020). Interestingly, the crude sclerotia extracts showed higher antimicrobial activity than that of carpophores. Likewise, the researchers observed that the tested pathogenic fungi (*C. albicans*, *Aspergillus fumigatus*, and *A. ochraceus*, MIC 3.13–6.25 mg/mL) were more sensitive to crude extracts of *P. tuber-regium* than bacteria.

Of the 35 tested wild mushrooms, *Trametes* spp. and *Microporus* spp. showed high antimicrobial activities against six bacterial pathogens and two yeast species (Gebreyohannes et al. 2019). In particular, *S. aureus*, *P. aeruginosa*, and MRSA appeared to be the most susceptible to chloroform extract of *Trametes* spp. with MIC values of 0.83 mg/mL, 1.00 mg/mL, and 1.17 mg/mL, respectively. The same extracts inhibited the growth of *C. albicans* and *C. parapsilosis* at a MIC value of 1.5 mg/mL. The authors noted that hot water extracts provided better antimicrobial activities against all of the tested organisms with MIC values of 0.67–1.0 mg/mL. Between two species of *Clitocybe*, *C. geotropa* expressed strong and better antimicrobial activity than *C. nebularis* (Kosanić et al. 2020a). MIC values of acetone extracts against *B. cereus*, *B. subtilis*, *E. coli*, *P. mirabilis*, *S. aureus* for *C.*

geotropa were in the range of 0.78–6.25 mg/mL and for *C. nebularis* from 3.12 to 25 mg/mL. Like in other studies (Alves et al. 2012; Bach et al. 2019), G+ bacteria were more susceptible to extracts than G- bacteria. In general, fungi were more resistant than bacteria. *C. geotropa* showed the lowest MIC values for fungal species against *Geotrichum candidum* (6.25 mg/mL), *A. fumigatus* (6.25 mg/mL), and *C. albicans* (6.25 mg/mL).

Rena et al. (2014) tested polysaccharides extracts of eight edible mushroom species for their ability to inhibit the growth of five common bacterial pathogens. Among them, an aqueous extract from *Pleurotus australis* inhibited the growth of *S. epidermidis* with a MIC value of 0.47 mg/mL while extract from *Cordyceps sinensis* inhibited the growth of *B. subtilis* and *S. epidermidis* with MIC values of 0.94 and 0.47 mg/mL, respectively. The recently published paper (Kosanić et al., 2020b) reports that acetone extract of *Lactarius piperatus* was active against both G+ and G- bacteria with the MIC values of 0.039 mg/mL for *S. aureus*, 0.078 mg/mL for *E. coli* and *B. cereus*, and 0.156 mg/mL for *B. subtilis* and *P. mirabilis*. The same mushroom extract inhibited cell growth of *C. albicans* (MIC - 2.5 mg/mL), *T. viride* (MIC - 5 mg/mL) as well as *A. niger*, *Mucor mucedo*, and *Penicillium italicum* with MIC of 10 mg/mL. Angelini et al. (2019) found out that ABA and AFA of methanolic extracts from the FB of medicinal mushroom *Inonotus hispidus* are rather higher than that of the mycelial culture. In particular, MIC of extracts from mushroom FB and mycelium against *C. albicans* and *Aspergillus tubingensis* were 1.71 and 2.56 mg/mL, respectively.

Of eight mushrooms tested, ethanol extract from *Hydnum repandum* exerted AFA toward five phytopathogenic fungal strains with MIC of 24.75 mg/ml (Spremo et al. 2017). The methanol extract of the same species exhibited activity to *Alternaria padwickii* (MIC 24.75 mg/ml) as well. Likewise, methanol extracts of *Stereum subtomentosum* and *Coprinellus truncorum* displayed AFA to all phytopathogenic fungi but with lower MIC (49.5–198.0 mg/mL). The authors consider these mushrooms as potentially efficient antifungal agents that can be used as biocontrol agents against phytopathogenic fungi. It is worth noting that the methanolic extract of *B. adusta* affected only on *Fusarium* (MIC 24.75–99.0 mg/mL), while the methanol extracts of *Coprinus comatus*, *T. versicolor*, *F. velutipes*, as well as ethanol extracts of *C. comatus*, *A. strobiliformis*, and the chloroform extract of *C. comatus* had antifungal effects on the phytopathogenic isolate *A. padwickii*. No antifungal effects on any of the tested phytopathogenic isolates were observed in the testing of the methanol extract of *Amanita strobiliformis* and chloroform extract of *C. micaceus*.

The presence of antimicrobial activity in *Ramaria* species is also well documented. Among them, *R. flava* (Liu et al. 2013) expressed activity against Gram+ bacteria but weak or no activity against Gram- bacteria. Thus, the ethanol extract from *R. flava* exhibited activity against bacteria *S. aureus*, *B. subtilis*, and *E. coli* with the MIC values of 6.25, 25, and 100 mg/ml, respectively, and toward pathogenic fungi, causing at the concentration of 2 mg/mL reduction of *Fusarium avenaceum*, *C. albo-maculans*, and *F. graminearum* growth by 36.64, 30.03, and 19.99% (as compared to standard drug clotrimazole), respectively (Liu et al. 2013). The ethanol extract of *Ramaria* sp. showed almost complete inhibition of four Gram+

and Gram- bacteria and two fungi (*Geotrichum candidum*, *Saccharomyces cerevisiae*) at 10 mg/mL (Bala et al. 2012). Moreover, at 1 mg/mL it retained activity against *B. cereus* and both fungi along with some moderate activity against *P. aeruginosa*.

Cyclohexane, dichlormethane, methanol, and aqueous extracts of *Fomes fomentarius* appeared to be very effective against nine Gram+ and Gram- bacterial strains with the MICs values in the range of 0.125–0.25 mg/mL (Kolundžić et al. 2016). Of particular interest is that methanol and aqueous extracts have shown inhibitory activity against *Helicobacter pylori* with MIC values between 0.004–0.03 mg/mL. The authors suggested that the high content of polyphenols and β -glucan in *F. fomentarius* is responsible for the significant antimicrobial activity of this basidiomycete.

2.2 Antimicrobial Activity of Mycelial Biomass and Culture Liquid

Overwhelming studies on mushroom antimicrobial activity dealt with the evaluation of extracts obtained from FB. However, wild mushrooms are seasonal and the formation of FB by some mushroom species depends on the host. Moreover, certain mushrooms are slow-growing and rare in nature. Commercial production of FB is a labor-intensive process that can last several months. Naturally, submerged cultivation is a promising alternative for the production of mycelial biomass and bioactive compounds (Aqueveque et al. 2010; Elisashvili 2012; Duvnjak et al. 2016). Indeed, both mycelia and filtrates of liquid cultures have proven to be good sources of antimicrobial compounds against various groups of microorganisms. For comparison, Tables 13.1 and 13.2 show diversity in the antibacterial and antifungal potential of fruit bodies, mycelia, and culture liquids of some mushrooms.

Suay et al. (2000) screened 204 Spain mushroom species against a range of human pathogens. It was observed that the methanol extracts from the fermentation broths of more than 40% of the total species exhibited antimicrobial activity and 20% showed AFA. Among active mushrooms, members of the *Ganodermatales* (73% of the isolates tested) followed by *Agaricales*, *Boletales*, *Poriales*, and *Stereales* (in the range of 46–49%) showed good inhibition against different microorganisms. Aqueveque et al. (2010) screened extracts from submerged cultures of 148 strains representing 68 species belonging to 44 genera and 22 families for the production of antimicrobial activities. Mushrooms of the order *Agaricales* accounted for 31.0% of active strains, followed by the orders *Polyporales* (20.6%), *Stereales* (18.3%), *Boletales* (11.4%), and *Cortinariales* (9.1%). However, no activity was observed in the representatives of the orders *Ganodermatales* and *Thelephorales*. Analyses showed that the AFA of tested mushrooms was more pronounced than ABA. Twelve extracts that exhibited strong antimicrobial activity showed MIC

Table 13.1 Antibacterial activity of extracts from some medicinal mushrooms

Mushroom	Target microorganisms, extracts/compounds, effect	References
<i>Agaricus bisporus</i> (FB)	Methanol extract (MIC, mg/mL): <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> – 15–20	Karnwal and Kaur (2020)
<i>Clitocybe geotropa</i> (FB)	Acetone extract (MIC, mg/ml): <i>Bacillus cereus</i> – 0.78, <i>B. subtilis</i> – 1.56, <i>E. coli</i> – 6.25, <i>Proteus mirabilis</i> – 6.25, <i>S. aureus</i> – 3.12	Kosanić et al. (2020a)
<i>Clitocybe nebularis</i> (FB)	Acetone extract (MIC, mg/ml): <i>B. cereus</i> – 3.12, <i>B. subtilis</i> – 6.25, <i>E. coli</i> – 25, <i>P. mirabilis</i> – 12.5, <i>S. aureus</i> – 6.25	Kosanić et al. (2020a)
<i>Coriolus versicolor</i> (culture liquid)	Exopolysaccharides (MIC, mg/mL): <i>Enterococcus faecalis</i> – 2.5, <i>B. cereus</i> – 5.0, <i>B. spizizeni</i> – 40.0, <i>S. aureus</i> – 2.5, <i>S. epidermidis</i> – 0.3, <i>Listeria monocytogenes</i> – 40.0, <i>L. ivanovii</i> – 10.0, <i>L. innocua</i> – 40.0; <i>P. mirabilis</i> – 40.0, <i>Proteus hauseri</i> – 40.0, <i>P. aeruginosa</i> – 40.0, <i>Salmonella enteritidis</i> – 40.0, <i>Salmonella typhimurium</i> – 40.0, <i>Shigella sonnei</i> – 20.0, <i>Yersinia enterocolitica</i> – 10.0, <i>Citrobacter freundii</i> – 40.0	Duvnjak et al. (2016)
<i>C. versicolor</i> (mycelium)	Methanol extract (MIC, mg/mL): <i>E. faecalis</i> – 20.0, <i>B. cereus</i> – 5.0, <i>B. spizizeni</i> – 0.3, <i>S. aureus</i> – 10.0, <i>S. epidermidis</i> – 0.3, <i>L. monocytogenes</i> – 10.0, <i>L. ivanovii</i> – 40.0; <i>P. mirabilis</i> – 20.0, <i>P. hauseri</i> – 10.0, <i>P. aeruginosa</i> – 20.0, <i>S. enteritidis</i> – 20.0, <i>S. typhimurium</i> – 40.0, <i>S. sonnei</i> – 20.0, <i>Y. enterocolitica</i> – 5.0, <i>C. freundii</i> – 10.0	Duvnjak et al. (2016)
<i>Flammulina velutipes</i> (culture liquid)	Ethyl acetate extract (IC ₅₀), enokipodin B: <i>B. subtilis</i> - 13.4 nmol/mL, <i>E. coli</i> – 67 nmol/mL, <i>S. aureus</i> - 406.3 nmol/mL; enokipodins A and C: <i>Plasmodium falciparum</i> – 0.002 and 0.001 mg/mL, respectively	Tabuchi et al. (2020)
<i>Fomitopsis pinicola</i> strains (FB)	Ethanol extract (MIC, mg/mL): <i>B. subtilis</i> – 0.031–0.125 mg/mL, <i>S. aureus</i> – 0.031–0.500 mg/mL	Dresch et al. (2015)
<i>F. pinicola</i> (FB)	Ethanol extract, <i>S. typhimurium</i> , <i>L. monocytogenes</i> , <i>P. aeruginosa</i> , <i>E. coli</i> , <i>E. faecalis</i> , <i>B. subtilis</i> : IZD = 9–17 mm; MIC = 12.5–50 mg/mL	Khadhri et al. (2017)
<i>F. pinicola</i> (FB)	Ethyl acetate extract, 100 mg/mL (IZD, mm): <i>B. subtilis</i> , <i>S. aureus</i> , <i>E. coli</i> , <i>P. vulgaris</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i> – 17.7–21.7	Pala et al. (2019)
<i>Ganoderma boninense</i> (FB)	Ethyl acetate extract, <i>Enterobacter</i> sp., <i>P. aeruginosa</i> , <i>P. mirabilis</i> , <i>Acinetobacter</i> sp., <i>K. pneumoniae</i> , <i>S. marcescens</i> , <i>S. pyogenes</i> , coagulase negative staphylococci, and MRSA: IZD = 9.17–14.20 mm, MIC = 1.25–2.50 mg/mL	Chan and Chong (2020)
<i>Ganoderma lucidum</i> (FB)	EPS, 1 mg/mL (IZD, mm): <i>S. aureus</i> – 12, <i>B. cereus</i> – 23, <i>B. subtilis</i> – 19, <i>Klebsiella</i> sp. – 9, <i>P. aeruginosa</i> – 10, <i>E. coli</i> – 19,	Mahendran et al. (2013)
<i>Ganoderma lucidum</i> (FB)	Aqueous extract (MIC, mg/mL): <i>Micrococcus luteus</i> – 0.75	Vazirian et al. (2014)

(continued)

Table 13.1 (continued)

Mushroom	Target microorganisms, extracts/compounds, effect	References
<i>Ganoderma lucidum</i> (FB)	Methanol extract (IZD, mm): <i>E. coli</i> – 18, <i>S. aureus</i> – 14, <i>B. subtilis</i> – 17, <i>P. aeruginosa</i> – 11, <i>E. aerogenes</i> – 9, <i>K. pneumoniae</i> – 19, <i>S. typhimurium</i> – 17	Mehta and Jandaik (2012)
<i>Ganoderma lucidum</i> (mycelium)	Methanol extract (IZD, mm): <i>E. coli</i> – 22, <i>S. aureus</i> – 16, <i>B. subtilis</i> – 11, <i>P. aeruginosa</i> – 24, <i>E. aerogenes</i> – 15, <i>K. pneumoniae</i> – 16, <i>S. typhimurium</i> – 10 Acetone extract (IZD, mm): <i>E. coli</i> – 30, <i>S. aureus</i> – 12, <i>B. subtilis</i> – 14, <i>P. aeruginosa</i> – 33, <i>E. aerogenes</i> – 24, <i>K. pneumoniae</i> – 24, <i>S. typhimurium</i> – 14	Mehta and Jandaik (2012)
<i>Hohenbuehelia</i> sp. (FB)	Aqueous extract, 1 mg/mL (GI): <i>B. cereus</i> – 40.1%, <i>Listeria monocytogenes</i> – 99.7%, <i>P. aeruginosa</i> – 0%, <i>Acinetobacter baumannii</i> – 90.3%; ethanol extract, 1 mg/mL (GI): <i>B. cereus</i> – 16.1%, <i>L. monocytogenes</i> – 0%, <i>P. aeruginosa</i> – 30.2%, <i>A. baumannii</i> – 31.5%	Bala et al. (2012)
<i>Inonotus hispidus</i> (FB)	Ethyl acetate extract, 100 mg/mL (IZD, mm): <i>B. subtilis</i> , <i>S. aureus</i> , <i>E. coli</i> , <i>P. vulgaris</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i> – 12-17.3	Pala et al. (2019)
<i>Inonotus hispidus</i> (FB)	Methanol extract (MIC, mg/mL): <i>B. cereus</i> , <i>S. aureus</i> , <i>E. faecalis</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , <i>S. typhi</i> – 0.17-0.86	Angelini et al. (2019)
<i>Inonotus hispidus</i> (mycelium)	Methanol extract (MIC, mg/mL): <i>B. cereus</i> , <i>S. aureus</i> , <i>E. faecalis</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , <i>S. typhi</i> – 0.32-2.03	Angelini et al. (2019)
<i>Lactarius piperatus</i> (FB)	Acetone extract (MIC, mg/mL): <i>S. aureus</i> – 0.039, <i>E. coli</i> and <i>B. cereus</i> – 0.078, <i>B. subtilis</i> and <i>P. mirabilis</i> – 0.156	Kosanić et al. (2020b)
<i>Lentinus edodes</i> (FB)	Phenolic extract (MIC, mg/mL): <i>B. cereus</i> – 12.5, <i>S. aureus</i> – 1.56, <i>S. enteritidis</i> – 100, <i>E. coli</i> – 100	Bach et al. (2019)
<i>Lentinus tigrinus</i> (FB)	Ethyl acetate extract, 100 mg/mL (IZD, mm): <i>B. subtilis</i> , <i>S. aureus</i> , <i>E. coli</i> , <i>P. vulgaris</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i> – 12.3-15.6	Pala et al. (2019)
<i>Lepista nuda</i> (FB)	Methanol/water (80: 20) extract (MIC, mg/mL): <i>Pasteurella multocida</i> – 5, <i>P. mirabilis</i> – 20; <i>Streptococcus agalactiae</i> – 10, <i>Streptococcus pyogenes</i> – 10	Alves et al. (2012)
<i>Laetiporus sulphureus</i> (FB)	The 60 °C water extract (MIC, mg/mL): <i>S. epidermidis</i> – 0.1	Hassan et al. (2019)
<i>Mircoporus</i> spp. (FB)	Water extract (MIC, mg/mL): <i>S. aureus</i> , MRSA, <i>K. pneumoniae</i> , <i>P. aeruginosa</i> , <i>E. coli</i> – 0.67–1.67; chloroform extract: 0.83–2.0	Gebreyohannes et al. (2019)
<i>Phellinus linteus</i> (FB)	Methanol extract (MIC, mg/mL): <i>S. aureus</i> – 0.12, <i>B. cereus</i> – 0.032, <i>M. flavus</i> – 0.048, <i>L. monocytogenes</i> – 0.048, <i>P. aeruginosa</i> – 0.13, <i>S. typhimurium</i> – 0.095, <i>E. coli</i> – 0.072, <i>E. cloacae</i> – 0.048	Reis et al. (2014)
<i>Pleurotus levis</i> (FB)	Hydroalcoholic extract (MIC, mg/mL): <i>B. subtilis</i> – 0.003, <i>L. monocytogenes</i> – >0.106, <i>S. aureus</i> – 0.026, <i>S. agalactiae</i> – 0.013, <i>E. coli</i> – >0.106, <i>P. aeruginosa</i> – >0.106, <i>S. typhi</i> – >0.106, <i>Stenotrophomonas</i> sp. – >0.106	Adebayo et al. (2018)

(continued)

Table 13.1 (continued)

Mushroom	Target microorganisms, extracts/compounds, effect	References
<i>Pleurotus ostreatus</i> (FB)	3-(2-aminophenylthio)-3-hydroxypropanoic acid purified from the water extract (MIC, mg/mL): <i>S. aureus</i> , <i>E. coli</i> – 0.02	Younis et al. (2015)
<i>Pleurotus ostreatus</i> (mycelium)	Hot water extract (IZD, mm): <i>S. enterica</i> , <i>B. thuringiensis</i> , <i>P. aeruginosa</i> , <i>S. dysenteriae</i> , <i>S. pyogenes</i> , <i>S. aureus</i> , <i>B. subtilis</i> , <i>E. coli</i> , <i>K. pneumoniae</i> - 12-17	Younis et al. (2015)
<i>Pleurotus tuber-regium</i> (FB)	Hydroalcoholic extract (MIC, mg/mL): <i>B. subtilis</i> – 0.048, <i>L. monocytogenes</i> – 0.024, <i>S. aureus</i> – 0.012, <i>S. agalactiae</i> – 0.006, <i>E. coli</i> – 0.048, <i>P. aeruginosa</i> – 0.012, <i>S. typhi</i> – 0.024, <i>Stenotrophomonas</i> sp. – 0.024	Adebayo et al. (2018)
<i>Pleurotus tuber-regium</i> (FB)	Hexane and chloroform extracts (MIC, mg/mL): <i>B. subtilis</i> , <i>E. faecalis</i> , <i>S. epidermidis</i> , <i>S. aureus</i> , <i>Mycobacterium smegmatis</i> , <i>E. cloacae</i> , <i>P. vulgaris</i> , <i>K. aerogenes</i> , <i>K. oxytoca</i> , <i>P. mirabilis</i> , <i>E. coli</i> – 6.25-12.5	Metsebing et al. (2020)
<i>Ramaria flava</i> (FB)	Ethanol extract (MIC, mg/mL): <i>S. aureus</i> – 6.25, <i>B. subtilis</i> – 25, <i>E. coli</i> - 100	Liu et al. (2013)
<i>Ramaria</i> sp. (FB)	Aqueous extract, 1 mg/mL (GI, %): <i>B. cereus</i> – 41.6, <i>L. monocytogenes</i> – 0, <i>P. aeruginosa</i> – 0, <i>Acinetobacter baumannii</i> – 0%; ethanol extract, 1 mg/mL (GI,%): <i>B. cereus</i> – 100, <i>L. monocytogenes</i> – 14.9, <i>P. aeruginosa</i> – 74.8, <i>A. baumannii</i> – 24.6	Bala et al. (2012)
<i>Sparassis latifolia</i> (FB)	Lectin (MIC, mg/mL): <i>E. coli</i> – 0.10, <i>P. aeruginosa</i> – 0.05, <i>S. typhimurium</i> – 0.025, <i>B. subtilis</i> – 0.05, <i>Listeria monocytogenes</i> – 0.10, <i>S. aureus</i> – 0.10	Chandrasekaran et al. (2016)
<i>Taiwanofungus salmonesus</i> (mycelium)	Ethanol extract (MIC, mg/mL): <i>B. cereus</i> , <i>L. monocytogenes</i> , <i>S. typhimurium</i> , <i>S. aureus</i> , <i>E. coli</i> – 6.25–12.50; hot water extract (MIC, mg/mL): 25–50	Chiang et al. (2013)
<i>Trametes</i> spp. (FB)	Chloroform extract (MIC, mg/mL): <i>E. coli</i> – 1.33, <i>K. pneumoniae</i> – 1.00, <i>P. aeruginosa</i> – 1.33, <i>S. aureus</i> – 0.67	Gebreyohannes et al. (2019)
<i>Tricholosporum goniospermum</i> (FB)	Methanol extract (MIC, mg/mL): <i>E. coli</i> – 0.198, <i>P. aeruginosa</i> – 0.63, <i>S. typhimurium</i> – 0.79, <i>B. cereus</i> – 0.157, <i>B. subtilis</i> – 0.314, <i>S. aureus</i> – 0.315	Angelini et al. (2020)
<i>Tricholosporum goniospermum</i> (mycelium)	Methanol extract (MIC, mg/mL): <i>E. coli</i> – 0.099, <i>P. aeruginosa</i> – 0.396, <i>S. typhimurium</i> – 0.62, <i>B. cereus</i> – 0.099, <i>B. subtilis</i> – 0.198, <i>S. aureus</i> – 0.198	Angelini et al. (2020)

values of 50 $\mu\text{L}/\text{mL}$ against *Bacillus brevis* and 25–50 $\mu\text{L}/\text{mL}$ against *Penicillium notatum* and *Paecilomyces variotii*.

Mehta and Jandaik (2012) compared ABA of methanol, acetone, and aqueous extracts of *G. lucidum* FB and mycelial biomass. The acetone extract showed maximum ABA followed by methanol and aqueous extract. Mycelial extract of the mushroom exhibited higher ABA as compared to fruiting body extract. The especially high inhibitory activity showed acetone extract of mycelial biomass against G- bacteria *P. aeruginosa* and *E. coli* with IZD of 33 and 30 mm, respectively (Table 13.1). At the same concentration, the least inhibitory effect was observed for

Table 13.2 Antifungal activity of extracts from medicinal mushrooms

Mushroom	Target microorganisms, extracts/compounds, effect	References
<i>Agaricus bisporus</i> (FB)	Methanol extract (IZD, mm): <i>Candida tropicalis</i> , <i>Candida albicans</i> – 11-14	Barros et al. (2008)
<i>Armillaria mellea</i> (FB)	Ethanol extract (IZD, mm): <i>C. albicans</i> – 19	Alves et al. (2013)
<i>Clitocybe geotropa</i> (FB)	Acetone extract (MIC, mg/ml): <i>Aspergillus flavus</i> – 12.5, <i>A. fumigatus</i> – 6.25, <i>C. albicans</i> – 6.25, <i>Geotrichum candidum</i> – 6.25, <i>Fusarium solani</i> – 12.5, <i>Penicillium chrysogenum</i> – 25, <i>Paecilomyces variotii</i> – 25, <i>Trichophyton mentagrophytes</i> – 12.5	Kosanić et al. (2020a)
<i>Clitocybe nebularis</i> (FB)	Acetone extract (MIC, mg/ml): <i>A. flavus</i> – 25, <i>A. fumigatus</i> – 25, <i>C. albicans</i> – 6.25, <i>G. candidum</i> – 25, <i>F. solani</i> – 12.5, <i>P. chrysogenum</i> – 25, <i>P. variotii</i> – 12.5, <i>T. mentagrophytes</i> – 12.5	Kosanić et al. (2020a)
<i>Flammulina velutipes</i> (mycelium)	Enokipodins: <i>A. fumigatus</i> , IC ₅₀ – 232 µM, MIC – 0.12 mg/mL	Wang et al. (2012)
<i>Fomitopsis pinicola</i> (FB)	Ethanol extract (MIC, mg/mL): <i>Absidia orchidis</i> , <i>A. flavus</i> , <i>A. fumigatus</i> , <i>Candida krusei</i> – 0.5-1.0	Dresch et al. (2015)
<i>Fomitopsis pinicola</i> (FB)	Ethyl acetate extract, 100 mg/mL (IZD, mm): <i>Saccharomyces cerevisiae</i> , <i>C. albicans</i> , <i>P. chrysogenum</i> , <i>A. fumigatus</i> – 15.7-19.7	Pala et al. (2019)
<i>Hohenbuehelia</i> sp. (FB)	Aqueous extract, 1 mg/mL (GI, %): <i>G. candidum</i> – 77.1, <i>S. cerevisiae</i> – 88.1; ethanol extract, 1 mg/mL (GI, %): <i>G. candidum</i> – 79.1, <i>S. cerevisiae</i> – 100	Bala et al. (2012)
<i>Hygrophorus agathosmus</i> (FB)	Chloroform extract: <i>Saccharomyces cerevisiae</i> , IZD = 11 mm, MIC = 0.25 mg/mL	Yamac and Bilgili (2006)
<i>Hericium</i> sp. (mycelium)	Compound 4 (MIC, mg/mL): <i>Cryptococcus neoformans</i> , <i>C. albicans</i> – 0.03-0.06	Song et al. (2020)
<i>Inonotus hispidus</i> (FB)	Ethyl acetate extract, 100 mg/mL (IZD, mm): <i>S. cerevisiae</i> , <i>Candida albicans</i> , <i>P. chrysogenum</i> , <i>A. fumigatus</i> – 13.3-18.7	Pala et al. (2019)
<i>Inonotus hispidus</i> (FB)	Methanol extract (MIC, mg/mL): <i>C. albicans</i> – 1.71, <i>C. tropicalis</i> – 0.86, <i>C. parapsilosis</i> – 0.68, <i>A. tubingensis</i> – 1.71, <i>A. minutusi</i> – 0.68	Angelini et al. (2019)
<i>Inonotus hispidus</i> (mycelium)	Methanol extract (MIC, mg/mL): <i>C. albicans</i> – 2.56, <i>C. tropicalis</i> – 2.03, <i>C. parapsilosis</i> – 2.03, <i>A. tubingensis</i> – 2.56, <i>A. minutusi</i> – 1.01	Angelini et al. (2019)
<i>Lactarius deliciosus</i> (FB)	Methanol extracts (MIC, mg/mL): <i>C. albicans</i> – 50, <i>C. neoformans</i> – 10	Barros et al. (2007)
<i>Lactarius piperatus</i> (FB)	Acetone extract (MIC, mg/mL): <i>C. albicans</i> – 2.5, <i>T. viride</i> – 5.0, <i>A. niger</i> , <i>Mucor mucedo</i> , <i>Penicillium italicum</i> – 10.0	Kosanić et al. (2020b)
<i>Morchella esculenta</i> (FB)	Butanol extract (IZD, mm): <i>C. albicans</i> – 18, <i>A. fumigatus</i> – 14, <i>A. niger</i> – 11; ethyl acetate extract: <i>A. fumigatus</i> – 18	Shameem et al. (2017)

(continued)

Table 13.2 (continued)

Mushroom	Target microorganisms, extracts/compounds, effect	References
<i>Oudemansiella mucida</i> (culture liquid)	Ethyl acetate extract, 40 mg/mL (IZD, mm): <i>Alternaria longipes</i> , <i>Alternaria brassicae</i> , <i>Gloesporium fructigenum</i> , <i>Fusarium graminearum</i> , <i>Alternaria alternata</i> – 11.36-13.11; (MIC, mg/mL): <i>A. longipes</i> , <i>A. brassicae</i> , and <i>F. graminearum</i> – 10.0, <i>G. fructigenum</i> and <i>A. alternata</i> – 5.0	Deng et al. (2020)
<i>Phellinus linteus</i> (FB)	Glucan fraction (MIC, mg/mL): <i>A. fumigatus</i> – 0.25, <i>A. versicolor</i> – 0.06, <i>Aspergillus ochraceus</i> – 0.12, <i>A. niger</i> – 0.22, <i>T. viride</i> – 0.11, <i>P. funiculosus</i> – 0.11, <i>Penicillium ochrochloron</i> – 0.27, <i>P. verrucosum</i> – 0.27	Reis et al. (2014)
<i>Pleurotus ostreatus</i> (FB)	Hot water extracts (IZD, mm): <i>C. albicans</i> , <i>Cryptococcus humicola</i> , and <i>Trichosporon cutaneum</i> – 30, <i>G. candidum</i> , <i>A. fumigatus</i> , and <i>Fusarium moniliforme</i> – 25, <i>A. niger</i> – 12, <i>Botrytis cinerea</i> – 0	Younis et al. (2015)
<i>Pleurotus ostreatus</i> (mycelium)	Hot water extracts (IZD, mm): <i>C. albicans</i> – 3, <i>C. humicola</i> – 10, <i>T. cutaneum</i> – 15, <i>G. candidum</i> – 3, <i>A. fumigatus</i> – 0, <i>F. moniliforme</i> – 3, <i>A. niger</i> – 0, <i>B. cinerea</i> – 0	Younis et al. (2015)
<i>Pleurotus ostreatus</i> (culture liquid)	Hot water extracts (IZD, mm): <i>C. albicans</i> – 12, <i>C. humicola</i> – 0, <i>T. cutaneum</i> – 0, <i>G. candidum</i> – 0, <i>A. fumigatus</i> – 7, <i>F. moniliforme</i> – 5, <i>A. niger</i> – 5, <i>B. cinerea</i> – 0	Younis et al. (2015)
<i>P. ostreatus</i> (FB)	3-(2-aminophenylthio)-3-hydroxypropanoic acid purified from the water extract (MIC mg/mL): <i>C. albicans</i> , <i>A. fumigatus</i> – 0.03	Younis et al. (2015)
<i>Ramaria flava</i> (FB)	Ethanol extract, 2 mg/mL (GI, %): <i>Fusarium avenaceum</i> – 36.64, <i>C. albo-maculans</i> – 30.03, <i>F. graminearum</i> – 19.99	Liu et al. (2013)
<i>Ramaria</i> sp. (FB)	Aqueous extract, 1 mg/mL (GI, %): <i>G. candidum</i> – 73.9, <i>S. cerevisiae</i> – 94.7; ethanol extract, 1 mg/mL (GI, %): <i>G. candidum</i> – 100, <i>S. cerevisiae</i> – 100	Bala et al. (2012)
<i>Schizophyllum commune</i> (mycelium)	Methanol extract (MIC, mg/mL): <i>Pycnoporus sanguineus</i> – 5.0, <i>Trametes feei</i> – 0.63, <i>Trametes menziesii</i> – 0.31, <i>Trametes elegans</i> – 0.63, <i>Gloephyllum trabeum</i> – 2.5, <i>Lentinus</i> sp. – 0.16, <i>Microporus affinis</i> – 0.31, <i>Microporus xanthopus</i> – 0.31	Teoh et al. (2015)
<i>Stereum hirsutum</i> (culture liquid)	Sterenin D from the ethyl acetate extract (MIC, mg/mL): <i>B. cinerea</i> – 20.0	Aqueveque et al. (2017)
<i>Tricholosporum goniospermum</i> (FB)	Ethyl acetate extract (MIC, mg/mL): <i>C. albicans</i> – 0.198, <i>C. tropicalis</i> – 0.157, <i>C. parapsilopsis</i> – 0.099	Angelini et al. (2020)
<i>T. Goniospermum</i> (mycelium)	Ethyl acetate extract (MIC, mg/mL): <i>C. albicans</i> – 0.051, <i>C. tropicalis</i> – 0.099, <i>C. parapsilopsis</i> – 0.079	Angelini et al. (2020)
<i>Verpa bohemica</i>	Butanol extract (IZD, mm): <i>C. albicans</i> – 22; ethyl acetate extract (IZD, mm): <i>A. fumigatus</i> – 14	Shameem et al. (2017)

Gram+ bacteria *B. subtilis* and *S. aureus* (14 and 12 mm, respectively). MIC value of mycelial biomass was found to be 4 mg/mL against *P. aeruginosa* followed by *E. coli* with a value of 6 mg/mL. In the case of the fruiting body, MIC was 15 mg/mL for *P. aeruginosa* and *E. coli*.

Comparison of antimicrobial activity of *P. ostreatus* FB and that of submerged culture mycelium and filtrate revealed that the mycelium extract had little or no inhibitory effect on the growth of bacteria, whereas the broth extract exhibited high antimicrobial activity against a wide spectrum of bacterial strains suggesting that synthesized antibacterial compounds were secreted by the mycelium (Younis et al., 2015). It is worth noting that water extracts from the mushroom FB and broth had much higher antibacterial activities in comparison to the ethanol and methanol extracts. The water extract of cultural broth gave 17 mm IZD against *S. enterica* and *B. thuringiensis*, followed by 13 mm on *P. aeruginosa*, *S. dysenteriae*, and *S. pyogenes*. However, no inhibition was observed against *B. megaterium*. Regarding anti-fungal activity of *P. ostreatus*, the water extracts from the mushroom FB had the widest spectrum and the highest growth inhibitory effect against tested fungi, especially against *C. albicans*, *C. humicola*, and *T. cutaneum* (Younis et al. 2015). Water extracts from cultural broth and mycelial biomass exhibited moderate activities against *C. albicans*, *C. dubliniensis*, *Curvularia clavata*, and *Fusarium moniliforme*, but the degrees of inhibition varied. It is interesting that while the mushroom broth extract inhibited *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, and *T. viride*, the mycelia extract had no effect at all against these species. On the other hand, the mycelia extract inhibited *Cryptococcus humicola*, *Trichosporon cutaneum*, *Geotrichum candidum*, and *Penicillium expansum*, whereas the broth extract was without an effect on them. However, *Penicillium roqueforti*, *Botrytis cinerea*, *Mucor rouxii*, and *Syncephalastrum racemosum* appeared to be resistant to the inhibition by any extracts from *P. ostreatus*.

The antimicrobial activity of 21 strains belonging to 18 species of basidiomycetes was evaluated after their submerged cultivation on 7 nutrient media (Dyakov et al. 2011). Antimicrobial substances were formed by 13 strains (81.25%) of 12 fungal species. Of them, all strains exhibited activity against Gram+ bacteria, 4 strains inhibited the growth of both Gram+ and Gram- bacteria, 4 strains were active toward *A. niger*. No activity against *P. aeruginosa* was revealed in any of the fungal strains. No antimicrobial activity was revealed in the culture fluid of the *P. aurivella*, *P. squarrosa* (three strains), and *S. crispa* strains, despite satisfactory growth of fungi.

Duvnjak et al. (2016) tested the sensitivity of a wide range of bacterial species to methanol extract and exopolysaccharides isolated from the submerged culture of *Coriolus versicolor*. Among the G+ strains, the most sensitive to EPS were *E. faecalis*, *S. aureus*, and *S. epidermidis* with the MIC values of 2.5 mg/mL, 2.5 mg/mL, and 0.3 mg/mL, respectively. For *B. cereus* and *L. ivanovii* MIC values were slightly higher (5 mg/mL and 10 mg/mL, respectively). Other G+ bacteria were equally sensitive with a MIC value of 40 mg/mL. Testing of G- bacteria revealed that *Y. enterocolitica* and *S. sonnei* were also susceptible to EPS but with higher MIC values ranging from 10 to 20 mg/mL, while the *E. coli* strains were resistant to the tested concentrations of EPS.

Krupodorova et al. (2016) compared ABA of 30 mushroom species mycelia and culture liquids. Among them, mycelium and culture liquid of *Phellinus igniarius* cultivated on glucose-peptone-yeast extract medium caused full inhibition of *E. coli*

growth. The antibacterial effect of this mushroom mycelium and culture liquid even exceeded the values of used antibiotics. Culture liquid of *Piptoporus betulinus* grown on synthetic medium showed the complete suppression of *B. subtilis* and *S. aureus* whereas culture liquid of *L. edodes* fully inhibited the growth of *B. subtilis*. It is worth noting that the mycelium of *Piptoporus betulinus* did not exhibit any ABA against the tested bacteria. On the whole, comparison of the ABA of mycelium and culture liquid showed that the culture liquid has a higher activity. G- bacteria *E. coli* was more resistant to tested antibiotics than G+ bacteria *B. subtilis* and *S. aureus*.

Twelve wood-degrading basidiomycetes from Malaysian forests were evaluated for antifungal activities and the possibility of using them as bio-fungicides for biological control in the rubberwood industry (Teoh et al. 2015). Among them, *S. commune* and *P. sanguineus* appeared to be the least sensitive to both aqueous and methanol extracts from mycelium of basidiomycetes (MIC values were >5 mg/mL). At the same time, the growth of *M. affinis* was inhibited by the water extract of *G. trabeum* at the MIC value of 0.63 mg/mL. The methanol extract of *P. sanguineus* exhibited considerable activities against all wood-degrading fungi tested, particularly against *Lentinus* with MIC of 0.1 mg/mL. Likewise, methanol extract of *S. commune* biomass possessed AFA with MIC values between 0.16 and 5 mg/mL against all tested wood-degrading fungi. Based on the results obtained, the authors concluded that methanol extracts from mycelia of all fungal strains provided better AFA compared with water extracts.

Angelini et al. (2020) compared activities of n-hexane, ethyl acetate, and methanol extracts from FB and liquid-cultured mycelia of *Tricholosporum goniospermum* against Gram+ and Gram- bacteria, clinical yeast, and fungal dermatophytes. All extracts showed antimicrobial activity, but with a different degree. In general, ethyl acetate and methanol extracts from mycelia exhibited rather higher antimicrobial activities as compared with those from FB. The strongest inhibition against *E. coli* was observed for ethyl acetate and methanol mycelia extracts (MIC 0.099 mg/mL), while the extracts obtained from FB showed MIC 0.157 and 0.198 mg/mL, respectively. However, methanol and ethyl acetate extracts from the mushroom FB were more efficient toward *B. cereus* and dermatophyte *T. mentagrophytes* (CCF 5930) growth, respectively. It is worth noting that the n-hexane extract of FB was the least effective against all the tested microorganisms. The authors established that the methanol extract from mycelia was the richest in gallic acid, whereas the ethyl acetate extract from FB was the sole extract containing catechin. They assumed that the antimicrobial activity of these extracts is related, at least in part, to the content of these compounds.

Dutta et al. (2019) analyzed the antimicrobial activities of *S. commune* culture filtrate against various plant pathogens. Adding even 1% of the filtrate to PDA inhibited the growth of *Alternaria solani*, *B. cinerea*, and *Colletotrichum gloeosporioides*. The IC₅₀ for *C. gloeosporioides* and *B. cinerea* were 20% and 15% CF, respectively. Moreover, treatment of pepper plants with the culture filtrate of *S. commune* in field experiments significantly reduced the symptoms of anthracnose at a concentration of 12.5%. However, the CL of *S. commune* was not effective against

Rhizopus stolonifer and the bacterial pathogens, *Ralstonia solanacearum* or *Pectobacterium carotovorum* subsp. *carotovorum* at any tested concentrations. The researchers identified an active compound responsible for the antifungal and disease control activity and verified it as schizostatin.

Four samples obtained from submerged culture of *Oudemansiella mucida* were evaluated for antimicrobial activity against five common phytopathogenic fungi (Deng et al. 2020). Culture broth inhibited the growth of *Alternaria longipes*, *Alternaria brassicae*, *Gloesporium fructigenum*, *Fusarium graminearum*, *Alternaria alternata* with inhibition zones of 11.36–13.11 mm. The ethyl acetate extract at the concentration of 40 mg/mL gave inhibition zones from 14.96 to 16.01 mm. The MIC values for *A. longipes*, *A. brassicae*, and *F. graminearum* were 10 mg/mL, while for *G. fructigenum* and *A. alternata* – 5 mg/mL.

Unlike most researchers, Teoh et al. (2012) tested AFA of water and methanol extracts of *S. commune* toward a range of wood-degrading fungi. *Lentinus* sp. followed by *Microporus affinis* and *Microporus xanthopus* appeared to be the most sensitive to the water extract with MIC values of 0.31–0.61 mg/mL, whereas weak activity was revealed toward *Pycnoporus sanguineus*, *Trametes versicolor*, *Lentinus sajor-caju*, and *Lentinus strigosus* with MIC higher than 5 mg/mL. The methanol extract was most active against *Lentinus* sp. with MIC of 0.16 mg/mL but reduced growth of *P. sanguineus* only at MIC of 5 mg/mL. It is worth noting that the water extract exhibited weak activity against *Trametes menziesii* (MIC = 5 mg/mL), while the methanol extract inhibited the growth of this fungus at an MIC value of 0.31 mg/mL.

In our study, wood-rotting and litter-decomposing basidiomycetes collected on the plains and in the mountains of Georgia and isolated in pure cultures have been screened for their ABA (Khardziani et al., 2020). On agar plates, the mycelium of *S. commune* exhibited the highest inhibitory activity against *E. coli*, *P. aeruginosa*, and *S. aureus* with IZD of 17, 19, and 19 mm, respectively. Moreover, this mushroom showed activity against *S. enteritidis* (11 mm) and *S. epidermidis* (12 mm). Ethanol extract from fungal biomass, as well as ethyl acetate and ethanol extracts from CL, when analyzed on a 96-well plate, showed significant ABA, especially against *S. aureus* with MIC of 1 mg/mL. Interestingly, the ethyl acetate extract from culture liquid inhibited the growth of *E. coli* with a MIC of 0.5 mg/mL. However, the cold water extract from *S. commune* biomass showed the lowest activity against *S. aureus* and *E. coli* with MIC of 5 and 7.5 mg/mL, respectively. All extracts, except ethyl acetate extract from culture liquid, appeared to be more effective against *S. aureus* than toward *E. coli*. In our other work (Metreveli et al., 2021), we showed that the ethanolic extracts from mycelium and culture liquid of *F. pinicola* were especially active against *E. coli* with MIC of 0.5 mg/mL, whereas the MIC values against *S. aureus* were, respectively, 15 and 4 times higher. At the same time, the hot water extract from *F. pinicola* biomass and ethyl acetate extract from culture liquid showed the lowest ABA against *E. coli* and *S. aureus* with MIC of 15 and 20 mg/mL, and 7.5 and 6.0 mg/mL, respectively. In this regard, it should be noted that in experiments performed by Dresch et al. (2015), ethanolic extracts of *F.*

pinicola strains showed excellent bacteriostatic and bactericidal activities with MICs of 0.031–0.125 mg/mL against *B. subtilis* and of 0.031–0.500 mg/mL against *S. aureus*.

Summarizing, we can see that basidiomycetous medicinal mushrooms exhibiting antimicrobial activity are geographically distributed on all continents of the planet. These fungi, belonging to different taxonomic, physiological, and ecological groups, have been isolated from a wide variety of ecological niches, climatic zones, environmental conditions, and growth substrates. Therefore, it is not surprising that there are qualitative and quantitative differences in antimicrobial activity identified among strains of the same species of mushrooms. Moreover, some examples presented in Tables 13.1 and 13.2 indicate that not only fungi belonging to different species, but even strains belonging to the same species can exhibit different antibacterial activity and have a different spectrum of target microorganisms. Thus, in the study by Younis et al. (2015), the broth extract of *P. ostreatus* exhibited high antimicrobial activity against a wide spectrum of bacterial strains, whereas in experiments performed by Owaïd et al. (2015) none of the filtrates of *P. ostreatus* (grey and white strains) showed any activity against pathogenic bacteria and yeast. Likewise, cordimin isolated from *Cordyceps militaris* efficiently inhibited the growth of *Bipolaris maydis*, *Mycosphaerella arachidicola*, *Rhizoctonia solani*, and *C. albicans* (IC₅₀ 50 µM, 10 µM, 80 µM, and 0.75 mM, respectively) but had no effects against *A. fumigatus*, *F. oxysporum* (Wong et al. 2011).

The available literature data evidence that most of the mushrooms extracts tested are more effective against Gram+ bacteria, but there are many mushroom strains exhibiting especially high activity against Gram- bacteria. Fungal organisms usually are more resistant (Pala et al. 2019; Kosanić et al. 2020b). The extracts obtained from the mushroom cap and stipe show significant differences in the activity and antimicrobial selectivity. Furthermore, the ABA of methanol extracts from FB of *I. hispidus* appeared to be higher than that of the mycelial culture (Angelini et al. 2019). On the contrary, ethyl acetate and methanol extracts of *T. goniospermum* mycelium exhibited rather higher against most tested bacteria as compared with those from FB (Angelini et al. 2020). However, extracts from the mushroom FB were more efficient toward *B. cereus* and a dermatophyte *T. mentagrophytes*.

3 Mushroom Extracts and Isolated Compounds with Antimicrobial Activity

Analysis of literature data indicates that the antimicrobial activity of extracts depends on the mushroom species and the source of antimicrobial products (fruiting body, mycelium, culture liquid). Intracellular and extracellular compounds and extracts from the fruiting body/mycelium or culture filtrate of the same mushroom species may contain chemically different antimicrobial compounds, such as anthraquinones, aromatics, fatty acids, organic acids, peptides, proteins, polysaccharides,

steroids, terpenes, etc. (Alves et al. 2013). Certainly, these compounds significantly differ in polarity and their solubility depends on the type of solvent used for the extraction of antimicrobial substances. Ethyl acetate and methanol extracts obtained, respectively, from culture liquid and mycelia of *Stereum hirsutum* and *Stereum rameale* had considerable AFA toward *Botrytis cinerea* suppressing mycelial growth and sporogenesis of the phytopathogenic fungus (Aqueveque et al. 2016). Plate diffusion assay showed that ethyl acetate extracts were more active than methanol extracts. Ethyl acetate extract produced by *S. hirsutum* inhibited 67% of the target fungus growth at a dose of 1 mg/mL and completely inhibited the sporulation at 500 mg/mL. Subsequently, Aqueveque et al. (2017) obtained ethyl acetate extract from culture liquid and methanol extract from mycelium of *S. hirsutum* after 10 days of fungus cultivation in a fermenter containing glucose, malt extract, and yeast extract-based medium. Only ethyl acetate extract showed AFA against *B. cinerea* and fractions exhibiting a marked AFA against grey mold were isolated. Of the compounds identified, Sterenin D exhibited the highest antifungal effects with an inhibition zone of 28 mm and showed MIC at 0.02 mg/mL.

Detailed work has been carried out with *Phellinus linteus* FB (Reis et al. 2014). The methanol and ethanol extracts revealed the highest activity against eight Gram+ and Gram- bacteria with MIC values lower than those in the presence of ampicillin and streptomycin (except in the case of *S. aureus*). The triterpenoids fraction was the strongest inhibitor of bacterial growth with MIC 0.16–0.28 mg/mL whereas the glucan fraction was the less effective. However, in an evaluation of the AFA, the glucan fraction appeared to be most effective against *A. versicolor*, *A. ochraceus*, *T. viride*, and *P. funiculosum* having lower MIC values than bifonazole and ketoconazole. The methanol extract had an extremely high activity against *T. viride* (MIC = 0.0045 mg/mL, against other fungi 0.16–0.72 mg/mL). Furthermore, Pala et al. (2019) evaluated the antimicrobial activity of *L. tigrinus*, *F. pinicola*, *I. hispidus*, and *R. formosa* against two Gram+, four Gram- bacteria, and four fungi. They observed no or insignificant antimicrobial activity of aqueous extracts of these mushrooms against the tested bacteria and fungi. Both methanolic and ethyl acetate extract of *F. pinicola* and *I. hispidus* showed significant antimicrobial activity against all the bacterial (Table 13.1) and fungal (Table 13.2) strains while the ethyl acetate extract of *L. tigrinus* and *R. formosa* showed significant antimicrobial activity against the bacterial strains but mild or no activity against the fungal strains.

The ABA of ethanol extracts from *Taiwanofungus salmoneus* was more effective than that of hot-water extracts (Chiang et al. 2013). Unlike ethanol extracts, hot-water extracts at 1 mg did not show any ABA, at 5 and 20 mg the inhibition zones of 5 bacterial species were 12.8–15.5 and 21.0–23.8 mm, respectively. At the same time, ethanol extracts showed effective and dose-dependent antibacterial activities with the IZD against tested pathogenic bacteria of 12.3–15.3 mm at a dose of 1 mg. The MIC of the hot-water extract against *B. cereus* was 25 mg/mL, whereas that against the rest of the bacteria was 50 mg/mL. The MIC of the ethanolic extract against pathogenic bacteria was 6.25 mg/mL, except for that against *S. typhimurium* which was 12.5 mg/mL. Both extracts were more effective against G+ bacteria than against G- bacteria. Mycelium of the submerged cultures of *Pleurotus* species

(*P. florida*, *P. citrinopileatus*, *P. sajor-caju*, *P. ostreatus*, and *P. eryngii*) grown in glucose-containing medium showed significant antibacterial properties (Özdal et al. 2019). The hot-water extracts of *P. ostreatus* and *P. florida* were highly antibacterial against *A. agilis* and *H. pylori*, whereas the extract of *P. eryngii* was highly antibacterial against *K. oxycota* and *X. campestris*. The extracts were distinguished with a high content of total phenolics (9.14 mg/g in *P. ostreatus* extract) and total flavonoids (3.1 mg/g in *Pleurotus sajor-caju* extract).

In early work, Barros et al. (2007) observed that antimicrobial activity of *L. deliciosus*, *T. portentosum*, and *S. imbricatus* directly correlates with the content in total phenols and flavonoids in the entire mushroom, the mushroom cap, and the stipe. The content in total phenols and flavonoids for the stipe methanolic extracts was always lower than in the other extracts. Phenolic (cinnamic and *p*-hydroxybenzoic) acids from *G. lucidum* revealed higher activity (MICs of 0.003–0.12 mg/mL) against *A. fumigatus*, *A. versicolor*, *A. ochraceus*, *A. niger*, *T. viride*, *P. funiculosum*, *P. ochrochloron*, and *P. verrucosum* than positive controls bifonazole (MIC = 0.15 mg/mL) and ketoconazole (MIC = 1.0 mg/mL) (Heleno et al. 2013). Triterpenoid favolon B was isolated from the fermentation broth of *Mycena* spp., it showed AFA toward *Botrytis cinerea*, *M. miehei*, *P. variotii*, and *P. notatum* (Aqueveque et al. 2005). Ethanol and methanol extracts from *Ganoderma* sp. containing lanostane triterpenes were active against the *Tubercular bacilli* with the MIC values in the range of 0.05–0.78 mg/mL (Isaka et al. 2016). Steroidal compounds isolated from FB of *G. lucidum* were effective against *S. aureus* and *B. subtilis* with a MIC value of 2.5–5 mg/mL (Vazirian et al. 2014).

Several antimicrobial compounds responsible for antimicrobial activity have been isolated earlier from higher basidiomycetes, including grifolin (Hirata and Nakanishi 1950), pleuromutilin (Kavanagh et al. 1951), striatins A, B, and C (Anke and Oberwinkler 1977), scorodonin (Anke et al. 1980), oudemansin (Anke et al. 1990), strobilurin C (Anke et al. 1983), ganomycins (Mothana et al. 2000), micaeol (Zahid et al. 2006), and other. Merulinic acids A, B, and C of polyketide origin were isolated from the FB of *Merulius tremellosus* and *Phlebia radiata* (Giannetti et al. 1978). These metabolites showed exceptionally high ABA with MIC values of 0.4–10 µg/mL against *Arthrobacter citreus*, *B. subtilis*, *Corynebacterium insidiosum*, *Micrococcus roseus*, and *Sarcina lutea*. Himanimide C (N-hydroxylated maleimide derivative) isolated from *Serpula himantoides* exhibited fungicidal effect against *Alternaria porri*, *Aspergillus ochraceus*, and *Pythium irregulare* from a concentration of 0.025 mg/mL whereas fungistatic effect was observed against *Absidia glauca*, *Cladosporium cladosporioides*, *Curvularia lunata*, *Zygorhynchus moelleri*, *Nadsonia fulvescens*, and *S. cerevisiae* (Aqueveque et al. 2002).

Eight strains of *F. velutipes* and four strains of *F. rossica* were examined for the production of enokipodins A–D, antimicrobial sesquiterpenes (after static cultivation in 3% malt extract, 0.3% peptone, at 24 °C, 46 days in the dark) (Tabuchi et al. 2020). It was found that *F. rossica* also produces enokipodins, whereas no enokipodins were detected in some strains of *F. velutipes*. Among enokipodins, enokipodin B showed the strongest growth inhibitory activity against *B. subtilis* (IC₅₀ was 13.4 nmol/mL) and *E. coli* (IC₅₀ was 67 nmol/mL), while enokipodins A and C

showed relatively selective anti-malarial activities against *Plasmodium falciparum* with IC_{50} of 2.4 and 1.1 $\mu\text{g/mL}$, respectively. It is worth noting that for *S. aureus* IC_{50} was more than 406.3 nmol/mL (100 $\mu\text{g/mL}$). Enokipodin B followed by enokipodin A exhibited the highest AFA against *P. oryzae* and *C. albicans*. According to Wang et al. (2012) sesquiterpenes, enokipodin F, G, and I isolated from *F. velutipes* mycelium presented low activity against *Aspergillus fumigatus* with IC_{50} values 229.1, 233.4, 235.1 μM , respectively.

Song et al. (2020) isolated and identified nine pure compounds (new erinacrin alkaloid, aldehyde derivative of 4-hydroxy chroman, four chlorinated orcinol derivatives, pyran, erinaceolactone, and erinacine the extract from the culture of *Hericium* sp. grown on the Cheerios substrate). Compound 4 (2-chloro-1,3-dimethoxy-5-methyl benzene) showed inhibition against *C. albicans* and *C. neoformans*, with MIC values of 62.5 and 31.25 $\mu\text{g/mL}$, respectively. Glucosylceramide was isolated from the fruiting body of *Pleurotus citrinopileatus* and its chemical composition was identified (Meng et al., 2012). The IC_{50} value of this compound for the growth of *E. coli* and *S. aureus* was 200 $\mu\text{g/ml}$ and 235 $\mu\text{g/ml}$, respectively.

An antifungal protein ganodermin was isolated from *G. lucidum* with activity against phytopathogenic toxin-producing fungi, such as *B. cinerea* (IC_{50} = 0.015 mM), *F. oxysporum* (IC_{50} = 0.012 mM), and *Physalospora paricola* (IC_{50} = 0.018 mM) (Wang and Ng 2006). Purified protein, clitocypin, from *Clytocybe geotropa* showed inhibition against *Clavibacter michiganensis* subsp. *sepedonicus* when tested on agar plates (Dreo et al. 2007). Likewise, antifungal proteins were obtained from FB of *Pleurotus eryngii* (eryngin- exhibiting activity) against *F. oxysporum* and *Mycosphaerella arachidicola* (Wang and Ng 2004) and many other mushrooms. The lectin (protein) isolated and purified from FB of the Korean cauliflower medicinal mushroom *Sparassis latifolia* displayed high activity against bacteria (resistant strains of *E. coli*, *S. aureus*, and *P. aeruginosa* with MIC of 0.1, 0.2, and 0.05 mg/mL , respectively), yeast cells of *C. albicans*, *C. catenulata*, *C. glabrata*, and *C. rugosa* and against hyphae-forming fungi of *F. oxysporum* and *F. solani* (Chandrasekaran et al. 2016).

Recently, antimicrobial peptides have gained increased interest due to their high efficacy and specificity, low drug interaction and toxicity, and the inability of developing resistance by the microorganisms (Boparai and Sharma 2020). An antifungal peptide cordymin was isolated from *C. militaris* inhibiting mycelial growth in *Mycosphaerella arachidicola*, *Bipolaris maydis*, *Rhizoctonia solani*, and *C. albicans* with the IC_{50} values of 0.01 mM, 0.05 mM, 0.08 mM, and 0.75 mM, respectively. An antifungal peptide isolated from FB of edible mushroom *Lentinus squarrosulus* appeared to be especially active against clinical isolates *Trichophyton mentagrophytes* and *T. rubrum*, with inhibition zone diameter of 25.7 mm and 22.8 mm, respectively, at a dosage of 30 $\mu\text{g/disc}$ (Poompouang and Suksomtip 2016). Two peptide fractions extracted from *G. lucidum* fruiting body and mycelium showed strong antibacterial activity against *E. coli* and *S. typhi* with MIC 0.06 mg and 0.052 mg, 0.042 mg and 0.036 mg, respectively (Mishra et al. 2018).

Demiri and Yamaç (2008) compared the antimicrobial activity of extracts from basidiocarps, submerged grown mycelia, and crude EPS precipitates of eight

mushroom species. Antimicrobial activities of *Lentinus strigosus* and *Clavariadelphus truncatus* FB were higher than those in other mushrooms tested. Especially heptane, chloroform, and dichloromethane extracts of *L. strigosus* showed higher antimicrobial activity as compared to the controls. The chloroform extract of *C. truncatus* basidiocarps and submerged mycelium extracts of *Cerrena unicolor* exhibited antimicrobial activity against both bacteria and yeasts. These extracts were the most active to inhibit the growth of *S. aureus*, *M. luteus*, and *E. faecium*. The activities of *C. unicolor* and *Polyporus arcularius* EPS against *E. faecium*, *S. aureus*, and *M. luteus* were the same as in the positive controls (vancomycine or fluconazole). However, *C. unicolor* did not show any activity against *B. subtilis*. Moreover, the activities of *Ganoderma carnosum* EPS also were higher than positive control against *M. luteus*, *E. faecium*, and *C. albicans*. However, exopolysaccharides of other basidiomycetes did not show any activity against the test cultures. Furthermore, EPS isolated from a culture liquid of *Coriolus versicolor* inhibited the growth of *S. epidermidis*, *S. aureus*, *E. faecalis*, and *B. cereus* with MIC of 0.3, 2.5, 2.5, and 5 mg/mL, respectively (Duvnjak et al. 2016). Other tested G+ bacteria were equally sensitive with a MIC value of 40 mg/mL. Among G- bacteria, *Y. enterocolitica* and *S. sonnei* were susceptible to EPS with MIC values of 10 and 20 mg/mL, respectively, whereas the *E. coli* strains were resistant to the tested concentrations of EPS.

4 Effect of Cultivation Conditions

In the literature, there is a large number of publications reporting on the effect of cultivation conditions and medium composition on mushroom growth and bioactive compounds production. Surprisingly, very limited information is available regarding the influence of cultivation parameters on the antimicrobial activity of basidiomycetes. At the same time, both submerged and solid-state fermentations are suitable cultivation techniques for the production of mushroom biomass and various metabolites. Especially, submerged cultivation in bioreactors provides a possibility to optimize the cultivation process for maximum accumulation (intracellularly or extracellularly) of the target primary or secondary metabolites. Naturally, the production of such metabolites is species-specific, associated with metabolic features of mushrooms and their physiological response to nutrient and environmental factors.

The antimicrobial properties of mushrooms can be significantly affected by nutritional and environmental factors changing mushroom metabolism and leading to variations in the production of their secondary metabolites (Barros et al. 2007; Shen et al. 2017). Vahidi et al. (2004) were among the first who studied the effect of cultivation conditions on growth and AFA of *Mycena leptcephala*. Good growth of the mushroom and AFA against *C. lipolytica* were observed when glucose (IZD – 15.2 mm) followed by fructose and maltose were used as carbon source, whereas sucrose and starch appeared to be poor carbon sources with IZD of 8.1 mm.

Likewise, a high level of antifungal activity was observed when a complex nitrogen source (yeast extract) was used as a nitrogen source (IZD – 14.8 mm), while NH_4Cl and NaNO_3 gave IZD of 8.2 and 7.6 mm, respectively. It was established that optimal growth and maximum AFA were observed when the mushroom cultivation was performed in a medium with an initial pH of 5.5 at 25 °C.

Comparative analysis of *Polyporus tricholoma* ABA showed that the inhibition of *S. aureus* growth by ethyl acetate extract of the culture filtrate obtained after the fungus cultivation on a malt extract-soy peptone medium was higher than that of a medium with potato dextrose (Vieira et al. 2008). The inhibitory activity of the fungus gradually increased during cultivation and achieved the maximum in the stationary phase of growth. Moreover, the authors established that lactose is a preferred carbon source as compared to glucose for the accumulation of antibacterial substances; an increase of lactose concentration from 1% to 4%, as well as substitution of static cultivation with agitation at 150 rpm favored the increase of the extract activity against *S. aureus*.

Extracts from the FB, mycelia grown in solid (Cheerios or rice substrates) and liquid media (malt or soy media), and culture supernatants of *Hericium* sp. were evaluated for antimicrobial activity by Song et al. (2020). The fruiting body extract was inactive against all of the strains tested. Only the crude extract from the Cheerios mycelial culture showed activity against both yeast pathogens *C. albicans* and *C. neoformans* with a MIC of less than 0.25 mg/mL. Extracts from soy and malt culture supernatants showed activity against *C. neoformans* with MICs comparable to the Cheerios extract, while extracts from rice cultures were active with MIC >0.50 mg/mL. Extracts from rice, malt mycelia, and supernatant, and soy supernatant exhibited weak ABA (MIC >0.50 mg/mL) against *S. aureus*; moreover, extract from the soy culture supernatant was also active against *E. faecalis* (MIC >0.50 mg/mL).

The antimicrobial activity of a wide range of basidiomycetes was evaluated under submerged cultivation on seven nutrient media (Dyakov et al. 2011). These authors showed that the tested mushrooms' activity in many cases depended on inoculum form and medium composition. Thus, *Panellus serotinus* did not produce antibiotic activity against *B. subtilis* in malt extract medium, but this mushroom expressed it in the same medium supplemented with soybean flour and distillery dregs. However, culture liquids from both media were active against *S. aureus*.

The influence of growth substrate for mushrooms antimicrobial compounds formation has been established in several studies. Thus, evaluation of ABA of *Lentinula edodes* grown in 14 different culture media revealed that rice bran, vermiculite, and molasses favor mycelial growth of mushroom and antibacterial metabolite production against *B. subtilis* (Hasegawa et al. 2005). By contrast, the sawdust-containing medium was not suitable for producing antibacterial substance(s). It is interesting that although pH 3.0–3.5 was most favorable for mushroom biomass production, the best ABA was observed at 4.5. This result indicated that incubation conditions that enhanced growth are not optimal for the ABA accumulation.

In experiments performed by Krupodorova et al. (2016), no ABA was revealed using mushroom mycelium obtained after cultivation of *P. betulinus* on

glucose-containing medium, the culture liquid completely inhibited the growth of *B. subtilis* and *S. aureus* but didn't inhibit the growth of *E. coli*. When this mushroom was cultivated on the amaranth flour containing medium mycelial suspension expressed a moderate activity (IZD = 10 mm) against *E. coli* and *B. subtilis*. Interestingly, both mycelium and culture liquid of *S. commune* grown on the glucose-based medium exhibited ABA against *B. subtilis*, whereas no activity was revealed in the culture grown on the amaranth flour containing medium. However, the opposite response of this mushroom to the medium composition was observed in testing ABA against *E. coli*.

Accumulation of EPS in submerged cultivation of *G. lucidum* and *Lysinibacillus fusiformis* only slightly depended on media containing 10 g/L glucose or 40 g/L malt extract (Mahendran et al. 2013). However, EPS isolated from malt extract-based medium exhibited significantly higher ABA against both Gram+ and Gram- bacteria than polysaccharides obtained from the glucose-containing medium. Thus, the inhibition zones of *G. lucidum* EPS from malt extract and glucose media against *E. coli* were 18 mm and 12 mm, while the inhibition zones of *L. fusiformis* EPS from the same media against *B. cereus* were 15 mm and 7 mm, respectively.

In experiments with *F. velutipes*, the type of culture medium influenced both the mycelia growth and the antimicrobial metabolite production (de Melo et al. 2009). This mushroom produces enokipodins in the stationary stage of *F. velutipes* mycelia development in malt extract broth (MEB). The PDB medium provided the best mycelial growth but not optimum production of the antimicrobial compound. At the same time, complete Pontecorvo's medium resulted in greater antibacterial metabolite production than the control MEB culture medium. The authors suggested that exhaustion of the carbon in the Pontecorvo's medium and/or the excess of some mineral and/or vitamin component activated the enokipodins biosynthesis. This study showed that there was no correlation between biomass and antimicrobial metabolite production, but there may be a correlation between culture medium composition and enokipodins biosynthesis. It should also be mentioned that a rise in temperature from 25° to 37 °C on the 15th day of *F. velutipes* mycelia cultivation in malt extract-peptone broth favored antimicrobial metabolite production. Likewise, evaluation of the antimicrobial activity of extracts from the culture broth of *L. sulphureus* grown under different culture conditions (temperature 20 and 28 °C, shaking and static conditions, medium pH 5, 6, and 7) showed that static cultivation of mushroom at medium pH 5 and 20 °C favored the accumulation of antimicrobial activity against *X. vesicatoria* and *S. aureus* (Barneche et al., 2016).

In our studies, we exploited physiological approaches to elucidate cultivation conditions enhancing the antimicrobial activity of selected mushrooms. In particular, among carbon sources tested for the cultivation of *S. commune*, xylose ensured the highest (70%) inhibition of *S. aureus* growth, whereas the highest inhibition activity (60%) against *E. coli* was detected when the fungus was grown in the medium containing glucose (Khardziani et al. 2020). Submerged fermentation of mandarin pomace by *S. commune* provided the highest ABA toward *S. aureus* (89% growth inhibition) and *E. coli* (90% inhibition). The banana peels fermentation ensured 54% and 35% inhibition of *S. aureus*. It is worth noting that no ABA was

observed after fermentation of corn cobs, ethanol production residue (EPR), and wheat bran by *S. commune*. However, the same samples of culture liquids showed ABA against *E. coli* with an 18–36% growth inhibition effect. Testing of abiotic controls obtained after sterilization of lignocellulose-containing media did not reveal any statistically significant ABA. In this study, we showed that the method of lignocellulosic materials fermentation affects antibacterial substances production by *S. commune*. Specifically, unlike submerged fermentation, 12%, 88%, and 76% inhibitions of *S. aureus* growth were revealed, respectively, in the corncobs, EPR, and wheat bran solid-state fermentation. Interestingly, the antifungal activity of *S. commune* against *Fusarium* sp., *Aspergillus* sp., and *Guinardia bidwellii* only a little depended on the carbon source used for the mushroom submerged cultivation (unpublished results). Another picture was revealed in submerged fermentation of lignocellulosic materials. Specifically, culture filtrate obtained after fermentation of mandarin pomace caused 55, 85, and 91% inhibition of *Fusarium* sp., *Aspergillus* sp., and *Guinardia bidwellii* growth, respectively. Wheat bran also provided the high antifungal activity of *S. commune*, whereas corn cobs and wheat straw appeared to be poor substrates for secretion of antifungal compounds.

5 Conclusions

Analysis of the published data in this chapter shows that basidiomycete medicinal mushrooms are promising sources of a wide range of chemically diverse biologically active compounds, including those with antimicrobial activity against a broad spectrum of pathogen microorganisms. To effectively exploit the mushroom potential in mycopharmacology is an exciting task. Therefore, we believe that future research should focus on several interrelated aspects. In particular, the expansion of search and bioprospecting of geographically, climatically, ecologically, and biologically diverse habitats is necessary to discover new promising producers of antimicrobial substances. Certainly, to correctly evaluate and compare the results on the antimicrobial potential of mushrooms reported by different authors standardization and unification of methods for the extraction of biologically active compounds and an assay of ABA and AFA against specific pathogens is necessary.

One of the main challenges for future research is to focus on fundamental physiological studies to develop innovative approaches and strategies for the sustainable production of inexpensive antimicrobials under controlled conditions. A better understanding of the factors that regulate fungal metabolism and affect not only fungal growth but also the production of antimicrobial compounds is critical to maximizing the profitability of producing targeted drugs. An interesting approach may be to create stressful cultivation conditions that can affect secondary metabolism, including the activation of silent genes involved in the production of bioactive compounds. Likewise, co-cultivating fungi with pathogens can be a promising strategy to increase their antimicrobial activity.

Finally, numerous extracts and individual compounds containing ABA and/or AFA have already been isolated from mushroom cultures. Similar to penicillin and other antibiotics, future research should establish the mechanisms of action of these natural compounds responsible for antimicrobial activity.

Acknowledgments We appreciate the support from the Shota Rustaveli National Science Foundation of Georgia (grant FR-19 -3719).

References

- Adebayo EA, Martínez-Carrera D, Morales P, Sobal M, Escudero H, Meneses ME, Avila-Nava A, Castillo I, Bonilla M (2018) Comparative study of antioxidant and antibacterial properties of the edible mushrooms *Pleurotus levis*, *P. ostreatus*, *P. pulmonarius* and *P. tuber-regium*. *Int J Food Sci Technol* 53:1316–1330
- Adongbede EM, Jaiswal YS, Davis SS, Randolph PD, Huo L, Williams LL (2020) Antioxidant and antibacterial activity of *Trametes polyzona* (Pers.) Justo. *Food Sci Biotechnol* 29:27–33
- Alves MJ, Ferreira ICFR, Martins A, Pintado M (2012) Antimicrobial activity of wild mushroom extracts against clinical isolates resistant to different antibiotics. *J Appl Microbiol* 113:466–475
- Alves MJ, Ferreira IC, Dias J, Teixeira V, Martins A, Pintado M (2013) A review on antimicrobial activity of mushroom (basidiomycetes) extracts and isolated compounds. *Planta Med* 78:1707–1718
- Angelini P, Girometta C, Tirillini B, Moretti S, Covino S, Cipriani M, D’Ellena E, Angeles G, Federici E, Savino E, Cruciani G, Venanzoni R (2019) A comparative study of the antimicrobial and antioxidant activities of *Inonotus hispidus* fruit and their mycelia extracts. *Int J Food Properties* 22:768–783
- Angelini P, Venanzoni R, Flores G, Tirillini B, Orlando G, Recinella L, Chiavaroli A, Brunetti L, Leone S, Di Simone SC, Ciferri MC, Zengin G, Ak G, Menghini L, Ferrante C (2020) Evaluation of antioxidant, antimicrobial and tyrosinase inhibitory activities of extracts from *Tricholosporum goniospermum*, an edible wild mushroom. *Antibiotics* 9:513
- Anke T, Oberwinkler F (1977) The striatins - new antibiotics from the basidiomycete *Cyathus striatus* (Huds. Ex Pers.). *Willd J Antibiot* 30:221–225
- Anke T, Kupka J, Schramm G, Steglich W (1980) Antibiotics from basidiomycetes.10. Scorodinin, a new anti-bacterial and antifungal metabolite from *Marasmius scorodonius* (Fr) Fr. *J Antibiot* 33:463–467
- Anke T, Besl H, Mocek U, Steglich W (1983) Antibiotics from basidiomycetes. Strobilurin c and oudemansin-b, 2 new anti-fungal metabolites from *Xerula species* (Agaricales). *J Antibiot* 36:661–666
- Anke T, Werle A, Bross M, Steglich W (1990) Antibiotics from basidiomycetes.23. Oudemansin-x, a new antifungal e-betamethoxyacrylate from *Oudemansiella radicata* (Relhan ex-Fr) sing. *J Antibiot* 43:1010–1011
- Aqueveque P, Anke T, Sterner O (2002) The himanimides, new bioactive compounds from *Serpula himantoides*. *Z Naturforsch* 57:257–262
- Aqueveque P, Anke T, Anke H, Sterner O, Becerra J, Silva M (2005) Favolon B, a new triterpenoid isolated from the Chilean *Mycena* sp. strain 96180. *J Antibiot* 58:61–64
- Aqueveque P, Anke T, Saéz K, Silva M, Becerra J (2010) Antimicrobial activity of submerged cultures of Chilean basidiomycetes. *Planta Med* 76:1787–1791
- Aqueveque P, Céspedes CL, Alarcón J, Schmeda-Hirschmann G, Cañumir JA, Becerra J, Silva M, Sterner O, Radrigán R, Aranda M (2016) Antifungal activities of extracts produced by liquid

- fermentations of Chilean *Stereum* species against *Botrytis cinerea* (grey mould agent). *Crop Prot* 89:95–100
- Aqueveque P, Céspedes CL, Becerra J, Aranda M, Sterner O (2017) Antifungal activities of secondary metabolites isolated from liquid fermentations of *Stereum hirsutum* (Sh134-11) against *Botrytis cinerea* (grey mould agent). *Food Chem Toxicol* 109:1048–1054
- Asatiani MD, Elisashvili VI, Wasser SP, Reznick AZ, Nevo E (2007) Free-radical scavenging activity of submerged mycelium extracts from higher basidiomycetes mushrooms. *Biosci Biotechnol Biochem* 71:3090–3092
- Asatiani MD, Sharvit L, Barseghyan GS, Chan JSL, Elisashvili V, Wasser SP (2018) Cytotoxic activity of medicinal mushroom extracts on human cancer cells. *SF J Biotechnol Biomed Eng* 1:1006
- Bach F, Zielinski AAF, Helm CV, Maciel GM, Pedro AC, Stafussa AP, Avila S, Haminiuk CWI (2019) Bio compounds of edible mushrooms: *in vitro* antioxidant and antimicrobial activities. *LWT Food Sci Technol* 107:214–220
- Badalyan SM, Barkhudaryan A, Rapior S (2019) Recent progress in research on the pharmacological potential of mushrooms and prospects for their clinical application. *Medicinal mushrooms: recent progress in research and development*. Springer Nature Pte Ltd Chapter 1, Singapore, pp 1–70
- Bala N, Aitken EAB, Cusack A, Steadman KJ (2012) Antimicrobial potential of australian macrofungi extracts against foodborne and other pathogens. *Phytother Res* 26:465–469
- Barneche S, Jorcin G, Cecchetto G, Cerdeiras MP, Vázquez A, Alborés S (2016) Screening for antimicrobial activity of wood rotting higher basidiomycetes mushrooms from Uruguay against phytopathogens. *Int J Med Mushr* 18:261–267
- Barros L, Calhelha RC, Vaz JA, Ferreira ICFR, Baptista P, Estevinho LM (2007) Antimicrobial activity and bioactive compounds of Portuguese wild edible mushrooms methanolic extracts. *Eur Food Res Technol* 225:151–156
- Barros L, Cruz T, Baptista P, Estevinho LM, Ferreira ICFR (2008) Wild and commercial mushrooms as source of nutrients and nutraceuticals. *Food Chem Toxicol* 46:2742–2747
- Barseghyan GS, Barazani A, Wasser SP (2015) Medicinal mushrooms with antiphytopathogenic and insecticidal properties. In: Petre P (ed) *Mushroom biotechnology: developments and applications*. Academic Press pp, Verlag, pp 137–154
- Boparai JK, Sharma PK (2020) Mini review on antimicrobial peptides, sources, mechanism and recent applications. *Protein Peptide Lett* 27:4–16
- Chan YS, Chong KP (2020) Antimicrobial activity and metabolite analysis of *Ganoderma boninense* fruiting body. *J Pure Appl Microbiol* 14:1213–1226
- Chandrasekaran G, Lee YC, Park H, Wu Y, Shin HJ (2016) Antibacterial and antifungal activities of lectin extracted from fruiting bodies of the Korean cauliflower medicinal mushroom, *Sparassis latifolia* (Agaricomycetes). *Int J Med Mushr* 18:291–299
- Chaturvedi VK, Agarwal S, Gupta KK, Ramteke PW, Singh MP (2018) Medicinal mushroom: boon for therapeutic applications. 3. *Biotech* 8:334
- Chiang SS, Wang LT, Chen SY, Mau JL (2013) Antibacterial and anti-inflammatory activities of mycelia of a medicinal mushroom from Taiwan, *Taiwanofungus salmoneus* (higher basidiomycetes). *Int J Med Mushr* 15:39–47
- Cohen N, Asatiani MD, Varshney VK, Yu HT, Yang YC, Li YH, Mau JL, Wasser SP (2014) Chemical composition and nutritional and medicinal value of fruit bodies and submerged cultured mycelia of culinary-medicinal higher basidiomycetes mushrooms. *Int J Med Mushr* 16:273–291
- de Melo MR, Paccola-Meirelles LD, de Jesus FT, Ishikawa NK (2009) Influence of *Flammulina velutipes* mycelia culture conditions on antimicrobial metabolite production. *Mycosci* 50:78–81
- Demiri MS, Yamaç M (2008) Antimicrobial activities of basidiocarp, submerged mycelium and exopolysaccharide of some native basidiomycetes strains. *J Appl Biol Sci* 2:89–93
- Deng X, Li R, Zhang Y, Li R (2020) Preliminary study on antimicrobial activity of fermentation broths of *Oudemansiella mucida*. *Earth Environ Sci* 440:022035

- Dreo T, Želko M, Scubic J, Brzin J, Ravnikar M (2007) Antibacterial activity of proteinaceous extracts of higher basidiomycetes mushrooms against plant pathogenic bacteria. *Int J Med Mushr* 9:226–227
- Dresch P, D'Agunno MN, Rosam K, Grienke U, Rollinger JM, Peintner U (2015) Fungal strain matters: colony growth and bioactivity of the European medicinal polypores *Fomes fomentarius*, *Fomitopsis pinicola* and *Piptoporus betulinus*. *AMB Express* 5:4–18
- Dutta S, Woo EE, Yu SM, Nagendran R, Yun BS, Lee YH (2019) Control of anthracnose and gray mold in pepper plants using culture extract of white-rot fungus and active compound schizostatin. *Mycobiol* 47:87–96
- Duvnjak D, Pantić M, Pavlović V, Nedović V, Lević S, Matijašević D, Sknepnek A, Nikšić M (2016) Advances in batch culture fermented *Coriolus versicolor* medicinal mushroom for the production of antibacterial compounds. *Innovative Food Sci Emerg Technol* 34:1–8
- Dyakov M, Kamzolkina OV, Shtær OV, Bis'ko NA, Poedinok NL, Mikhailova OB, Tikhonova OV, Tolstikhina TE, Vasil'eva BF, Efremenkova OV (2011) Morphological characteristics of natural strains of certain species of basidiomycetes and biological analysis of antimicrobial activity under submerged cultural conditions. *Microbiol* 2:274–285
- Elisashvili V (2012) Submerged cultivation of medicinal mushrooms: bioprocesses and products (review). *Int J Med Mushr* 14:211–239
- Fukushima-Sakuno E (2020) Bioactive small secondary metabolites from the mushrooms *Lentinula edodes* and *Flammulina velutipes*. *J Antibiot* 73:687–696
- Gargano ML, van Griensven LJJ, Isikhuemhen OS, Lindequist U, Venturella G, Wasser SP, Zervakis GI (2017) Medicinal mushrooms: valuable biological resources of high exploitation potential. *Plant Biosyst* 151:548–565
- Gebreyohannes G, Nyerere A, Bii C, Sbhatu DB (2019) Determination of antimicrobial activity of extracts of indigenous wild mushrooms against pathogenic organisms. *Evidence-Based Complement Alternat Med*:6212673
- Genilloud O (2012) Current challenges in the discovery of novel antibacterials from microbial natural products. *Recent Pat Antiinfect Drug Discov* 7:189–204
- Giannetti BM, Steglich W, Quack W, Anke T, Oberwinkler F (1978) Antibiotika aus basidiomyceten, VI. [1] Merulinsäuren A, B und C, neue Antibiotika aus *Merulius tremellosus* Fr. und *Phlebia radiata* Fr. *Z Naturforsch C Biosci* 33:807–816
- Hassan F, Ni S, Becker TL, Kinstedt CM, Abdul-Samad JL, Actis LA, Kennedy MA (2019) Evaluation of the antibacterial activity of 75 mushrooms collected in the vicinity of Oxford, Ohio (USA). *Int J Med Mushr* 21:131–141
- Hassegawa RH, Kasuya MCM, Vanetti MCD (2005) Growth and antibacterial activity of *Lentinula edodes* in liquid media supplemented with agricultural wastes. *Electron J Biotechnol* 8:212–217
- Heleno SA, Ferreira ICFR, Esteves AP, Ciric A, Glamoclija J, Martins A, Sokovic M, Queiroz MJRP (2013) Antimicrobial and demelanizing activity of *Ganoderma lucidum* extract, p-hydroxybenzoic and cinnamic acids and their synthetic acetylated glucuronide methyl esters. *Food Chem Toxicol* 58:95–100
- Hetland G, Tangen JM, Mahmood F, Mirlashari MR, Nissen-Meyer LSH, Nentwich I, Therkelsen SP, Tjonnfjord GE, Johnson E (2020) Antitumor, anti-inflammatory and antiallergic effects of *Agaricus blazei* mushroom extract and the related medicinal basidiomycetes mushrooms, *Hericium erinaceus* and *Grifola frondosa*: a review of preclinical and clinical studies. *Nutrients* 12:1339
- Hirata Y, Nakanishi K (1950) Grifolin, an antibiotic from a basidiomycete. *J Biol Chem* 184:135–144
- Isaka M, Chinthanom P, Sappan M, Danwisetkanjana K, Boonpratuang T, Choeyklin R (2016) Antitubercular lanostane triterpenes from cultures of the basidiomycete *Ganoderma* sp. *BCC* 16642. *J Nat Prod* 79:161–169
- Jonathan SG, Awotona FE (2010) Studies on antimicrobial potentials of three *Ganoderma* species. *Afr J Biomed Res* 13:133–139

- Karnwal A, Kaur M (2020) Assessment of *Agaricus bisporus* S-II extract as a bio-controlling agent against human pathogenic bacterial species. *Archiv Razi Instit* 75:123–130
- Kavanagh F, Hervey A, Robbins WJ (1951) Antibiotic substances from basidiomycetes *Pleurotus multilus* (Fr.) Sacc. And *Pleurotus passeckerianus* Pilat. *Proc Natl Acad Sci of the USA* 37:570–574
- Khadhri A, Aouadhi C, Aschi-Smiti S (2017) Screening of bioactive compounds of medicinal mushrooms collected on Tunisian territory. *Int J Med Mushr* 19:127–135
- Khardziani T, Metreveli E, Didebulidze K, Elisashvili V (2020) Screening of Georgian medicinal mushrooms for their antibacterial activity and optimization of cultivation conditions for the split gill medicinal mushroom, *Schizophyllum commune* BCC64 (Agaricomycetes). *Int J Med Mushr* 22:659–669
- Kolundžić M, Grozdanić ND, Dodevska M, Milenković M, Sisto F, Miani A, Farronato G, Kundaković T (2016) Antibacterial and cytotoxic activities of wild mushroom *Fomes fomentarius* (L.) Fr., Polyporaceae. *Industr Crops Prod* 79:110–115
- Kosanić M, Petrović N, Stanojković T (2020a) Bioactive properties of *Clitocybe geotropa* and *Clitocybe nebularis*. *Food Measure* 14:1046–1053
- Kosanić M, Petrović N, Milošević-Djordjević O, Grujičić D, Tubić J, Marković A, Stanojković T (2020b) The health promoting effects of the fruiting bodies extract of the peppery milk cap mushroom *Lactarius piperatus* (Agaricomycetes) from Serbia. *Int J Med Mushr* 22:347–357
- Krupodorova TA, Barshteyn VY, Zabeida EF, Pokas EV (2016) Antibacterial activity of macromycetes mycelia and culture liquid. *Microbiol Biotechnol Lett* 44:246–253
- Lindequist U, Niedermeyer THJ, Julich WD (2005) The pharmacological potential of mushrooms. *eCAM* 2:285–299
- Liu K, Wang J, Zhao L, Wang Q (2013) Anticancer, antioxidant and antibiotic activities of mushroom *Ramaria flava*. *Food Chem Toxicol* 58:375–380
- Mahendran S, Saravanan S, Vijayabaskar P, Anandapandian KTK, Shankar T (2013) Antibacterial potential of microbial exopolysaccharide from *Ganoderma lucidum* and *Lysinibacillus fusiformis*. *Int J Rec Sci Res* 4:501–505
- Mehta S, Jandaik S (2012) In vitro comparative evaluation of antibacterial activity of fruiting body and mycelial extracts of *Ganoderma lucidum* against pathogenic bacteria. *J Pure Appl Microbiol* 6:1997–2001
- Meng TX, Ishikawa H, Shimizu K, Ohga S, Kondo R (2012) A glucosylceramide with antimicrobial activity from the edible mushroom *Pleurotus citrinopileatus*. *J Wood Sci* 58:81–86
- Metreveli E, Khardziani T, Didebulidze K, Elisashvili V (2021) Improvement of *Fomitopsis pinicola* (Sw.) P. karst. BCC58 antibacterial activity in fermentation of lignocellulosic materials. *Int J Med Mushr* 23. (in press)
- Metsebing BP, Oba R, Mossebo DC, Fonkui TY, Tsigain FT, Fotsing MCD, Mungoh TC, Ndinteh DN (2020) Comparative assessment of antifungal and antibacterial activities of crude extracts of the king tuber culinary–medicinal mushroom, *Pleurotus tuber-regium* (Agaricomycetes) from Cameroon. *Int J Med Mushr* 22:359–366
- Mishra J, Rajput R, Singh K, Puri S, Goyal M, Bansal A, Misra K (2018) Antibacterial natural peptide fractions from Indian *Ganoderma lucidum*. *Int J Peptide Res Therapeut* 24:543–554
- Mothana RAA, Jansen R, Julich WD, Lindequist U (2000) Ganomycins a and B, new antimicrobial farnesyl hydroquinones from the basidiomycete *Ganoderma pfeifferi*. *J Nat Prod* 63:416–418
- Owaid MN, Al-Saeedi SSS, Al-Assaffii IAA (2015) Antimicrobial activity of mycelia of oyster mushroom species (*Pleurotus* spp.) and their liquid filtrates (*in vitro*). *J Med Bioeng* 4:376–380
- Owaid MN, Al-Saeedi SSS, Al-Assaffii IAA (2017a) Antifungal activity of cultivated oyster mushrooms on various agro-wastes. *Summa Phytopat* 43:9–13
- Owaid MN, Al-Saeedi SSS, Abed IA, Shahbazi P, Sabaratnam V (2017b) Antifungal activities of some *Pleurotus* species (higher basidiomycetes). *Walailak J Sci Tech* 14:215–224
- Özdal M, Gülmez Ö, Gür Özdal Ö, Algur ÖF (2019) Antibacterial and antioxidant activity of mycelial extracts of different *Pleurotus* species. *Food Health* 5:12–18

- Pala SA, Wani AH, Ganai BA (2019) Antimicrobial potential of some wild macromycetes collected from Kashmir Himalayas. *Plant Sci Today* 6:137–146
- Panico A, Serio F, Bagordo F, Grassi T, Idolo A, Giorgi M, Guido M, Congedo M, Donno A (2019) Skin safety and health prevention: an overview of chemicals in cosmetic products. *J Prev Med Hyg* 60:50–57
- Piotrowski J, Jędrzejewski T, Kozak W (2015) Immunomodulatory and antitumor properties of polysaccharide peptide (PSP). *Postepy Hig Med Dosw* 69:91–97
- Poompouang S, Suksumtip M (2016) Isolation and characterization of an antifungal peptide from fruiting bodies of edible mushroom *Lentinus squarrosulus* Mont. *Malaysian J Microbiol* 12:43–49
- Rai M, Gaikwad S, Nagaonkar D, dos Santos CA (2015) Current advances in the antimicrobial potential of species of genus *Ganoderma* (higher basidiomycetes) against human pathogenic microorganisms (review). *Int J Med Mushr* 17:921–932
- Reis FS, Barreira JCM, Calhella RC, van Griensven LJJD, Ćirić A, Glamočlija J, Soković M, Ferreira ICFR (2014) Chemical characterization of the medicinal mushroom *Phellinus linteus* (Berkeley & Curtis) Teng and contribution of different fractions to its bioactivity. *LWT - Food Sci Technol* 58:478–485
- Rena L, Hemar Y, Perera CO, Lewis G, Krissansen GW, Buchanan PK (2014) Antibacterial and antioxidant activities of aqueous extracts of eight edible mushrooms. *Bioactive Carbohydr Dietary Fibre* 3:41–51
- Sanchez C (2017) Reactive oxygen species and antioxidant properties from mushrooms. *Synth Syst Biotechnol* 2:13–22
- Shameem N, Kamili AN, Ahmad M, Masoodi FA, Parray JA (2017) Antimicrobial activity of crude fractions and morel compounds from wild edible mushrooms of north western Himalaya. *Microb Pathogen* 105:356–360
- Shen HS, Shao S, Chen JC, Zhou T (2017) Antimicrobials from mushrooms for assuring food safety. *Compr Rev Food Sci Food Saf* 16:316–329
- Song X, Gaascht F, Schmidt-Dannert C, Salomon CE (2020) Discovery of antifungal and biofilm preventative compounds from mycelial cultures of a unique north American *Hericium* sp. fungus. *Molecules* 25:963
- Spremo NR, Tesnović KD, Rakić MS, Janušević LN, Ignjatov MV, Bjelić DD, Karaman MA (2017) Antifungal activity of macrofungi extracts on phytopathogenic fungal strains of genera *Fusarium* sp. and *Alternaria* sp. *Matica Srpska J Nat Sci Novi Sad* 133:231–240
- Suay I, Arenal F, Asensio FJ, Basilio A, Gorrochategui J, Hernandez P, Pelaez F, Vicente MF (2000) Screening basidiomycetes for antimicrobial activities. *A Van Leeuw J* 78:129–139
- Tabuchi A, Fukushima-Sakuno E, Osaki-Oka K, Futamura Y, Motoyama T, Osada H, Ishikawa NK, Nagasawa E, Tokimoto K (2020) Productivity and bioactivity of enokipodins A–D of *Flammulina rossica* and *Flammulina velutipes*. *Biosci Biotechnol Biochem* 84:876–886
- Teoh YP, Don MM, Ujang S (2012) Nutrient improvement using statistical optimization for growth of *Schizophyllum commune*, and its antifungal activity against wood degrading fungi of rubberwood. *Biotechnol Prog* 28:232–241
- Teoh YP, Mashitah MD, Salmiah U (2015) Antifungal activities of selected wood-degrading fungi of rubberwood. *J Trop Forest Sci* 27:325–333
- Vahidi H, Kobarfard F, Namjoyan F (2004) Effect of cultivation conditions on growth and antifungal activity of *Mycena leptoccephala*. *African J Biotechnol* 3:606–609
- Vazirian M, Faramarzi MA, Ebrahimi SES, Esfahani HRM, Samadi N, Hosseini SA, Asghari A, Manayi A, Mousazadeh SA, Asef MR (2014) Antimicrobial effect of the Lingzhi or Reishi medicinal mushroom, *Ganoderma lucidum* (higher basidiomycetes) and its main compounds. *Int J Med Mushrooms* 16:77–84
- Venturini ME, Rivera CS, Gonzales C, Blanco D (2008) Antimicrobial activity of extracts of edible wild and cultivated mushrooms against foodborne bacterial strains. *J Food Protect* 71:1701–1706

- Vieira GRT, Liebl M, Tavares LBB, Paulert R, Júnior AS (2008) Submerged culture conditions for production of mycelial biomass and antimicrobial metabolites by *Polyporus tricholoma* Mont. Brazilian J Microbiol 39:561–568
- Venturella G, Saporita P, Gargano ML (2019) The potential role of medicinal mushrooms in the prevention and treatment of gynecological cancers: a review. Int J Med Mushr 21:225–235
- Wang H, Ng TB (2004) Eryngin, a novel antifungal peptide from fruiting bodies of the edible mushroom *Pleurotus eryngii*. Peptides 25:1–5
- Wang H, Ng TB (2006) Ganodermin, an antifungal protein from fruiting bodies of the medicinal mushroom *Ganoderma lucidum*. Peptides 27:27–30
- Wang Y, Bao L, Yang X, Li L, Li S, Gao H, Yao XS, Wen H, Liu HW (2012) Bioactive sesquiterpenoids from the solid culture of the edible mushroom *Flammulina velutipes* growing on cooked rice. Food Chem 132:1346–1353
- Wasser SP (2011) Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. Appl Microbiol Biotechnol 89:1323–1332
- Wasser SP (2014) Medicinal mushroom science: current perspectives, advances, evidences, and challenges. Biom J 37:345–356
- Wasser SP (2017) Medicinal mushrooms in human clinical studies. Part I. anticancer, oncoimmunological, and immunomodulatory activities: a review. Int J Med Mushr 19:279–317
- Wong JH, Ng TB, Wang H, Sze SCW, Zhang KY, Li Q, Lu X (2011) Cordymin, an antifungal peptide from the medicinal fungus *Cordyceps militaris*. Phytomed 18:387–392
- Xue Z, Ma Q, Chen Y, Lu Y, Wang Y, Jia Y, Zhang M, Chen H (2020) Structure characterization of soluble dietary fiber fractions from mushroom *Lentinula edodes* (Berk.) Pegler and the effects on fermentation and human gut microbiota *in vitro*. Food Res Int 129:108870
- Yamac M, Bilgili F (2006) Antimicrobial activities of fruit bodies and/or mycelial cultures of some mushroom isolates. Pharm Biol 44:660–667
- Younis AM, Wu FS, El Shikh HH (2015) Antimicrobial activity of extracts of the oyster culinary medicinal mushroom *Pleurotus ostreatus* (higher basidiomycetes) and identification of a new antimicrobial compound. Int J Med Mushr 17:579–590
- Zahid S, Udenigwe CC, Ata A, Eze MO, Segstro EP, Holloway P (2006) New bioactive natural products from *Coprinus micaceus*. Nat Prod Res 20:1283–1289

Chapter 14

Anti-quorum Sensing Properties of Mushrooms



Zdenka Bedlovičová and Imrich Strapáč

Abstract The increasing resistance of pathogens to conventionally used antibiotics forces the scientific community to look for new ways of treatment for infectious diseases. It is the pressing need to find out the new sources of effective drugs. Nature is an unlimited source of substances with health-promoting properties. The discovery of new drugs, in general, is based on current knowledge of natural medicine, chemistry, and biology with the combination of modern technologies. Mushrooms are appreciated as naturally occurring source of compounds with nutritional, chemical, and medicinal qualities, so they can be used for the development of natural medicines and as a source of anti-infective agents. Quorum sensing is known as an intercellular mechanism of communication between microbes and is very important for life of microorganisms and population growth. These facts have made quorum sensing inhibition an interesting target in the development of the new antibacterial drugs. Mushrooms offer a variety of chemical compounds inhibiting quorum sensing such as flavonoids, phenolics, quinones, terpenoids, or vitamins and polysaccharides. The aim of this chapter is to summarize the results in the field of quorum sensing inhibition by mushrooms as a response in fighting with the microbial resistance.

Keywords Mushrooms · Quorum sensing · Microbial resistance · Antimicrobial activity

Abbreviations

AHL	Acyl homoserine lactone
AIPs	Autoinducing peptides
AIs	Autoinducers
GC	Gas chromatography
LC	Liquid chromatography

Z. Bedlovičová (✉) · I. Strapáč
Department of Chemistry, Biochemistry and Biophysics, University of Veterinary Medicine
and Pharmacy, Košice, Slovakia
e-mail: zdenka.bedlovicova@uvlf.sk

MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MS	Mass spectrometry
QQ	Quorum quenching
QS	Quorum sensing
QSI	Quorum sensing inhibition

1 Introduction

The increasing resistance in pathogens is a relevant reason to find out the new anti-infective agents. The researchers are forced to identify new chemical structures to develop novel drugs to treat microbial infections. Diseases caused by microorganisms (viruses, bacteria, fungi) are relatively common cause of mortality of patients worldwide (in the EU, there were 33,100 deaths during the years 2011–2012; in the United States, 50,000 people per year die on MRSA (methicillin-resistant *Staphylococcus aureus*) infections), but the alarming situation is in developing countries (Asfour 2018; Cassini et al. 2019; Chokshi et al. 2019).

Bacterial cells are capable of social interactions including quorum sensing (QS) as intercellular communication possibility. QS controls variety of extracellular functions, such as virulence, biofilm production, nutrient scavenging, and population growth. Inhibition of quorum sensing (QS) as the way of communication between bacterial cells then plays a noble target for developing new antibiotics and biocides (Asif and Acharya 2012; Azimi et al. 2020). Inhibition of QS can be executed by interfering with signalling pathways and/or intercepting with the signal molecules of quorum sensing (Zhang and Dong 2004; Rasmussen and Givskov 2006; Williams 2007). Naturally occurred chemical compounds represent a promising way to develop antibacterial drugs based on the QS disruption, for example, flavonoids and phenolics have been studied as inhibitors of virulence factors production and biofilm generation (Nazzaro et al. 2013).

In that context, the mushrooms are good candidate to be a source of bioactive compounds with anti-QS properties. They represent a valuable resource of bioactive compounds such as proteins, saccharides, fatty acids, vitamins, phenolic compounds, flavonoids, carotenoids, terpenes, lycopenes, anthraquinones, and minerals, indicating antioxidant, antimicrobial, antitumour, antiviral, and otherwise beneficial properties (Borchers et al. 2004; Obi et al. 2009; Bedlovičová et al. 2016; Lee-Hoon et al. 2020).

The aim of this chapter is to briefly introduce the readers into quorum sensing, quorum quenching, and capability of mushrooms to inhibit this intercellular communication.

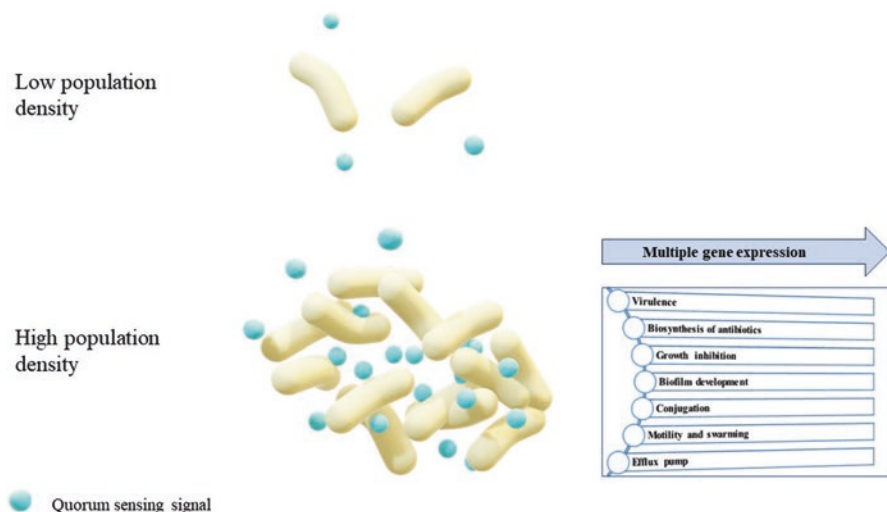


Fig. 14.1 The quorum sensing: at low cell density, the AIs are produced at essential concentrations, but when the cell density is increased, the signal molecules are produced at increasing concentrations to reach the quorum (threshold density) of cells. At this stage, the gene expression leads to accumulation of signals followed by population growth to induct of quorum sensing-dependent genes and to switch on QS-controlled features. These features differ between bacteria species

2 Quorum Sensing

The quorum sensing (QS), or cell-to-cell communication, is understood as social interaction of bacterial cells. Bacteria are able to co-operate and sense the information from other cells in the population to coordinate activities of every single cell when they reach a quorum (threshold concentration). This process is usually achieved through formation of small signal molecules (autoinducers) which are responsible for gene expression regulation and then controlling density of bacterial cell population. When the sufficient bacteria cell concentration is reached, the density of population increases, the synthesis of autoinducers (AIs) rises in the environment leading to threshold concentration of AIs followed by activation of repress target genes (Fig. 14.1) (Williams 2007; Deep et al. 2011; Wu and Luo 2021). Thus, mechanism of quorum sensing is based on the biosynthesis, release, and uptake of autoinducers accumulated in the environment.

Autoinducers regulate the expression of genes in another bacterial cells leading to control of bacterial responses, including variety of physiological processes such as virulence, formation of biofilm, antibiotics biosynthesis, etc. (Asfour 2018). The signal molecules are divided into three main groups. The first is a group of *N*-acyl homoserine lactones (AHLs) synthesized by Gram-negative bacteria to control density of population; the second class of AIs are oligopeptides (autoinducing peptides, or AIPs) consisting of 5–34 amino acids produced by Gram-positive bacteria for intercellular communication, and finally, the third main group of signalling

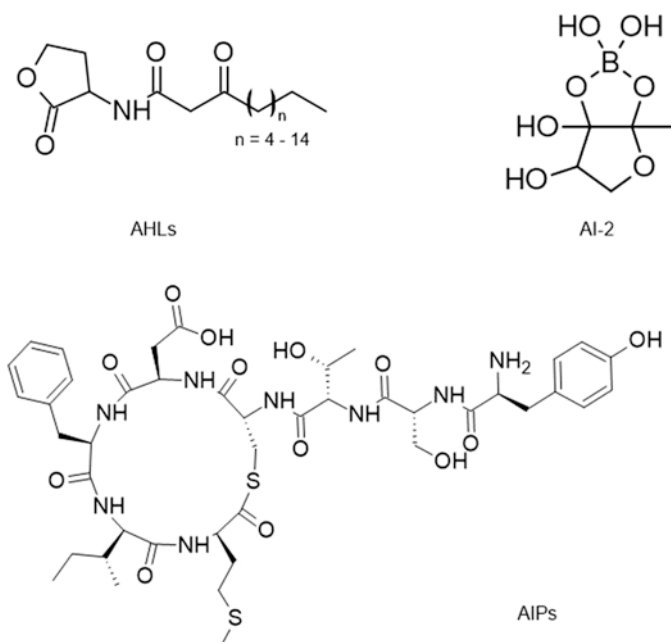


Fig. 14.2 The chemical structures of signalling molecules AHLs, AIPs, and AI-2

molecules are AI-2 (identified as a furanosylborate diester produced by members of the proteins of LuxS family) generated by both Gram-negative and Gram-positive bacterial cells for communications between different species (Xavier and Bassler 2003; Azimi et al. 2020) (Fig. 14.2).

According to the type of bacteria, various mechanisms of quorum sensing are proceeded. In Gram-positive bacteria, the precursors of autoinducing peptides are modified and transported by ATP-binding complex into extracellular environment. As the concentration of AIPs achieves the threshold level, the kinase protein is activated and the response-controlling protein is phosphorylated. Finally, this protein interacts with the target leading to the QS gene regulation. On the other side, in Gram-negative bacteria, signalling molecules directly diffuse into extracellular matrix. Signal molecules are accumulated and bind to the receptor and then form AI-receptor complex. This complex is ultimately bound to the target promoter leading to the QS gene regulation (Asfour 2018). It is necessary to mention that the concentration of signalling molecule increases with the bacterial cell population growth, but when the concentration reaches a certain level, molecules are diffused back into the intracellular matrix to regulate specific genes, for example, biofilm formation, production of antibiotics, or virulence factors (Finch et al. 1998; Zaki et al. 2013).

3 Compounds Inhibiting Quorum Sensing

A broad spectrum of compounds inhibiting QS has been reported. Several mechanisms of quorum sensing inhibition (referred as quorum quenching) were identified: (a) inhibition of the signal molecules (autoinducers) synthesis; (b) degradation of AIs by enzymes; (c) scavenging the signal molecules by antibodies and macromolecules; (d) competition with AIs in binding to receptor; (e) interfering with the binding of AIs to gene promoters leading to inhibition of gene expression (Kato et al. 2007; Morohoshi et al. 2007; Kalia and Purohit 2011; Kalia et al. 2014; Glamočlija et al. 2015a; Paluch et al. 2020).

3.1 Quorum Quenching

Quorum quenching is defined as inhibition mechanism of quorum sensing process. In general, it serves as effective help in inhibition of microbial communication, mainly when standard antibiotics and anti-infectives are inefficient due to resistance of microorganisms.

Quorum quenching as mechanism of disruption of the bacterial communication can decrease or definitely inhibit the virulence factors, for example, production of pyocyanin in *Pseudomonas aeruginosa* or violacein in *Chromobacterium violaceum* (Morohoshi et al. 2008, 2010; Mion et al. 2021).

Production of pyocyanin can be avoided by various compounds, for example, quaternary ammonium salts containing lipophilic alkyl chains (Piecuch et al. 2016), quinolin-2(1*H*)-ones (Morkunas et al. 2016), heterocycles including aminopyridine (Miller et al. 2015), or thiazolidine-2,4-diones (Froes et al. 2020). Violacein biosynthesis may be reduced by furanones (Morohoshi et al. 2007), secondary metabolites of *Halobacillus salinus* (Teasdale et al. 2009), maniwamycins (Fukumoto et al. 2016), etc.

The QQ mechanism is based on the enzymatic degradation of quorum sensing signalling molecules to avoid the cumulation of autoinducers and finally to inhibit expression of genes. For example, the enzyme AHL-lactonase produced by *Bacillus cereus* VT96 can directly degrade AHLs molecules by cleaving the lactone ring, so it is able to control the virulence of *P. aeruginosa* and *P. carotovorum* (Rajesh and Rai 2016). Another quorum quenching enzyme, MomL, isolated from marine *Muricauda olearia* Th120, has also been investigated as a novel type of AHL-lactonase (Wang et al. 2019). AHL-acylase (Sio et al. 2006) and/or oxidoreductase (Terwagne et al. 2012) can also degrade AHL signal molecules (Fig. 14.3).

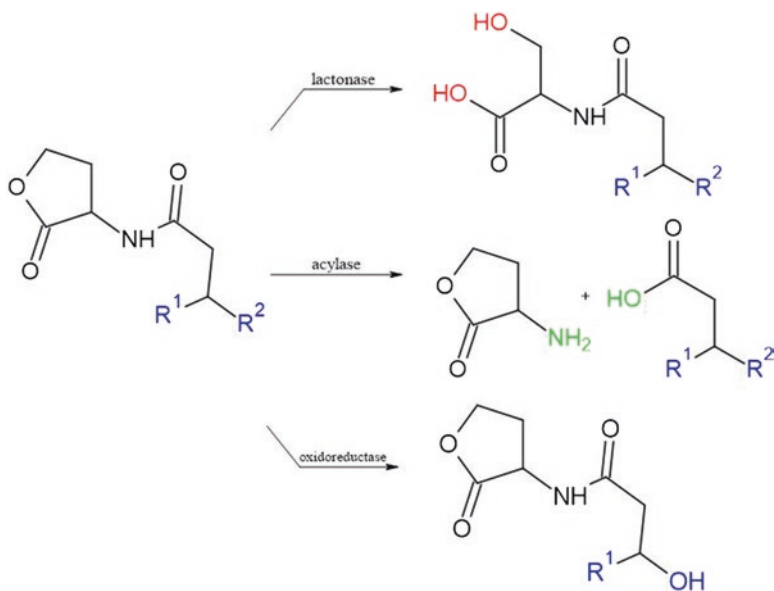


Fig. 14.3 AHL-deactivating enzymes – lactonases, acylases, and oxidoreductases – redrawn according to (Chen et al. 2013)

3.2 Methods of Determination of Quorum Quenching

As the knowledge of quorum sensing/quenching increased, the scientists are focused on finding new active quorum sensing inhibitors and investigating their properties. Many molecules have been successfully characterized and examined, but the finding of a single molecule which will inhibit all the mentioned quorum sensing mechanisms is improbable. Nevertheless, Kalia (2013) proposed some criteria for selecting an efficient QS inhibitor. The molecule should be small and chemically stable. A good QS inhibitor should be able to reduce gene expression regulated by QS. The inhibitor should also be highly specific for QS regulator, then it must not have any negative effect on the bacterial or host cells, and should be longer than native AHL (Kalia 2013).

The qualitative and quantitative measurements of QQ are proceeded using various methods, which can be classified as direct and indirect (biosensors are necessary). Most of the methods are based on the detection of autoinducers reacting with specific chemicals leading to color reaction which can be quantitatively determined (for example, by colorimetry) or have luminescence or fluorescence ability. Other analytical methods are capillary electrophoresis, TLC (thin-layer chromatography), HPLC (high performance liquid chromatography), and GC (gas chromatography) (Shaw et al. 1997; Teplitski et al. 2003; Yang et al. 2006). Liquid and gas chromatography coupled with mass spectrometry (LC-MS/MS; GC-MS) have been successfully used for the detection of AHLs (Cataldi et al. 2004; Purohit et al. 2013;

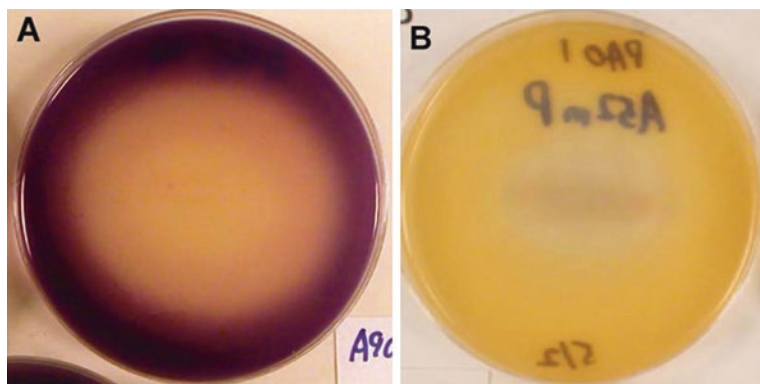


Fig. 14.4 Influence of C4 HSL and 3-oxo-C12 HSL production from *P. aeruginosa* PAO-1 on *C. violaceum* 12,472 (a) and *P. aureofaciens* 30–84 (b) overlay. (Permissions by Elsevier (McLean et al. 2004))

Patel et al. 2016; Huang et al. 2020), for example farnesol, and tyrosol produced by *Candida albicans* (Greguš et al. 2010; Pilařová et al. 2020), or peptides (Debunne et al. 2018).

Techniques based on bacterial biosensors have also been studied for AHLs detection. Bacterial biosensors represent a fast tool for detection of specific signalling molecules. Biosensors are genetically modified organisms of various species (*Pseudomonas aeruginosa*, *Vibrio fischeri*) which have the ability to detect quorum sensing molecules by proteins and bacterial pathways. These proteins are usually detected by optical or electrochemical methods. Most of the QS biosensors express a reporter gene from a quorum sensing response promoter. This promoter is getting activated immediately as a complex of signal molecule and quorum sensing transcriptional activator binds to the promoter (Rai et al. 2015). The detection of AHLs can also be applied by using of genetically modified bacterial strains producing bioluminescence. The most usually used assay is bioluminescence *Vibrio harveyi* BB170 method. This *V. harveyi* strain is disabled to produce AHLs and AI-2 due to deleted luxN gene, which encodes LuxN protein. The result of these mutations is that the bioluminescence is detected only if the exogenous AI-2 molecule is present in bacterial environment (O'Connor et al. 2016). The approach of using genetic modifications to create bacterial strains serving as quorum quenchers is also applicable (Oh et al. 2017).

Measurement of enzymatic activity is also the way of quantification of quorum sensing inhibition. As we mentioned, the quorum quenching inducing enzymes represent AHL-acylase, AHL-oxidoreductase, and AHL-lactonase (Chen et al. 2013). The capability of quorum quenching enzymes to decrease virulence of bacteria can also be examined by genome modification (Chen et al. 2013).

Biosensor strains, such as *Chromobacterium violaceum* CV026, *Pseudomonas aureofaciens* 30–84, or *Agrobacterium tumefaciens* A136, are quite commonly and

successfully used for the detection of QQ (Shaw et al. 1997; McLean et al. 2004; Zhu et al. 2012; Tang et al. 2013; Zaki et al. 2013; Tabbouche et al. 2017). The *C. violaceum* and *P. aureofaciens* methods are based on inhibition of the produced pigment violacein and phenazine, respectively (Fig. 14.4).

Some limitations were observed for these methods, they are time-consuming, low QQ is undetectable, and measuring of inhibition zones can be inaccurate (Liu et al. 2010; Tang et al. 2013; Lee et al. 2016).

4 Mushrooms as Quorum Sensing Inhibitors

Mushrooms are rich source of various compounds including fatty acids, amino acids, polysaccharides (in general β -glucans), minerals, secondary metabolites such as phenolics, flavonoids, β -carotenes, lycopenes, vitamins, terpenes, steroids, anthraquinones, benzoic acid derivatives, quinolines, organic acids, or high-molecular-weight molecules (peptides, proteins, nucleic acids) occurring in fruiting bodies, mycelia, and spores (Reis et al. 2012; Bedlovičová et al. 2016; Strapáč et al. 2019; Omer and Alfaig 2020). Mushroom-derived compounds possess a variety of biological activities, including antimicrobial properties (Petrović et al. 2014; Soković et al. 2014; Kostić et al. 2017; Strapáč et al. 2019). The presence of mentioned molecules is varying depending on the particular species of mushrooms, but in general, these compounds are based on phenolics, flavonoids, lactones, chitosans, quinones, coumarins, terpenoids, polysaccharides, and alkaloids (Glamočlija et al. 2015a; Bedlovičová et al. 2016).

De Carvalho et al. isolated coprinuslactone [(3*R*,4*S*)-2-methylene-3,4-dihydroxypentanoic acid 1,4-lactone] from edible mushroom *Coprinus comatus*, which interferes with QS and disperses biofilms of *Pseudomonas aeruginosa* and *Staphylococcus aureus* (de Carvalho et al. 2016). Melanin from edible jelly mushroom (*Auricularia auricula*) has shown the antibiofilm activity regulated by QS (Bin et al. 2012).

Related studies showed that extracts of edible mushrooms are able to inhibit quorum sensing, but there is a problem to find out the mechanism of QSI (quorum sensing inhibition) because extracts are complex mixtures of different chemical compounds of various types. Some authors suggest that QSI is probably associated with the presence of phenolic compounds (Hossain et al. 2017; Strapáč et al. 2019; Vunduk et al. 2019), others proposed furanone-like derivatives (Zhu and Sun 2008), but in general, the exact compounds presented in extracts, which are responsible for anti-quorum sensing properties, are still unknown, so relevant studies are needed to clarify the mechanism of QS inhibition (Petrović et al. 2014; Glamočlija et al. 2015a, 2015b; Tabbouche et al. 2017; Gurgun et al. 2018; Yıldız et al. 2019).

As already mentioned, several studies related to the QSI by extracts of mushrooms were released (Table 14.1).

An interesting study was revealed by Koc et al. (2020), in which an extract of mushroom *Tricholoma terreum* was used as chitosan-based film producer.

Table 14.1 Quorum sensing inhibition by extracts of mushrooms

Mushroom species	Extraction reagent	Bacteria species	Method of QSI activity	References
<i>Tricholoma terreum</i>	Water	<i>Chromobacterium violaceum</i> CV026	Inhibition of violacein pigment production	Koc et al. (2020)
<i>Agaricus bisporus</i> <i>Clitocybe nuda</i> <i>Lactarius volemus</i> <i>Macrolepiota procera</i> <i>Xerocomellus chrysenteron</i>	Water	<i>Pseudomonas aeruginosa</i> 119, 44	Microtiter plate method	Strapáč et al. (2019)
<i>Pleurotus flabellatus</i>	Water	MRSA <i>Escherichia coli</i> <i>Pseudomonas aeruginosa</i> <i>Proteus mirabilis</i> <i>Enterococcus faecalis</i>	Microtiter plate method	Vunduk et al. (2019)
<i>Agaricus bisporus</i> <i>Laccaria bicolor</i> <i>Bovista plumbea</i> <i>Lactarius deliciosus</i> <i>Boletus edulis</i>	Supercritical CO ₂	<i>Chromobacterium violaceum</i>	Inhibition of violacein pigment production	Yıldız et al. (2019)
<i>Pleurotus ostreatus</i> <i>Geastrum fornicatum</i> <i>Agaricus arvensis</i> <i>Amanita pantherina</i>	Methanol	<i>Chromobacterium violaceum</i>	Inhibition of violacein pigment production	Gurgen et al. (2018)
<i>Amanita rubescens</i> <i>Lactarius</i> sp.	Ethanol	<i>Chromobacterium violaceum</i>	Inhibition of violacein pigment production	Tabbouche et al. (2017)
<i>Armillaria mellea</i>	Methanol	<i>Pseudomonas aeruginosa</i> PAO1	Biofilm inhibition Twitching and flagella motility inhibition Pyocyanin production inhibition	Kostić et al. (2017)
<i>Polyporus squamosus</i>	Methanol	<i>Pseudomonas aeruginosa</i> PAO1	Biofilm inhibition Twitching and flagella motility inhibition Pyocyanin production inhibition	Fernandes et al. (2016)

(continued)

Table 14.1 (continued)

Mushroom species	Extraction reagent	Bacteria species	Method of QSI activity	References
<i>Agaricus bisporus</i> <i>Agaricus bitorquis</i> <i>Agaricus campestris</i> <i>Agaricus macrosporus</i>	Methanol	<i>Pseudomonas aeruginosa</i> PAO1	Biofilm inhibition Twitching and flagella motility inhibition Disc-diffusion method	Glamočlija et al. (2015b)
<i>Inonotus obliquus</i>	Water Ethanol	<i>Pseudomonas aeruginosa</i> PAO1	Twitching and flagella motility inhibition Swarming Pyocyanin production inhibition	Glamočlija et al. (2015a)
<i>Agrocybe aegerita</i>	Methanol	<i>Pseudomonas aeruginosa</i> PAO1	Biofilm inhibition Twitching and flagella motility inhibition Disc-diffusion method Pyocyanin production inhibition	Petrović et al. (2014)
<i>Agaricus blazei</i>	Water	<i>Pseudomonas aeruginosa</i> PAO1	Biofilm inhibition Twitching and flagella motility inhibition Pyocyanin production inhibition Disc-diffusion method	Soković et al. (2014)
<i>Pleurotus florida</i>	Methanol Chloroform	<i>Pseudomonas aeruginosa</i>	Swarming motility AHL inhibition Biofilm inhibition	Silambarasan et al. (2014)
<i>Phellinus igniarius</i>	Fermentation	<i>Chromobacterium violaceum</i>	Inhibition of violacein pigment production	Zhu et al. (2012)

(continued)

Table 14.1 (continued)

Mushroom species	Extraction reagent	Bacteria species	Method of QSI activity	References
<i>Phellinus igniarius</i> <i>Auricularia auricula</i> , <i>Cordyceps sinensis</i> <i>Coriolus versicolor</i> , <i>Ganoderma lucidum</i> , <i>Inonotus obliquus</i> <i>Antrodia camphorata</i> <i>Lentinus edodes</i> <i>Pleurotus ostreatus</i> <i>Flammulina velutipes</i> <i>Sparassis crispa</i> <i>Agrocybe aegerita</i> <i>Agaricus bisporus</i> <i>Auricularia polytricha</i>	Fermentation	<i>Chromobacterium violaceum</i>	Inhibition of violacein pigment production	Zhu et al. (2011)
<i>Tremella fuciformis</i>	75% methanol	<i>Chromobacterium violaceum</i>	Inhibition of violacein pigment production	Zhu and Sun (2008)

Anti-quorum sensing activities of prepared chitosan-mushroom extract films were tested against various types of bacteria (*Escherichia coli*, *Salmonella typhimurium*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Bacillus thuringiensis*). The results showed that the combination of chitosan film with mushroom extracts is a good method for increasing anti-quorum sensing activity (26 ± 1 mm), due to much more inhibition capability of violacein production than gentamicin (12 ± 1 mm) or chitosan film without extract of *Tricholoma terreum* (9.1 ± 1 mm) (Koc et al. 2020).

Methanolic extracts of two different samples of *Polyporus squamosus*, a wild mushroom obtained from Serbia and Portugal, were subjected to the study of quorum sensing inhibition of *P. aeruginosa* by three methods. The first was antibiofilm activity tested at subinhibitory level (0.5 and 0.125 MIC). Inhibition of biofilm formation was observed only for extract of sample obtained from Serbia at the value of $88.3 \pm 0.65\%$, $84.30 \pm 0.55\%$, respectively. The inhibition of biofilm formation was better than ampicillin and streptomycin standards. The second QSI technique was study of inhibition of the twitching and flagella motility of *P. aeruginosa*. The sample from Serbia showed better activity than from Portugal, and also than the standard antibiotics. Pyocyanin production inhibition by *P. aeruginosa* PAO1 was the third method of anti-QS activity studies. *P. squamosus* extract of Portuguese sample showed higher ability to reduce pyocyanin production than Serbian sample and standard antibiotics. The strain of *P. aeruginosa* PAO1 produced a significant amount of pyocyanin (83.12%) and the methanolic extract of studied mushroom from Portugal inhibited this production to an amount of 44.5%. The QSI mechanism of action is unclear nevertheless the authors also determined chemical composition of extracts (fructose, rhamnose, mannitol, trehalose, fatty acids, organic acids, tocopherols) (Fernandes et al. 2016).

These methods for QSI study were also used for methanolic extract of *Armillaria mellea* (honey mushroom). The effect of honey mushroom on *P. aeruginosa* biofilm formation was studied using 0.5, 0.25, and 0.125 MIC. The obtained results showed that the extracts were more effective than standard antibiotics (streptomycin and ampicillin), and biofilm inhibition was in a concentration-dependent manner (for 0.5, 0.25, and 0.125 MIC, the inhibition was determined at the values of 69.8, 45.89, and 17.01%). *A. mellea* methanolic extract also reduced the twitching motility of *P. aeruginosa*. The anti-quorum sensing activity of extract was also studied against pyocyanin production. The highest ability to inhibit pyocyanin production was observed for extract of 0.5 MIC concentration (38.47%), whereas the streptomycin exhibited 10.96% and ampicillin 15.84% reduction. The chemical composition of honey mushroom was also measured. The main components were carbohydrates, sugars (mannitol, trehalose, D-xylose, D-glucose, D-galactose), fatty acids, organic acids (malic, citric, fumaric, oxalic), polyphenols, and tocopherols. The authors claimed that the role of molecules in QS mechanism is elaborate, and there are more factors affecting the mechanism, so that it is important to study different mechanisms of action and specifically with the biomolecules present in the species of *A. mellea* (Kostić et al. 2017).

Ethanol extracts of *Agaricus* species (*A. bisporus*, *A. bitorquis*, *A. campestris*, *A. macrosporus*) were also tested against quorum sensing. All the samples showed anti-biofilm effects (reduction was observed in the range of 53–87%), the best results were obtained for *A. macrosporus*. The reduction of biofilm formation by standard antibiotics was detected for streptomycin in 51% and for ampicillin in 31%. The QS inhibition zones obtained by disc diffusion method showed comparable results as ampicillin standard. On the other side, the streptomycin standard possessed the best anti-QS activity.

All the extracts also showed a promising inhibition of twitching of *P. aeruginosa* and flagella motility (Glamočlija et al. 2015b).

The methanolic extract of *Agrocybe aegerita* also possessed antibiofilm activity of *P. aeruginosa*. The tested extract at subMIC concentrations (0.5, 0.25, and 0.125 MIC) showed better ability to reduce biofilm formation than standard streptomycin and ampicillin antibiotics. The best results were observed for 0.5 MIC extract which reduced formation of biofilm in 82.24%, whereas ampicillin and streptomycin reduced biofilm generation by 30.84% and 50.60%, respectively. The QS zones of inhibition were designated by disc diffusion technique. The extracts of all concentrations showed a better anti-QS effect between 7.70–10.30 mm of inhibition zone, while ampicillin standard possessed lower activity, but at higher concentration (7.60 mm). On the other side, the streptomycin activity was much higher (15.50–22.06 mm). Pyocyanin pigment reduction was observed for all the *Agrocybe aegerita* extracts in concentration-depending manner. The best results were noticed for 2 MIC concentration of extract, and all the extracts showed better reduction of pigment than standard antibiotics used for determination. In addition, authors were also focused on the twitching and flagella motility inhibition, which are responsible for initializing the formation of biofilm by *P. aeruginosa*. They observe reduction of

twitching and flagella motility by the extract, streptomycin reduced flagellas absolutely, ampicillin did not affect the flagella formation (Petrović et al. 2014).

Another study demonstrated that hot water extracts of *Agaricus blazei* reduced *P. aeruginosa* biofilm formation more effectively than commercial antibiotics (streptomycin and ampicillin). The QS-inhibiting zones were also observed in the range of 7.0–17.7 mm. Water extracts of *A. blazei* also much more efficiently reduced pyocyanin pigment formation at subMIC concentrations and are able to reduce motility of flagella and twitching (Soković et al. 2014).

Chaga mushroom (*Inonotus obliquus*) is a known medicinal mushroom. Glamočlija et al. studied its chemical composition and anti-quorum sensing properties (Glamočlija et al. 2015a). The organic acids presented in the extracts were oxalic acid, phenolic acids, such as gallic acid, protocatechuic, and *p*-hydroxybenzoic acid. All the extracts exhibited unequivocal activity against *P. aeruginosa* PAO1 biofilm formation, pyocyanin productions, and twitching and flagella motility (Glamočlija et al. 2015a).

Methanolic extracts of three cultivated mushrooms of *Pleurotus ostreatus* and three wild mushrooms (*Gastrum fornicatum*, *Agaricus arvensis*, *Amanita pantherine*), ethanolic extracts of *Amanita rubescens*, and *Lactarius* sp. collected in Turkey were subjected to anti-quorum sensing activity study by the method of inhibition of violacein pigment production by *Chromobacterium violaceum*. The authors found out that all the extracts of studied mushrooms demonstrated anti-QS activity due to inhibition of pigment formation without change of the bacterial count (Tabbouche et al. 2017; Gorgen et al. 2018).

Five edible mushrooms (*Agaricus bisporus*, *Clitocybe nuda*, *Lactarius volemus*, *Macrolepiota procera*, and *Xerocomellus chrysenteron*) were studied regarding their anti-quorum sensing properties using *E. coli* JM109 with pSB1142 plasmid reporter strain against *P. aeruginosa*. All the extracts showed significant anti-quorum sensing activity without affecting the growth of *P. aeruginosa* (Strapáč et al. 2019).

Zhu et al. (2011) tested 14 mushrooms against inhibition of violacein produced by *C. violaceum*. All the tested supernatants obtained by fermentation inhibited violacein production without affecting bacterial growth (Zhu et al. 2011, 2012).

Tremella fuciformis dimethyl sulfoxide extract was successfully subjected to violacein inhibition study. The studied mushroom extract inhibited violacein production without affecting the growth of *C. violaceum* (Zhu and Sun 2008).

In the study of Yıldız et al. (2019), four wild mushroom extract (*Lactarius deliciosus*, *Laccaria bicolor*, *Bolista plumbea*, and *Boletus edulis*) and one cultivated mushroom extract (*Agaricus bisporus*) prepared by extraction using supercritical CO₂ were tested. Three of four wild mushroom extracts possessed anti-quorum sensing activity using violacein pigment inhibition method. *Lactarius deliciosus*, *Boletus edulis*, and *Laccaria bicolor* remarkably reduced production of pigment produced by *C. violaceum*. The growth of bacteria was unvaried or only slightly affected. QSI was not noticed for cultivated *A. bisporus* (Yıldız et al. 2019).

Pleurotus florida methanolic and chloroform extracts were studied as anti-QS agents. Authors demonstrated that *P. florida* has the potential to inhibit signalling

molecules produced by *P. aeruginosa* and obstruct its virulence factors. A study of swarming motility indicated that extracts are able to reduce motility. Authors also determined inhibition of AHL (acyl-homoserine lactone) and biofilm formation in concentration-dependent manner. Inhibition of AHL for methanolic and chloroform extracts was in the range of 37.89–58.94% and 50.05–70.05%, respectively. These results are in correlation with biofilm formation inhibition study, when the methanolic extracts decreased the formation of biofilm in the range of 33.9–83.9%, while using chloroform extracts, it was between 60.7 and 82.1%. The authors declared that both types of the extracts showed considerable ability to inhibit QS, and chloroform extracts exhibited a higher percentage of inhibition of AHL and biofilm production (Silambarasan et al. 2014).

These findings propose that mushrooms have the ability to produce compounds serving as a source of anti-quorum sensing agents, but the key molecule and mechanism of action have not been clarified yet.

5 Conclusions

The problem of microbial resistance is the reality of the current world. This fact forced the research communities around the world to exploit new and alternative strategies to fight against harmful resistant, or lethal microbes. The good and promising approach is quorum sensing inhibition.

A broad spectrum of compounds inhibiting QS have been reported, and various mechanisms of inhibition quorum sensing were reported. Mushrooms as quorum sensing inhibitors are also studied, due to broad spectrum of pharmacological activities (antimicrobial, antiviral, immunomodulatory, or antioxidant). Mushrooms represent rich source of bioactive compounds, namely polysaccharides, proteins, peptides, or secondary metabolites, such as phenolic compounds, flavonoids, vitamins, terpenes, steroids, anthraquinones, benzoic acid derivatives, and quinolines, organic acids, which are perspective antimicrobial substances. The various mushroom extracts underwent the study of anti-quorum sensing activity by various methods, but with perspective and promising results. But, on the other hand, there is a quite difficult challenge to find a single molecule responsible for quorum sensing inhibition of mushroom extract, and finally to clarify the mechanism of action.

References

- Asfour HZ (2018) Anti-Quorum sensing natural compounds. *J Microbiol Ultrastruct* 6:1–10. <https://doi.org/10.4103/JMAU.JMAU>
- Asif M, Acharya M (2012) Quorum sensing: a nobel target for antibacterial agents. *Avicenna J Med* 2:97–99. <https://doi.org/10.4103/2231-0770.110743>
- Azimi S, Klementiev AD, Whiteley M, Diggle SP (2020) Bacterial quorum sensing during infection. *Annu Rev Microbiol* 74:201–219. <https://doi.org/10.1146/annurev-micro-032020-093845>

- Bedlovičová Z, Smrčová M, Strapáč I, Šteffeková Z (2016) Mushrooms as the source of potential antimicrobial agents: a review. *Curr Org Chem* 20:2620–2632. <https://doi.org/10.2174/1385272820666160608101058>
- Bin L, Wei L, Xiaohong C et al (2012) In vitro antibiofilm activity of the melanin from *Auricularia auricula*, an edible jelly mushroom. *Ann Microbiol* 62:1523–1530. <https://doi.org/10.1007/s13213-011-0406-3>
- Borchers AT, Keen CL, Gershwin EM (2004) Mushrooms, tumors, and immunity: an update. *Exp Biol Med* 229:393–406
- Cassini A, Högberg LD, Plachouras D et al (2019) Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. *Lancet Infect Dis* 19:56. [https://doi.org/10.1016/S1473-3099\(18\)30605-4](https://doi.org/10.1016/S1473-3099(18)30605-4)
- Cataldi TRI, Bianco G, Frommberger M, Schmitt-Kopplin P (2004) Direct analysis of selected N-acyl-L-homoserine lactones by gas chromatography/mass spectrometry. *Rapid Commun Mass Spectrom* 18:1341–1344. <https://doi.org/10.1002/rcm.1480>
- Chen F, Gao Y, Chen X et al (2013) Quorum quenching enzymes and their application in degrading signal molecules to block quorum sensing-dependent infection. *Int J Mol Sci* 14:17477–17500. <https://doi.org/10.3390/ijms140917477>
- Chokshi A, Sifri Z, Cennimo D, Horng H (2019) Global contributors to antibiotic resistance. *J Glob Infect Dis* 11:36–42. https://doi.org/10.4103/jgid.jgid_110_18
- de Carvalho MP, Gulotta G, do Amaral MW et al (2016) Coprinuslactone protects the edible mushroom coprinus comatus against biofilm infections by blocking both quorum-sensing and mur a. *Environ Microbiol* 18:4254–4264. <https://doi.org/10.1111/1462-2920.13560>
- Debunne N, Verbeke F, Janssens Y et al (2018) Chromatography of quorum sensing peptides: an important functional class of the bacterial Peptidome. *Chromatographia* 81:25–40. <https://doi.org/10.1007/s10337-017-3411-2>
- Deep A, Chaudhary U, Gupta V (2011) Quorum sensing and bacterial pathogenicity: from molecules to disease. *J Lab Phys* 3:4–11. <https://doi.org/10.4103/0974-2727.78553>
- Fernandes Â, Petrović J, Stojković D et al (2016) *Polyporus squamosus* (Huds.) Fr from different origins: chemical characterization, screening of the bioactive properties and specific antimicrobial effects against *Pseudomonas aeruginosa*. *LWT-Food Sci Technol* 69:91–97. <https://doi.org/10.1016/j.lwt.2016.01.037>
- Finch RG, Pritchard DI, Bycroft BW et al (1998) Quorum sensing: a novel target for anti-infective therapy. *J Antimicrob Chemother* 42:569–571. <https://doi.org/10.1093/jac/42.5.569>
- Froes TQ, Guido RVC, Metwally K, Castilho MS (2020) A novel scaffold to fight *Pseudomonas aeruginosa* pyocyanin production: early steps to novel antivirulence drugs. *Future Med Chem* 12:1489–1503. <https://doi.org/10.4155/fmc-2019-0351>
- Fukumoto A, Murakami C, Anzai Y, Kato F (2016) Maniwamycins: new quorum-sensing inhibitors against *Chromobacterium violaceum* CV026 were isolated from *Streptomyces* sp. TOHO-M025. *J Antibiot (Tokyo)* 69:395–399. <https://doi.org/10.1038/ja.2015.126>
- Glamočlija J, Čirić A, Nikolić M et al (2015a) Chemical characterization and biological activity of Chaga (*Inonotus obliquus*), a medicinal “mushroom”. *J Ethnopharmacol* 162:323–332. <https://doi.org/10.1016/j.jep.2014.12.069>
- Glamočlija J, Stojković D, Nikolić M et al (2015b) Comparative study on edible *Agaricus* mushrooms as functional foods. *Food Funct* 6:1900–1910
- Greguš P, Vlčková H, Buchta V et al (2010) Ultra high performance liquid chromatography tandem mass spectrometry analysis of quorum-sensing molecules of *Candida albicans*. *J Pharm Biomed Anal* 53:674–681. <https://doi.org/10.1016/j.jpba.2010.05.029>
- Gurgen A, Yildiz S, Can Z et al (2018) Antioxidant, antimicrobial and anti-quorum sensing activities of some wild and cultivated mushroom species collected from Trabzon, Turkey. *Fresenius Environ Bull* 27:4120–4131
- Hossain MA, Lee SJ, Park NH et al (2017) Impact of phenolic compounds in the acyl homoserine lactone-mediated quorum sensing regulatory pathways. *Sci Rep* 7:1–16. <https://doi.org/10.1038/s41598-017-10997-5>

- Huang S, Zhang H, Albert Ng TC et al (2020) Analysis of N-Acy-L-homoserine lactones (AHLs) in wastewater treatment systems using SPE-LLE with LC-MS/MS. *Water Res* 177:115756. <https://doi.org/10.1016/j.watres.2020.115756>
- Kalia VC (2013) Quorum sensing inhibitors: an overview. *Biotechnol Adv* 31:224–245. <https://doi.org/10.1016/j.biotechadv.2012.10.004>
- Kalia VC, Purohit HJ (2011) Quenching the quorum sensing system: potential antibacterial drug targets. *Crit Rev Microbiol* 37:121–140. <https://doi.org/10.3109/1040841X.2010.532479>
- Kalia VC, Wood TK, Kumar P (2014) Evolution of resistance to quorum-sensing inhibitors. *Microb Ecol* 68:13–23. <https://doi.org/10.1007/s00248-013-0316-y>
- Kato N, Tanaka T, Nakagawa S et al (2007) Control of virulence factor expression in opportunistic pathogens using cyclodextrin immobilized gel. *J Incl Phenom Macrocycl Chem* 57:419–423. <https://doi.org/10.1007/s10847-006-9228-5>
- Koc B, Akyuz L, Cakmak YS et al (2020) Production and characterization of chitosan-fungal extract films. *Food Biosci* 35:100545. <https://doi.org/10.1016/j.fbio.2020.100545>
- Kostić M, Smiljković M, Petrović J et al (2017) Chemical, nutritive composition and a wide range of bioactive properties of honey mushroom: *Armillaria mellea* (Vahl: Fr.) Kummer. *Food Funct* 8:3239–3249. <https://doi.org/10.1039/c7fo00887b>
- Lee-Hoon H, Noroul AZ, Thuan-Chew T (2020) Edible mushroom: nutritional properties, potential nutraceutical values, and its utilisation in food product development Lee-Hoon. In: Passari AK, Sánchez S (eds) *An introduction to mushrooms*, 1st edn. Intech Open, London, p 38
- Lee S, Park SK, Kwon H et al (2016) Crossing the border between laboratory and field: bacterial quorum quenching for anti-biofouling strategy in an MBR. *Environ Sci Technol* 50:1788–1795. <https://doi.org/10.1021/acs.est.5b04795>
- Liu CX, Zhang DR, He Y et al (2010) Modification of membrane surface for anti-biofouling performance: effect of anti-adhesion and anti-bacteria approaches. *J Memb Sci* 346:121–130. <https://doi.org/10.1016/j.memsci.2009.09.028>
- McLean RJC, Pierson LS, Fuqua C (2004) A simple screening protocol for the identification of quorum signal antagonists. *J Microbiol Methods* 58:351–360. <https://doi.org/10.1016/j.mimet.2004.04.016>
- Miller LC, O Loughlin T, Zhang Z et al (2015) Development of potent inhibitors of pyocyanin production in *Pseudomonas aeruginosa*. *J Med Chem* 58:1298–1306. <https://doi.org/10.1021/jm5015082.Development>
- Mion S, Carriot N, Lopez J et al (2021) Disrupting quorum sensing alters social interactions in *Chromobacterium violaceum*. *NPJ Biofilms Microbi* 7:40. <https://doi.org/10.1038/s41522-021-00211-w>
- Morkunas B, Gal B, Galloway WRJD et al (2016) Discovery of an inhibitor of the production of the *Pseudomonas aeruginosa* virulence factor pyocyanin in wild-type cells. *Beilstein J Org Chem* 12:1228–1233. <https://doi.org/10.3762/bjoc.12.137>
- Morohoshi T, Fukamachi K, Kato M et al (2010) Regulation of the violacein biosynthetic gene cluster by acylhomoserine lactone-mediated quorum sensing in *chromobacterium violaceum* ATCC 12472. *Biosci Biotechnol Biochem* 74:2116–2119. <https://doi.org/10.1271/bbb.100385>
- Morohoshi T, Kato M, Fukamachi K et al (2008) N-Acylhomoserine lactone regulates violacein production in *Chromobacterium violaceum* type strain ATCC 12472. *FEMS Microbiol Lett* 279:124–130. <https://doi.org/10.1111/j.1574-6968.2007.01016.x>
- Morohoshi T, Shiono T, Takidouchi K et al (2007) Inhibition of quorum sensing in *Serratia marcescens* AS-1 by synthetic analogs of N-acylhomoserine lactone. *Appl Environ Microbiol* 73:6339–6344. <https://doi.org/10.1128/AEM.00593-07>
- Nazzaro F, Fratianni F, Coppola R (2013) Quorum sensing and phytochemicals. *Int J Mol Sci* 14:12607–12619. <https://doi.org/10.3390/ijms140612607>
- O'Connor G, Knecht LD, Salgado N et al (2016) Whole-cell biosensors as tools for the detection of quorum-sensing molecules: uses in diagnostics and the investigation of the quorum-sensing mechanism. *Adv Biochem Eng Biotechnol* 154:181–200. https://doi.org/10.1007/10_2015_337

- Obi RK, Nwanebu FC, Ndbubuisi UU, Orji NM (2009) Antibacterial qualities and phytochemical screening of the oils of *Curcubita pepo* and *Brassica nigra*. *J Med Plants Res* 3:429–432. <https://doi.org/http://www.academicjournals.org/journal/JMPR/article-abstract/506FA1E15270>
- Oh HS, Tan CH, Low JH et al (2017) Quorum quenching bacteria can be used to inhibit the biofouling of reverse osmosis membranes. *Water Res* 112:29–37. <https://doi.org/10.1016/j.watres.2017.01.028>
- Omer III, Alfaig EAA (2020) Chemical composition and nutritional value of some type of wild mushrooms in Blue Nile state. *Int J Food Sci Biotechnol* 5:22–30. <https://doi.org/10.11648/j.ijfsb.20200502.12>
- Paluch E, Rewak-Soroczyńska J, Jędrusik I et al (2020) Prevention of biofilm formation by quorum quenching. *Appl Microbiol Biotechnol* 104:1871–1881. <https://doi.org/10.1007/s00253-020-10349-w>
- Patel NM, Moore JD, Blackwell HE, Amador-Noguez D (2016) Identification of unanticipated novel N-Acyl L-homoserine lactones (AHLs) using a sensitive non-targeted LC-MS/MS method. *PLoS One* 11:1–20. <https://doi.org/10.1371/journal.pone.0163469>
- Petrović J, Glamočlija J, Stojković D et al (2014) Bioactive composition, antimicrobial activities and the influence of *Agrocybe aegerita* (Brig.) sing on certain quorum-sensing-regulated functions and biofilm formation by *Pseudomonas aeruginosa*. *Food Funct* 5:3296–3303. <https://doi.org/10.1039/c4fo00819g>
- Piecuch A, Lamch PE et al (2016) Biofilm prevention by dicephalic cationic surfactants and their interactions with DNA. *J Appl Microbiol* 121:682–692. <https://doi.org/10.1111/jam.13204>
- Pilařová V, Kočová Vlčková H, Jung O et al (2020) Unambiguous determination of farnesol and tyrosol in vaginal fluid using fast and sensitive UHPLC-MS/MS method. *Anal Bioanal Chem* 412:6529–6541. <https://doi.org/10.1007/s00216-020-02699-1>
- Purohit AA, Johansen JA, Hansen H et al (2013) Presence of acyl-homoserine lactones in 57 members of the Vibrionaceae family. *J Appl Microbiol* 115:835–847. <https://doi.org/10.1111/jam.12264>
- Rai N, Rai R, Venkatesh KV (2015) Quorum sensing biosensors. In: *Quorum sensing vs quorum quenching: a battle with no end in sight*. Springer, India, pp 173–183
- Rajesh PS, Rai VR (2016) Inhibition of QS-regulated virulence factors in *Pseudomonas aeruginosa* PAO1 and *Pectobacterium carotovorum* by AHL-lactonase of endophytic bacterium *Bacillus cereus* VT96. *Biocatal Agric Biotechnol* 7:154–163. <https://doi.org/10.1016/j.bcab.2016.06.003>
- Rasmussen TB, Givskov M (2006) Quorum sensing inhibitors: a bargain of effects. *Microbiology* 152:895–904. <https://doi.org/10.1099/mic.0.28601-0>
- Reis FS, Martins A, Barros L, Ferreira ICFR (2012) Antioxidant properties and phenolic profile of the most widely appreciated cultivated berry species: a comparative study. *Food Chem Toxicol* 50:1201–1207. <https://doi.org/10.2478/fhort-2020-0008>
- Shaw PD, Ping G, Daly SL et al (1997) Detecting and characterizing N-acyl-homoserine lactone signal molecules by thin-layer chromatography. *Proc Natl Acad Sci U S A* 94:6036–6041. <https://doi.org/10.1073/pnas.94.12.6036>
- Silambarasan P, Sumathy R, Kumuthakalavalli R (2014) Anti-quorum sensing activity of an oyster mushroom, *Pleurotus florida* (Mont.) against *Pseudomonas aeruginosa*. *Elixir Appl Biol* 69:22947–22949
- Sio CF, Otten LG, Cool RH et al (2006) Quorum quenching by an N-acyl-homoserine lactone acylase from *Pseudomonas aeruginosa* PAO1. *Infect Immun* 74:1673–1682. <https://doi.org/10.1128/IAI.74.3.1673-1682.2006>
- Soković M, Ćirić A, Glamočlija J et al (2014) *Agaricus blazei* hot water extract shows anti quorum sensing activity in the nosocomial human pathogen *Pseudomonas aeruginosa*. *Molecules* 19:4189–4199. <https://doi.org/10.3390/molecules19044189>
- Strapáč I, Bedlovičová Z, Čuvalová A et al (2019) Antioxidant and anti-quorum sensing properties of edible mushrooms. *J Food Nutr Res* 58:146–152

- Tabbouche SA, Gürgen A, Yildiz S et al (2017) Antimicrobial and anti-quorum sensing activity of some wild mushrooms collected from Turkey. *MSU Fen Bil Dergi*, Cilt 5:453–457. <https://doi.org/10.18586/msufbd.347692>
- Tang K, Zhang Y, Yu M et al (2013) Evaluation of a new high-throughput method for identifying quorum quenching bacteria. *Sci Rep* 3:1–9. <https://doi.org/10.1038/srep02935>
- Teasdale ME, Liu J, Wallace J et al (2009) Secondary metabolites produced by the marine bacterium *Halobacillus salinus* that inhibit quorum sensing-controlled phenotypes in gram-negative bacteria. *Appl Environ Microbiol* 75:567–572. <https://doi.org/10.1128/AEM.00632-08>
- Teplitski M, Eberhard A, Gronquist MR et al (2003) Chemical identification of N-acyl homoserine lactone quorum-sensing signals produced by *Sinorhizobium meliloti* strains in defined medium. *Arch Microbiol* 180:494–497. <https://doi.org/10.1007/s00203-003-0612-x>
- Terwagne M, Bonnot S, Letesson JJ (2012) *Brucella* quorum sensing: much more than sensing quorum. *Brucella Mol Microbiol Genomics*:163–178
- Vunduk J, Wan-Mohtar WAAQI, Mohamad SA et al (2019) Polysaccharides of *Pleurotus flabellatus* strain Mynuk produced by submerged fermentation as a promising novel tool against adhesion and biofilm formation of foodborne pathogens. *LWT* 112:108221. <https://doi.org/10.1016/j.lwt.2019.05.119>
- Wang J, Lin J, Zhang Y et al (2019) Activity improvement and vital amino acid identification on the marine-derived quorum quenching enzyme MOML by protein engineering. *Mar Drugs* 17:300. <https://doi.org/10.3390/md17050300>
- Williams P (2007) Quorum sensing, communication and cross-kingdom signalling in the bacterial world. *Microbiology* 153:3923–3938. <https://doi.org/10.1099/mic.0.2007/012856-0>
- Wu L, Luo Y (2021) Bacterial quorum-sensing systems and their role in intestinal bacteria-host crosstalk. *Front Microbiol* 12:1–11. <https://doi.org/10.3389/fmicb.2021.611413>
- Xavier KB, Bassler BL (2003) LuxS quorum sensing: more than just a numbers game. *Curr Opin Microbiol* 6:191–197. [https://doi.org/10.1016/S1369-5274\(03\)00028-6](https://doi.org/10.1016/S1369-5274(03)00028-6)
- Yang YH, Lee TH, Kim JH et al (2006) High-throughput detection method of quorum-sensing molecules by colorimetry and its applications. *Anal Biochem* 356:297–299. <https://doi.org/10.1016/j.ab.2006.05.030>
- Yıldız S, Gürgen A, Tabbouche S et al (2019) Comparison of quorum sensing inhibition and antimicrobial properties of some commercial and wild mushrooms extracted with supercritical CO₂. *Celal Bayar Üniversitesi Fen Bilim Derg* 15:193–198. <https://doi.org/10.18466/cbayarfb.514436>
- Zaki AA, Shaaban MI, Hashish NE et al (2013) Assessment of anti-quorum sensing activity for some ornamental and medicinal plants native to Egypt. *Sci Pharm* 81:251–258. <https://doi.org/10.3797/scipharm.1204-26>
- Zhang LH, Dong YH (2004) Quorum sensing and signal interference: diverse implications. *Mol Microbiol* 53:1563–1571. <https://doi.org/10.1111/j.1365-2958.2004.04234.x>
- Zhu H, Liu W, Wang SX et al (2012) Evaluation of anti-quorum-sensing activity of fermentation metabolites from different strains of a medicinal mushroom, *Phellinus igniarius*. *Chemotherapy* 58:195–199. <https://doi.org/10.1159/000338383>
- Zhu H, Sun SJ (2008) Inhibition of bacterial quorum sensing-regulated behaviors by *Tremella fuciformis* extract. *Curr Microbiol* 57:418–422. <https://doi.org/10.1007/s00284-008-9215-8>
- Zhu H, Wang SX, Zhang SS, Cao CX (2011) Inhibiting effect of bioactive metabolites produced by mushroom cultivation on bacterial quorum sensing-regulated behaviors. *Chemotherapy* 57:292–297. <https://doi.org/10.1159/000329525>

Chapter 15

Beehives as a Natural Source of Novel Antimicrobials



Jelena Suran

Abstract Honey bee products have been used since ancient times as food and therapeutics. There is increasing knowledge on their content and molecular mechanism of action. Their bioactive compounds are a combination of both honey bee and plant origin. Plant immune response effectors are secondary metabolites (polyphenols, terpenes, antimicrobial peptides), and they are responsible for the antimicrobial effects of honey bee products like honey, propolis, and bee pollen. Honey bee innate immunity effectors are antimicrobial peptides, like defensin 1 and 2, apidaecins, abaecins, and hymenoptaecin, and some of them have been found in royal jelly, honey, and pollen. Plant secondary metabolites and honey bee antimicrobial peptides combine in beehive mixtures with synergistic antimicrobial activity and undoubtedly represent an interesting alternative to standard antibiotics. Further research should elucidate their interactions in honey bee products as well as their potential biotechnology applications.

Keywords Honey bees · Honey · Propolis · Royal jelly · Bee pollen · Plant secondary metabolites · Immunity · Antimicrobial peptides

Abbreviations

10-acetooxy-2-DEA	10-Acetoxydecanoic acid
10-HDA	10-Hydroxy-2-decenoic acid
3,10-HDA	3,10-Dihydroxy-decanoic acids
AAMP	Anionic antimicrobial peptides
AMPs	Antimicrobial peptides
CAMP	Cationic antimicrobial peptides
CAPE	Caffeic acid phenethyl ester

J. Suran (✉)

Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

e-mail: jelena.suran@vef.hr

CNS	Coagulase-negative staphylococci
CPPs	Cell-penetrating peptides
CS $\alpha \beta$	Cysteine-stabilized $\alpha \beta$ motif
Cys	Cysteine
Gly	Glycine
HIV	Human immunodeficiency virus
HSV	Herpes simplex virus
Imd	Immune deficiency pathway
Jak/STAT	Janus kinase/signal transducer and activator of transcription
JNK	c-Jun N-terminal kinase
JV	Junín virus
MAPK	Mitogen-activated protein kinases
MIC	Minimum inhibitory concentration
MRJP	Major royal jelly protein
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MSSA	Methicillin-sensitive <i>Staphylococcus aureus</i>
NB-LR	Nucleotide-binding leucine-rich repeat containing resistance proteins
NF- κ B	Nuclear factor kappa B
P/MAMP	Pathogen/microbe-associated molecular pattern molecules
PR	Pathogenesis-related proteins
Pro	Proline
PRRs	Pattern recognition receptors
RJ	Royal jelly
RLP/Ks	Receptor-like proteins or -kinases
RNAi	RNA interference
RSV	Respiratory syncytial virus
SM	Secondary metabolites
TMV	Tobacco mosaic virus
VRE	Vancomycin-resistant enterococci
VSV	Vesicular stomatitis virus
VZV	Varicella-zoster virus

1 Introduction

In recent decades, as antimicrobial resistance is being increasingly recognized as a global public health threat, natural mixtures with antimicrobial effects such as products from the honey bee *Apis mellifera* are being re-discovered by mainstream medicine.

Beehives have been used as a resource of food and medicines since ancient times. The oldest evidence of humans collecting honey from wild bees dates back to 10,000 years ago (Dams and Dams 1977). Beekeeping started in the early Neolithic period (Roffet-Salque et al. 2016), while according to Crane (1999), domestication of bees was depicted in Egyptian art from around 4500 years ago. Honey was used

in the past in different parts of the world to improve wound and gut healing (Zumla and Lulat 1989). Even the Muslim prophet Mohammed and Aristotle (350 BC) recommended the use of honey for medical purposes (Molan 1999). In Ancient Egypt, propolis was first recognized as an adhesive for sealing cracks in wood, while Aristotle was one of the first to refer to it as a healing agent. In addition, Aristotle was the first to recognize how royal jelly promotes physical strength and intellectual capacity (Fratini et al. 2016a).

Centuries later, with the advent of science, these products have been extensively studied; their composition is analyzed with advanced instrumental methods, while their biological activity is studied in different *in vitro* and *in vivo* assays. As the knowledge about their molecular mechanisms of action grows, we become more aware of their complexities.

The beehive can be viewed as a melting pot of plant and insect defense mechanisms (Fig. 15.1). These defense mechanisms can be extracted in the form of beehive products used as antimicrobial agents. These products are honey, propolis royal jelly, bee pollen, beeswax, and bee venom. Each honey bee product is specific for its content of active compounds, and many of them have a plethora of effects – from antioxidant to antimicrobial.

The compounds vital for plant defense are plant secondary metabolites (SM), abundant in honey bee products. Polyphenols are a huge and versatile group of SM, and many of them can be used as representative markers of honey bee products like propolis. Along with polyphenols, there are terpenoids and plant antimicrobial peptides (AMP). The possible interactions among these compounds yet have to be

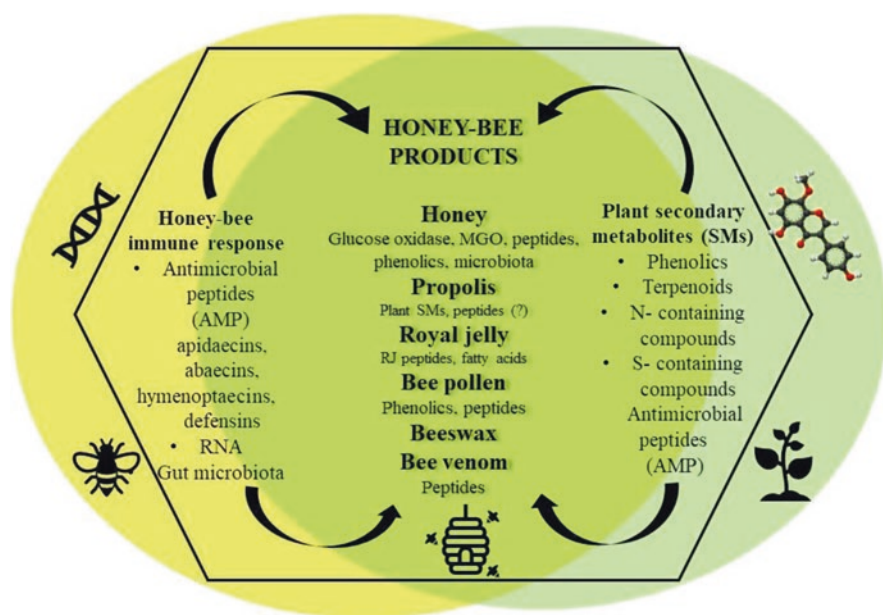


Fig. 15.1 The beehive as the melting-pot of honey bee and plant defense mechanisms

elucidated. Honey bees' defense is based on individual innate immunity and social, collective immunity. Plant and animal material that honey bees integrate into honey bee products is an essential part of the latter. Still, at the same time, these products work through the former – by acting on the intracellular mechanisms vital for individual innate immunity.

In this chapter, I present some of the most relevant antimicrobial compounds that build the defense system of the beehive. These compounds are divided according to their origin, with their role, and antimicrobial effects. Next, honey bee products are described, followed by numerous studies of their antimicrobial efficacy. Undoubtedly, beehives are rich resources of potent antimicrobial compounds, just waiting to be utilized to fight against antimicrobial resistance.

2 Plant Origin of Antimicrobial Substances in the Beehive

Using the beehive as a resource of antimicrobial compounds means considering the immune strategies of insects like honey bees and the vast array of plant–host defense mechanisms. These mechanisms work synergistically as plant, and insect-derived material is combined in honeybee products. Here is where the bees, with all their capabilities, concentrate the abundance of substances from plants and their own, such as polyphenols (flavonoids and phenolic acids), glycoproteins, and antimicrobial peptides, in fighting and resisting various pathogens.

2.1 Plant Immune Response

Plants respond to infection using a two-branched or two-level innate immune system (Jones and Dangl 2006) that needs to be versatile and effective, since plants lack the mobility and a somatic adaptive immune system from animals. The first branch recognizes and responds to molecules common to many classes of microbes, including non-pathogens through defense- receptor-like proteins or -kinases (RLP/Ks) as pattern recognition receptors (PRRs), which can detect conserved pathogen/microbe-associated molecular pattern (P/MAMP) molecules, considered to be an early warning system for the presence of pathogens and the timely activation of plant defense mechanisms (Jones and Dangl 2006; Dubery et al. 2012). A second line of plant defense includes the response to pathogen virulence factors, either directly or through their effects on host targets (Jones and Dangl 2006) via intracellular nucleotide-binding leucine-rich repeat (NB-LR)-containing resistance proteins, which recognize isolate-specific pathogen effectors once the cell wall has been compromised (Dubery et al. 2012).

Proteins and peptides involved in these mechanisms can be found in plant material collected by honey bees and integrated in honey and royal jelly products. One of the most studied antimicrobial peptides, defensins, found in bees, honey, and royal jelly could be partly of plant origin. Furthermore, plant polyphenols are highly

versatile secondary plant metabolites, allowing plants to respond promptly to unpredictable stress agents of different origins (Wink 2008).

2.2 Plant Secondary Metabolites (SMs)

General resistance in plants is achieved by the production of secondary metabolites (SMs), a highly diverse group of organic molecules which are not necessary for the actual metabolism or physiology of the plants producing them. These compounds serve as protective agents against various pathogens: bacteria, fungi, viruses, and insects (Wink 2008). There are several different classes of SMs: phenolic compounds (flavonoids, tannins), terpenoids, N-containing compounds (non-protein amino acids, cyanogenic glucosides alkaloids), and S-containing compounds (pathogenesis-related (PR) proteins, phytoalexins) (Wink 2008; Jamwal et al. 2018). In nature, these metabolites always come in complex mixtures.

Polyphenols

One of the most abundant groups of SMs in honey bee products is polyphenols. Polyphenols can be divided into several classes: flavonols, flavones, flavanones, anthocyanidins, flavanols, and isoflavones (Daglia 2012). Polyphenols were studied mostly because of their antioxidant effect as the basis for chronic disease prevention, but with the increase of antimicrobial resistance, their antimicrobial potential came into focus as well.

In general, flavonoids have shown stronger antimicrobial activity than non-flavonoid compounds. Flavan-3-ols, flavonols, and tannins were extensively studied due to their wide spectrum and higher antimicrobial activity compared to other polyphenols. Most of them can suppress many microbial virulence factors (such as inhibition of biofilm formation, reduction of host ligands adhesion, and neutralization of bacterial toxins) and show synergism with antibiotics (Daglia 2012). Although weaker than flavonoids, non-flavonoids such as phenolic acids (caffeic and ferulic acids) showed activity against Gram-positive (*Staphylococcus aureus*, *Listeria monocytogenes*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) (Daglia 2012).

There are several mechanisms of polyphenol antimicrobial activity (Olchowik-Grabarek et al. 2020): the damage of the cell membrane and cell wall (Funatogawa et al. 2004; Yi et al. 2010; Adnan et al. 2017), inhibition of energy metabolism (Li et al. 2017), production, secretion, structure, and activity of released toxins (Hisano et al. 2003; Shah et al. 2008; Lee et al. 2012; Dong et al. 2013; Verhelst et al. 2013; Wang et al. 2015; Song et al. 2016; Shimamura et al. 2015; Chang et al. 2019; Tang et al. 2019) and biofilm formation (Lin et al. 2011; Trentin et al. 2013). Polyphenols also act at the level of target cells, increasing their resistance to toxins (Olchowik-Grabarek et al. 2020). Regarding polyphenol interaction with cell structures, it was

hypothesized that the polyphenols rich in gallate moieties might attach to the cell surface, serve as bridges between surfaces of two neighbor cells, and initiate cell-binding and formation of similar clusters in the membrane of the opposite cell (Tarahovsky 2008). Phan et al. (2014) confirmed that an increase in the number of hydrophilic side chains (galloyl, hydroxyl, glucoside, gallate) increased the reactivity of the polyphenols with cell membranes. Due to their polarity, they are not able to pass the cell membrane through passive diffusion, so it is assumed that they pass through the membranes with the help of other plant SMs (Wink 2008).

The interactions of polyphenols with proteins and peptides are interesting, not only for a better understanding of their action on cell surfaces and signal transduction pathways but to understand how these molecules will interact with each other in a natural mixture like those found in the beehive. Peptides and polyphenols form noncovalent (hydrogen, hydrophobic, and ionic bonds) and covalent bonds (between oxidized phenolic compounds and peptides) (Sun and Udenigwe 2020).

While forming ionic bonds, negatively charged phenolate ions interact with positively charged amino acids. Depending on their size, a single polyphenol can bind even several proteins simultaneously (Wink 2008). Bourvellec and Renard (2012) describe how, at the same time when hydrophobic bonds form between polyphenol aromatic rings and hydrophobic residues of amino acids, hydrogen bonds are also formed between the hydroxyl groups of polyphenols and the acceptor site for hydrogen ions in the proteins (Bourvellec and Renard 2012). The primary factors affecting the protein–polyphenol interaction are conformation and type of both proteins and polyphenols. Other factors are environmental conditions, like temperature and pH (Quan et al. 2019). It is assumed that the phenolic binding can affect protein activity or even protect proteins from proteolytic cleavage (Wink 2008). When polyphenols oxidize to reactive quinones, they form covalent bonds with proteins in honey, and this complexation can lead to decreased antioxidant, enzymatic, or antimicrobial activity (Brudzynski and Maldonado-Alvarez 2015).

On the other hand, there is growing evidence that the formation of protein/peptide conjugates results in increased antioxidant activity and stability in food (Quan et al. 2019). Possibly, the same logic could be applied to their antimicrobial activity, and we assume that polyphenols could increase the stability and the activity of antimicrobial peptides.

Volatile SMs (Terpenoids)

The volatile SMs necessary for plant defense are complex mixtures of hydrocarbons and oxygenated hydrocarbons from the isoprenoid pathways, primarily monoterpenes and sesquiterpenes (Bankova et al. 2014). They are produced and secreted by glandular trichomes; specialized secretory tissues diffused onto the surface of plant organs, particularly flowers and leaves (Bankova et al. 2014).

Plant Antimicrobial Peptides (AMPs)

Plants produce PR proteins/peptides with numerous defense-related properties, including antibacterial, antifungal, antiviral, antioxidative activity, chitinase, and proteinase inhibitory activities (Tam et al. 2015). Antimicrobial peptides (AMPs) interact with cell membrane phospholipids and cell-penetrating peptides (CPPs), which introduce certain cargoes in the cell (Nawrot et al. 2014).

AMPs have been isolated from all parts of plants and can be divided into anionic (AAMPs) and cationic (CAMPs) peptides. These groups have shown activity against pathogenic microorganisms (bacteria, viruses, and fungi) and even neoplastic cells (Montesinos 2007; Nawrot et al. 2014). Antimicrobial peptides (AMP) found in plants are rich in Cys, enabling disulfide bonds. This contributes to their stability and resistance to enzymatic degradation. (Tam et al. 2015). According to Nawrot et al. (2014), there are six groups of plant AMPs: thionins, defensins, lipid transfer proteins, cyclotides, hevein-like proteins, and knottin-type proteins.

AMPs mechanism of antimicrobial action has been described through several types of models of membrane pore formation, which leads to cell content leakage and death. AMPs act on the microorganism cell membrane due to their negative charge, which attracts cationic peptides. In the bacterial membrane, negatively charged molecules, and thus main receptors of CAMPs are phospholipids. While in fungal membranes, these are glucosylceramides and sphingolipids. In addition, many CAMPs appear to target internal anionic cell constituents, such as DNA, RNA, or cell wall components (Diamond et al. 2009). AMPs exhibit broad-spectrum activity, and thus far, it appears as though bacteria do not develop resistance as quickly as with conventional antibiotics (Diamond et al. 2009).

While the mechanisms of CAMPs are better understood, those of AAMPs are less so. There is evidence suggesting they increase plasma membrane permeability by binding to lipids, disrupting the envelope integrity by attaching to chitin, and damaging intracellular structures, such as DNA. It is also proposed that AAMPs participate in the plant innate immune response and act synergistically with CAMPs (Prabhu et al. 2013). Prabhu et al. (2013) conclude that cyclotides are the plant AAMPs with the greatest potential for therapeutic and biotechnical development. Cyclotides are named after the cyclic peptide backbone and a knotted arrangement of three conserved disulfide bonds. Due to those bonds, they are relatively stable to thermal, chemical, and enzymatic degradation and can be modified by residue substitutions (Prabhu et al. 2013). One of the best-studied cyclotides, kalata B2, was found to have potent antibacterial activity against *Salmonella enterica*, *E. coli*, and *S. aureus* (Gran et al. 2008; Pranting et al. 2010), but also against parasites like gastrointestinal nematodes *Haemonchus contortus* and *Trichostrongylus colubriformis* (Colgrave et al. 2008). Other known antimicrobial cyclotides with antibacterial activity are vaby D (Pranting et al. 2010) and cycloviolacin O24 (Ireland et al. 2006) and cycloviolacins Y1, Y4, and Y5 which exhibit anthelmintic properties (Colgrave et al. 2008) and antiviral activity (Wang et al. 2008).

The two most prominent plant CAMP families are thionins and defensins. There are several common traits of these two CAMP families between various species (microbes, plants, animals), and those include their amphipathic nature, positive charge, and molecular structure. These peptides are membrane-active, while other families of AMPs have a different mechanism of action – from enzyme inhibition to lipid transfer. Thionins are AMPs with a small molecular weight (~5 kDa) rich in arginine, lysine, and cysteine residues (Nawrot et al. 2014). There are two groups of thionins, α -/ β - and γ -thionins (based on their structure, γ -thionins are considered to be a part of the defensin family of peptides). They are toxic against phytopathogenic bacteria, fungi (Ebrahimesbat et al. 1989), and yeasts, and also some animal and plant cells (Evans et al. 1989). They interact with the protein receptors or lipids in membranes (Osorio e Castro and Vernon 1989; Florack and Stiekema 1994; Garcia-Olmedo et al. 1998; Stec 2006) with their hydrophobic residues and positive surface charge to cause cell leakage and lysis (Majewski and Stec 2001; Tam et al. 2015). Thionins isolated from black seed (*Nigella sativa*) showed bactericidal and fungicidal effects on *Bacillus subtilis*, *S. aureus*, and *Candida albicans* (Vasilchenko et al. 2017).

Defensins are well-known and abundant AMP in plants, vertebrates, and invertebrate animals (Nawrot et al. 2014; Tam et al. 2015) and fungi (Wu et al. 2014). They are also of small molecular weight (~5 kDa), cysteine rich and cationic peptides with broad-spectrum antimicrobial activity; antibacterial, antifungal, antiviral, proteinase, and insect amylase inhibitor (Nawrot et al. 2014). Their previously described mechanisms of antimicrobial activity are based on membrane lysis. Still, there are other processes by which they disrupt, such as interfering with cell signaling, intracellular trafficking, blocking the receptor binding, and cell entry (Weber 2020). Plant defensins are ancient and conserved; therefore, they are similar to honey bees and vertebrate animals (Nawrot et al. 2014). They also act as immunomodulators by attracting immune cells and modulating adaptive immune responses (Weber 2020).

Despite having only identified and isolated AMPs from honey bees and their products, one cannot exclude the possibility that some of these peptides are of plant origin since there is a certain amount of plant material in the beehive. One cannot also exclude the possible relevance of these peptides, such as in the case of polyphenols and other secondary plant metabolites that have been identified in honey, pollen, or propolis.

3 Honey Bee Defense Mechanisms

Honey bees are social insects with a collective “social immunity” and an individual innate immunity, which consists of humoral and cellular effectors (Evans et al. 2006).

3.1 *Honey Bee Individual Immunity*

Cells involved in individual honey bee immune response are phagocytes and hemocytes and humoral-induced effectors such as AMPs, thioester linkage proteins, melanization, and coagulation proteins (Larsen et al. 2019). Antiviral intracellular defense mechanisms include RNA interference (RNAi), endocytosis, melanization, encapsulation, autophagy, and conserved immune pathways including Jak/STAT (Janus kinase/signal transducer and activator of transcription), JNK (c-Jun N-terminal kinase), MAPK (mitogen-activated protein kinases), and the NF- κ B mediated Toll and Imd (immune deficiency) pathways (McMenamin et al. 2018). Interestingly, RNAi is the key resistance mechanism against viruses, not only for individual honey bees but also for the whole beehive's immune response (Maori et al. 2019). Similarly, Toll, Imd, Janus kinase (JAK)/STAT, and JNK are signaling pathways induced by bacterial cell wall lipopolysaccharides or peptidoglycans (Boutros 2002; Evans et al. 2006) and result in the release of antimicrobial effectors, peptides, such as hymenoptaecin, defensin 1, and abaecin at the end of the cascade (Evans et al. 2006; Gättschenberger et al. 2013). As in plants, AMPs are considered the key component of honey bee innate immunity (Daníhlík et al. 2015).

3.2 *Honey Bee AMPs*

Both honey bee products and antimicrobial peptides (AMPs) have been recognized as resources of promising alternatives to conventional antibiotics. AMPs have been described as ancient evolutionary weapons produced by many living organisms as a part of their nonspecific immune response. Thus, they are effective against many microorganisms (Baltzer and Brown 2011). AMPs exhibit a multimodal mechanism of action, specifically responding to various intracellular targets and binding to lipopolysaccharides of the bacterial membrane with different, concentration-dependent affinity (Baltzer and Brown 2011; Hughes et al. 2000; Li et al. 2012).

As plant AMPs, insect AMPs form pores on the cell membrane of bacteria in different ways (Li et al. 2012). They can also bind to different intracellular targets (DNA, RNA, and proteins) once inside the cell and inhibit their synthesis (Lan et al. 2010; Li et al. 2012). Moreover, insect AMPs can interfere with bacterial cytokinesis by cell filamentation, using unique translocation mechanisms to alter the cytoplasmic membrane septum formation (Brown and Hancock 2006; Lan et al. 2010; Li et al. 2012).

Not only do they have broad-spectrum activity against microorganisms, but AMPs are also able to bypass the common resistance mechanisms that render conventional antibiotics ineffective (Wang et al. 2016). Apart from antimicrobial activity, AMPs also modulate the immune system via cytokine activity or angiogenesis (Li et al. 2012). Potential novel therapeutics such as AMPs could be

implemented using natural mixtures that may have antimicrobial and immunomodulatory activity due to their complexity and molecular synergism.

Based on their structure, insect AMPs can be divided into four categories: α -helix (cecropin and moricin), Cys-rich (insect defensin and drosomycin), Pro-rich (apidaecin, drosocin, and lebocin), and Gly-rich peptides (attacin) (Bulet and Stöcklin 2005; Yi et al. 2014). Honey bees pathogens induce four families of AMPs; apidaecins, abaecins, hymenoptaecins, and defensins. These families have a broad spectrum of antimicrobial activity in the hemolymph (Xu et al. 2009). Besides the active AMPs in adult honey bee lymph, inactive peptide precursors can be found in bee larvae (Casteels et al. 1989). Apidaecins were found to be very selective and active against human and animal Gram-negative bacteria (*E. coli*, *Salmonella*, and *Shigella* species) (Casteels et al. 1989), while abaecins are more active against Gram-positive bacteria (Casteels et al. 1990). To be more specific, in comparison to abaecins, apidaecins showed 200-fold more activity against *Agrobacterium*, *Erwinia*, and *E. coli* strains (Casteels et al. 1990). In the same study, abaecins showed the highest specific activity against plant pathogen *Xanthomonas campestris*. This was expected since honey bees are often exposed to plant-associated microorganisms whilst gathering food, pollen, and nectar. Hymenoptaecin is active against Gram-negative and Gram-positive bacteria, including several human pathogens (Casteels et al. 1993). Its bactericidal effect against *E. coli* results from sequential permeabilization of the outer and inner membranes (Casteels et al. 1993). When combined in immune lymph, hymenoptaecin, and apidaecin, as the two predominant factors, had a strong bactericidal effect against a broad spectrum of Gram-negative (*Bordetella bronchiseptica*, *Enterobacter cloacae*, *Haemophilus influenzae*, *Yersinia enterocolitica*, etc.) and some Gram-positive bacteria. Defensins killed Gram-positive bacteria (e.g., *Clostridium* and *Streptococcus* species) that were unaffected by their combination. As Casteels et al. (1993) concluded, “it is clear that the broad-spectrum antibacterial activity of immune lymph is the result of an amazing complementarity.”

As in plants, defensins are the most abundant group of AMPs in insects. In general, insect defensins have an N-terminal loop and an α -helical fragment followed by an antiparallel β -structure, connected by two of the three disulfide bridges. These form so-called cysteine-stabilized $\alpha\beta$ (CS $\alpha\beta$) motif (Cornet et al. 1995). Defensins have antibacterial activity against Gram-positive bacteria, including *S. aureus*, *Micrococcus luteus*, and *Aerococcus viridans* (Yi et al. 2014; Li et al. 2017). Two types of defensins have been identified in honey bees. Defensin 1 is synthesized in salivary glands and plays an important role in social immunity, while defensin 2 is synthesized by cells of body fat and lymph, which is an important factor in the system of the honey bee individual immunity (Ilyasov et al. 2013).

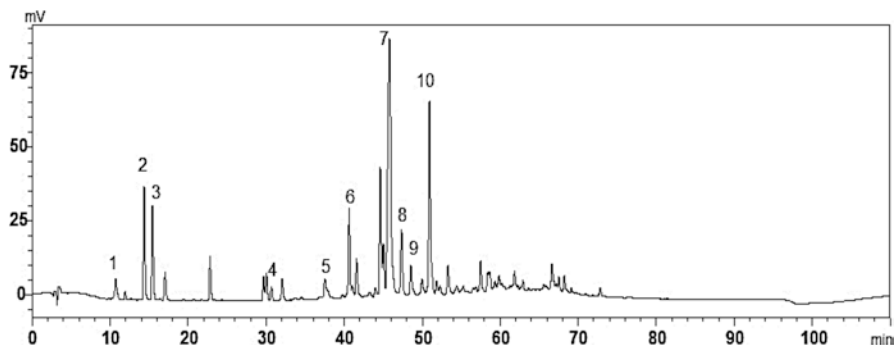


Fig. 15.2 The typical HPLC-UV chromatogram of propolis extracts obtained in our laboratory. Ten biomarkers are used for analysis: (1) caffeic acid, (2) p-coumaric acid, (3) ferulic acid, (4) trans-cinnamic acid, (5) kaempferol, (6) apigenin, (7) chrysin, (8) pinocembrin, (9) CAPE, (10) galangin

3.3 Honey Bee Social Immunity

Honey bees use social immunity as a collective defense against pathogens (DeGrandi-Hoffman and Chen 2015). This type of response is based on behavioral cooperation (Evans and Spivak 2010) during small tasks that have a colony-wide impact on reducing pathogenic invasion, for example, necrophoric and hygienic behavior (removing the dead adults or diseased brood from the colony), or thermoregulatory activity (workers produce high temperature) against heat-sensitive pathogens (DeGrandi-Hoffman and Chen 2015). The previously mentioned transmissible RNA pathway through the royal jelly and worker jelly also has an important role in social immunity and signaling between hive members. It protects bees against viruses and the *Varroa* mite (Maori et al. 2019).

Nutrition is a key factor in honey bees' social and individual immunity (DeGrandi-Hoffman and Chen 2015). Honey bees use plants as their food but also as a form of their external, collective immunity. Bee pollen is a primary source of food for the beehive, entirely of plant origin. Honey is produced partly from the sugary secretions of plants (floral nectar). The most effective honey bee product with immunomodulatory, antimicrobial, antioxidative activity is propolis. Propolis is a resin derived from plants combined with animal origin substances – honey bee saliva and beeswax – rich in polyphenols from plants (Bankova et al., 2021). These polyphenols are used as markers of the biological activity of propolis (Fig. 15.2).

As previously mentioned, to protect themselves against consumption by herbivores and pathogens, plants use complex mixtures of numerous secondary compounds (SM) (Wink 2008). The action of these compounds in mixtures can be synergistic or antagonistic. Mechanisms of activity are pleiotropic and interact with many targets at the same time. As such, these compounds have many advantages

over mono-target compounds (Wink 2008). Some common mechanisms include modulation of the structure and function of proteins, interference with gene expression, and changing membrane permeability. Most of these SMs have been found in the beehive in honey bee products.

4 Honey Bee Products as Beehive Defense Resources

There are six main products from the beehive with antimicrobial effect described in the scientific literature: honey, propolis, royal jelly, pollen, beeswax, and bee venom. Of these, honey and propolis antimicrobial activities have been studied the most and have the greatest potential in treating systemic or local infectious diseases.

4.1 Honey

The first product from the beehive used for its antimicrobial properties (besides the nutritional) in folk medicine was honey. Honey is the end product of nectar digestion and is stored in honeycomb cells. In terms of content, honey is made up of a supersaturated aqueous solution. This solution is comprised of 80% sugars, mostly fructose, and glucose.

It is known that natural unheated honey has some broad-spectrum antibacterial activity when tested against methicillin-resistant *S. aureus* (MRSA), *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, vancomycin-resistant enterococci (VRE), extended-spectrum β -lactamase-producing (ESBL) *Proteus mirabilis*, and *E. coli*. There are numerous studies on the antimicrobial activity of different types of honey. In one study, the MICs of Tualang honey ranged 8.75%–25% compared with those of manuka honey (8.75%–20%) against the wound and enteric microorganisms: *S. pyogenes*, CNS, MRSA, *Streptococcus agalactiae*, *S. aureus*, *Stenotrophomonas maltophilia*, *Acinetobacter baumannii*, *S. enterica* Serovar *typhi*, *P. aeruginosa*, *P. mirabilis*, *Shigella flexneri*, *E. coli*, and *E. cloacae* (Tan et al. 2009). In time-kill studies, antibiotic susceptible and resistant isolates of *S. aureus*, *S. epidermidis*, *Enterococcus faecium*, *E. coli*, *P. aeruginosa*, *E. cloacae*, and *Klebsiella oxytoca* were killed within 24 h by 10–40% (v/v) honey (Mandal and Mandal 2011). Several types of honey were tested against planktonic and biofilm-grown bacteria and showed 100% bactericidal efficacy against planktonic forms. The bactericidal rates for the Sidr and two types of Manuka honey against MSSA, MRSA, and *P. aeruginosa* biofilms were 63–82%, 73–63%, and 91–91%, respectively (Alandejani et al. 2009).

Different types of honey also displayed specific antiviral effects. Manuka and clover honey showed activity against varicella-zoster virus (VZV) in concentrations ranging from 0% to 6% wt/vol (Shahzad and Cohrs 2012). In addition, a randomized controlled trial on the efficacy of honey compared to acyclovir showed comparable

success rates of topical application of medical-grade kanuka honey and 5% aciclovir in the treatment of herpes labialis (Semprini et al. 2019).

These antimicrobial effects are attributed to a wide array of compounds found in honey, such as oligosaccharides (Cornara et al. 2017), glucose oxidase, and non-peroxide factors with antibacterial activity, like methyl syringate, methylglyoxal (MGO), peptides from honey bees (defensin-1) (Cornara et al. 2017), and honey glycoproteins (glps). Honey glycoproteins showed sequence identity with the major royal jelly proteins 1 (MRJP1) precursor (Brudzynski and Sjaarda 2015), and also the concentration-dependent antibacterial activity against Gram-positive *Bacillus subtilis* and Gram-negative *E. coli*. These glycoproteins bind and agglutinate bacterial cells and also cause membrane permeabilization (Brudzynski and Sjaarda 2015). Glucose oxidase is added by bees, which, by low dilution, converts glucose into H_2O_2 and gluconic acid.

Active compounds of plant origin that are found in honey differ based on the botanical origin of their nectar. Some types of honey are being marketed as specific regarding their antimicrobial effects and so-called unique factors. What they all have in common is supersaturation (high osmolarity, osmotic effect), low water activity, and low pH. These factors cultivate an unfavorable environment for microbial growth (Tan et al. 2009).

Microbiota from honey is also believed to be responsible for its antibacterial activity. Fourteen bacterial isolates of *Bacillus* sp. showed antimicrobial activity against *C. albicans*, *E. coli*, and *S. aureus* has been found in honey (Jia et al. 2020).

4.2 Propolis

Honey bees primarily use propolis as a construction material but also to maintain beehive health. Propolis is also used as an important part of social immunity due to its natural antiseptic properties (Bankova et al., 2018; Bankova et al., 2021). It is a resinous mixture of both animal and plant origin—bees collect it from exudates and plant buds, where it is further mixed with wax and saliva enzymes (Bankova et al., 2021). Its chemical composition varies depending on the geographical and botanical origin: the most common type of propolis in Europe is poplar-type, from *Populus nigra*. The most prevalent types of Brazilian propolis are green due to plant *Baccharis dracunculifolia* and red, from plant *Dalbergia ecastophyllum*. Brown Cuban propolis, the principal type of Cuban propolis, is derived from *Clusia rosea*. Each type of propolis contains about 300 bioactive compounds (Sforcin and Bankova 2011; Pellati et al. 2013); triterpenes (50% w/w), waxes (25–30%), volatile mono- and sesquiterpenes (8–12%) and phenolics (5–10%) (Huang et al. 2014).

Most active compounds are of plant origin and are believed to be responsible for the antimicrobial, antioxidant, immunomodulatory, and anti-inflammatory activities of propolis (Sforcin and Bankova 2011). The antimicrobial activity of propolis was confirmed when tested against bacteria, viruses, yeasts, and even parasites. Propolis extracts are highly active against Gram-positive (MRSA, VRE, *Streptococcus*

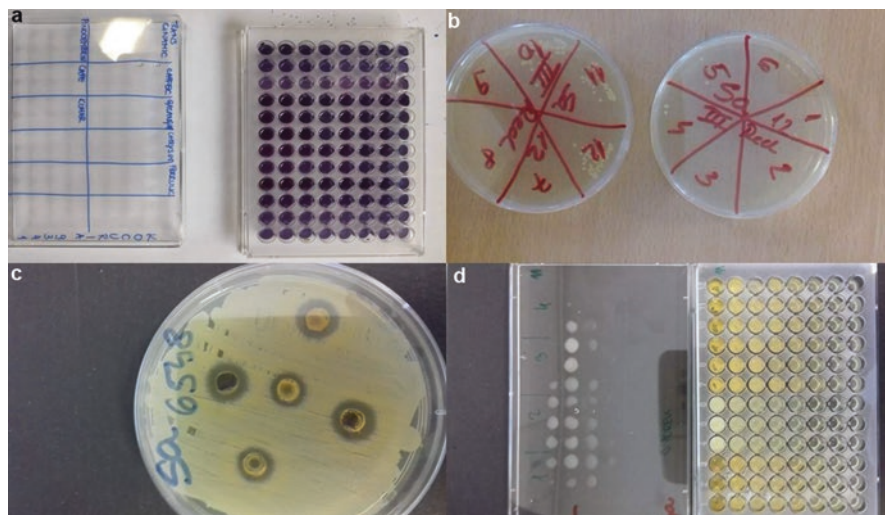


Fig. 15.3 Antimicrobial susceptibility testing: minimal biofilm eradication concentration (MBEC) determination for different (separate) propolis biomarkers (a), and propolis extracts minimum inhibitory concentrations (MICs) determination by subcultivation on agar plates (b), agar well diffusion (c), and broth microdilution (d) method. (With courtesy of Dr. Josipa Vlainić)

species, *B. subtilis*, *S. aureus*, *Enterococcus faecalis*) and less active against Gram-negative bacteria like *E. coli*. However, they have bactericidal activity on *P. aeruginosa* (Kosalec et al. 2005, Przybyłek and Karpiński 2019). Propolis is also active against yeasts like *Candida* species (Kosalec et al. 2005) and many viruses *in vitro* and *in vivo* (Berretta et al. 2020; Nolkemper et al. 2010; Schnitzler et al. 2010). The mechanism of action depends on inhibition of the virus' entry into cells and disruption of viral replication, which destroys RNA before or after its release in the cells (Búfalo et al. 2009; Sforzin 2016). Propolis components have inhibitory effects on the ACE2, TMPRSS2, and PAK1 signaling pathways and can potentially interfere with the host cell invasion by SARS-CoV-2 (Berretta et al. 2020).

It is presumed that the antimicrobial activity depends on the presence of flavonoids such as galangin, pinocembrin, rutin, quercetin, naringenin, and CAPE, since these compounds are known to increase bacterial membrane permeability. Some of those compounds (galangin, pinocembrin, CAPE) also inhibit bacterial RNA polymerase (Cornara et al. 2017). It is, therefore, clear that the antimicrobial activity of propolis is a result of the mixture effect and synergy between the flavonoid compounds and that the resultant antimicrobial actions are understood so far as complex mechanisms. Due to this complexity, propolis is active against multidrug-resistant bacteria (Pamplona-Zomenhan et al. 2011; Przybyłek and Karpiński 2019).

We confirmed this synergy when we compared the MIC values of propolis extracts with different amounts of active markers (p-coumaric acid, trans-ferulic acid, caffeic acid, CAPE, cinnamic acid, chrysin, pinocembrin, galangin, apigenin, kaempferol) (Fig. 15.3).

An interesting and completely unexpected result is that the mixture of these active substances in small concentrations is more effective than that of much higher concentrations of certain (pure) active substances alone (work in progress) (Fig. 15.3). It seems that the synergy effect between these compounds follows the Goldilocks principle.

There are certainly other compounds relevant to the investigation of propolis-mediated antimicrobial activity. These may not just be of plant, but honey bee origin, such as antimicrobial peptides found in other honey bee products. Based on the previously posited interaction pathways between peptides and polyphenols (Wink 2008; Quan et al. 2019), peptides in propolis could exert great stability and possibly enhanced therapeutic potential.

Surprisingly, the idea of propolis as a natural source of stable AMPs has never been tested before. Our preliminary and currently ongoing research confirmed peptides like MRJP1 and some peptides related to *Populus* genus in raw propolis samples. There remains a wealth of other detected peptides yet to be sequenced.

4.3 Royal Jelly as a Resource of Antimicrobials

Royal jelly (RJ) is a food for all bee larvae for the first 3 days of their life. For the queen bee, RJ serves as the source of all subsequent nutrition throughout her lifespan. RJ is a white-yellow, colloidal, slightly acidic secretion produced from the hypopharyngeal and mandibular salivary glands of young bees (nurse, aged between 5 and 14 days) (Fujita et al. 2013; Fratini et al. 2016a). It consists of 60–70% water, 11%–23% carbohydrates, 9–18% proteins, 4–8% lipids, and the remaining 0.8–3% are vitamins, minerals, and even phenolic compounds, presumably from plants (Sabatini et al. 2009; Fratini et al. 2016a). The composition varies based on the season and nutrition of the bees.

Bioactive peptides and proteins identified in royal jelly are the families of major royal jelly proteins (MRJPs), royalisin, glycoproteins jelleins, apolipoprotein III-like protein, glucose oxidase (Fratini et al. 2016a), defensin, apidaecins and hymenoptaecin (Han et al. 2014). Interesting components of royal jelly with antibacterial activity are unsaturated fatty acids, such as 10-hydroxy-2-decenoic (10-HDA), also known as queen-bee acid (Fratini et al. 2016a).

MRJPs have a significant role in honey bee nutrition since they account for 82–90% of total larval jelly proteins and contain essential amino acids. There are seven members of the MRJP family (MRJP 1–7) that have health-promoting effects and two members without these healthful advantages (Ahmad et al. 2020). MRJP1 occurs as a monomer (mono MRJP1 or royalactin), or can also appear as an oligomer known as apisin, when polymerized with apisimin (Ahmad et al. 2020). MRJP1 has been shown to modulate biological function in a broad range of species and can maintain pluripotency by activating a ground-state pluripotency-like gene network (Wan et al., 2018). However, it seems that MRJP1 does not display specific antimicrobial properties (Bucekova and Majtan 2016).

Nevertheless, jelleins, peptides isolated from MRJP1, showed a broad spectrum of activity against Gram-positive (*B. subtilis*, *S. aureus*, *Paenibacillus larvae*), Gram-negative bacteria (*E. coli*, *P. aeruginosa*), and against *C. albicans*. The MICs of synthetic jelleins varied between 2.5 µg/ml against *E. coli* and 15 µg/ml against *S. saprophyticus* (Brudzynski and Sjaarda 2015). Jellein I and Jellein II were active against *S. aureus*, *Staphylococcus saprophyticus*, and *B. subtilis* among the Gram-positive bacteria, and *E. coli*, *Enterobacter cloacae*, *K. pneumoniae*, and *P. aeruginosa* among the Gram-negative bacteria (Romanelli et al. 2011). Jellein III showed a narrower spectrum of general activity (Romanelli et al. 2011) but was the strongest in reacting against *S. epidermidis* (Capparelli et al. 2012).

MRJP2 and MRJP4 act as antimicrobial agents and have a wide range of activity against bacteria (Gram-positive and Gram-negative), fungi, and yeasts (Ahmad et al. 2020). They kill microorganisms by attaching to, and damaging, the cell wall of fungi, yeast, and bacteria (Kim et al. 2019; Park et al. 2019).

Royalisin is strongly active against Gram-positive bacteria strains of *Bifidobacterium*, *Clostridium*, *Corynebacterium*, *Lactobacillus*, *Leuconostoc*, *Staphylococcus*, and *Streptococcus* genera, with inhibitory efficacy comparable to that of antibiotics (Fratini et al. 2016a). Apolipoprotein III-like proteins (lipid transport proteins) and phosphorylated icarapin (venom protein-II) are the components of royal jelly that promote immune response (Ahmad et al. 2020).

The antifungal properties of royal jelly are not limited only to their peptide properties but can also be attributed to fatty acids, such as 3,10-HDA, 10-HDA, and 10-acetoxy-2-DEA, that inhibit the growth of *Candida tropicalis*, *C. albicans*, and *Candida glabrata* (Meliou and Chinou 2005).

Antiviral effects of royal jelly are not attributed to certain peptides but to the product as a whole. Honey, royal jelly, and acyclovir have the highest inhibitory effects on HSV-1 at concentrations of 500, 250, and 100 µg/mL, respectively (Hashemipour et al. 2014).

4.4 Honey Bee Pollen

Honey bee pollen is used as a raw material to produce bee bread. Bee bread is the main protein source for the bee colony and the source of nutritional and mineral substances for royal jelly produced by worker bees (Komosinska – Vassev et al. 2015). Pollen is also important for the production and expression of antimicrobial peptides—apidaecins and abaecin—in honey bees, not just due to its microbiota, but possibly to certain immunomodulatory protein factors that yet have to be determined (Daníhlík et al. 2018).

Honey bee pollen composition varies depending on the botanical and geographical origin of the pollen grains. Generally, pollen consists of proteins, amino acids, carbohydrates, lipids, fatty acids, phenolic compounds, enzymes, and coenzymes, and vitamins and elements. There are approximately 200 substances from different plant species found in pollen grains (Komosinska – Vassev et al. 2015). It is believed

that plant SMs like flavonoids and phenolic acids are responsible for pollen antioxidant and antimicrobial activity (Bridi et al. 2019). These effects are also possibly mediated by glucose oxidase activity, deriving from honey bee secretion (Cornara et al. 2017).

Bee pollen extract showed antibacterial activity against Gram-positive bacteria like *Streptococcus pyogenes* (Bridi et al. 2019), *S. aureus*, Gram-negative bacteria, including *E. coli*, *K. pneumoniae*, *Pseudomonas aeruginosa*, and on fungi such as *C. albicans* (Komosinska – Vassev et al. 2015).

Bee pollen is a component of honey and propolis and, as such, adds to their antimicrobial efficacy. When compared by their pollen content, heterofloral honey samples from Turkey, with pollen dominantly from *Chenopodiaceae/Amaranthaceae*, *Trifolium*, *Trigonella*, *Cyperaceae*, *Zea mays*, and *Anthemis* taxa, had the highest antibacterial activity against *P. aeruginosa*, *E. coli*, and *S. aureus* (Mercan et al. 2007). However, in our MIC study on Gram-positive and Gram-negative bacteria, we found no bactericidal or bacteriostatic activity of *Cistus* pollen extracts.

4.5 Beeswax

Honey bees secrete beeswax in order to build honeycombs. Beeswax is a complex mixture (more than 300 components) of hydrocarbons, free fatty acids, esters of fatty acids and a fatty alcohol, diesters, and exogenous substances (Tulloch, 1980), which are mainly residues of propolis, pollen, small pieces of floral component factors, and pollution (Hepburn et al. 1991).

Several studies report antimicrobial activity of crude beeswax against *S. aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *B. subtilis*, *P. aeruginosa*, *E. coli*, *S. enterica*, *C. albicans*, and *Aspergillus niger* (Fratini et al. 2016b). Similarly, beeswax methanolic and ethanolic extracts showed inhibitory activity on *L. monocytogenes*, *S. enterica*, *E. coli*, *A. niger*, *C. tropicalis*, *C. glabrata*, and *C. albicans* (Fratini et al. 2016b).

Beeswax also has good antimicrobial activity in synergy with other natural products, like propolis, honey, or olive oil (Fratini et al. 2016b).

4.6 Bee Venom (Apitoxin)

Honey bee venom glands secrete the venom and inject it through a stinger. Bee venom is rich in amphipathic polycationic peptides, melittin and apamin, enzymes such as phospholipase A2, and low-molecular-weight compounds including active bioamines such as histamine and catecholamines (Cornara et al. 2017). This complex mixture causes local inflammation, anticoagulant effect, and immune response in victims (Cornara et al. 2017).

Melittin, a peptide of 26 amino acid residues, has been recognized as a peptide with an antiviral effect. It has inhibited the viral replication of *Herpes simplex* virus (HSV), human immunodeficiency virus-1 (HIV-1), and Junín virus (JV), and it also has shown to reduce the infectivity of *Coxsackie* virus and other enteroviruses (*Picornaviridae*), Influenza A viruses (*Orthomyxoviridae*), respiratory syncytial virus (RSV; *Pneumoviridae*), vesicular stomatitis virus (VSV; *Rhabdoviridae*), and the plant virus tobacco mosaic virus (TMV; *Virgaviridae*) (Memariani et al. 2020). Melittin also showed effective antibacterial activity against *Streptococcus salivarius*, *Streptococcus sobrinus*, *Streptococcus mutans*, *Streptococcus mitis*, *Streptococcus sanguinis*, *Lactobacillus casei*, and *E. faecalis* with MIC values ranging from 4 to 40 µg/mL (Leandro et al. 2015). Although melittin has many therapeutic potentials, the systematic administration is followed by many side effects, and its biotechnological applications are limited to topical formulations (Moreno and Giral 2015).

5 Conclusion

Honey bee products result from combining the honey bee and plant-origin compounds in the beehive, and as such, have been used as food and therapeutics since ancient times. They are abundant in sugars, secondary plant metabolites, and honey bee proteins and peptides with antimicrobial activity. With the help of powerful modern technologies stemming from molecular biology, proteomics, and chemistry, the evidence and mechanisms of their antimicrobial activity are being elucidated increasingly. However, one must bear in mind the effect of the mixture and synergy between the components in natural products.

Acknowledgments Our preliminary results mentioned in this chapter were obtained as part of the project named *Beehive as a natural resource for antibiotic alternatives* financed by private Sweden foundation Ekhagastiftelsen, founded by Gösta Videgårds. I would like to thank them for all the support in our research work on antibiotic alternatives.

References

- Adnan SN, Ibrahim N, Yaacob WA (2017) Disruption of methicillin-resistant *Staphylococcus aureus* protein synthesis by tannins. *Germs* 7:186–192
- Ahmad S, Campos MG, Fratini F, Altaye SZ, Li J (2020) New insights into the biological and pharmaceutical properties of Royal Jelly. *Int J Mol Sci* 21:382
- Alandejani T, Marsan J, Ferris W, Slinger R, Chan F (2009) Effectiveness of honey on *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms. *Otolaryngol Head Neck Surg* 141:114–118
- Baltzer SA, Brown MH (2011) Antimicrobial peptides: promising alternatives to conventional antibiotics. *J Mol Microbiol Biotechnol* 20:228–235
- Bankova V, Popova M, Trusheva B (2014) Propolis volatile compounds: chemical diversity and biological activity: a review. *Chem Cent J* 8:28

- Bankova V, Popova M, Trusheva B (2018) The phytochemistry of the honeybee. *Phytochemistry* 155:1–11
- Bankova V, Trusheva B, Popova M (2021) Propolis extraction methods: a review. *J Apic Res.* <https://doi.org/10.1080/00218839.2021.1901426>
- Berretta AA, Silveira MAD, Capcha JMC, de Jong (2020) Propolis and its potential against SARS-CoV-2 infection mechanisms and COVID-19 disease: Running title: Propolis against SARS-CoV-2 infection and COVID-19. *Biomed Pharmacother* 131:110622. <https://doi.org/10.1016/j.biopha.2020.110622>
- Bourvellec CL, Renard CMGC (2012) Interactions between polyphenols and macromolecules: quantification methods and mechanisms. *Crit Rev Food Sci Nutr* 52:213–248
- Boutros M (2002) Sequential activation of signaling pathways during innate immune responses in drosophila. *Dev Cell* 3:711–722
- Bridi R, Atala E, Núñez Pizarro P, Montenegro G (2019) Honeybee pollen load: phenolic composition and antimicrobial activity and antioxidant capacity. *J Nat Prod* 82:559–565
- Brown KL, Hancock REW (2006) Cationic host defense (antimicrobial) peptides. *Curr Opin Immunol* 18:24–30
- Brudzynski K, Sjaarda C (2015) Honey glycoproteins containing antimicrobial peptides, Jelleins of the major Royal Jelly Protein 1, are responsible for the cell wall lytic and bactericidal activities of honey. *PLoS One* 10(4):e0120238
- Brudzynski K, Maldonado-Alvarez L (2015) Polyphenol-protein complexes and their consequences for the redox activity, structure and function of honey. A current view and new hypothesis – a review. *Polish J Food Nutr Sci* 65:71–80
- Bucekova M, Majtan J (2016) The MRJP1 honey glycoprotein does not contribute to the overall antibacterial activity of natural honey. *Eur Food Res Technol* 242:625–629
- Búfalo MC, Figueiredo AS, de Sousa JP, Candeias JM, Bastos JK, Sforcin JM (2009) Antipoliiovirus activity of *Baccharis dracunculifolia* and propolis by cell viability determination and real-time PCR. *J Appl Microbiol* 107:1669–1680
- Bulet P, Stöcklin R (2005) Insect antimicrobial peptides: structures, properties and gene regulation. *Protein Pept Lett* 12:3–11
- Capparelli R, De Chiara F, Nocerino N, Montella RC, Iannaccone M, Fulgione A, Romanelli A, Avitabile C, Blaiotta G, Capuano F (2012) New perspectives for natural antimicrobial peptides: application as antiinflammatory drugs in a murine model. *BMC Immunol* 13:61
- Casteels P, Ampe C, Jacobs F, Vaeck M, Tempst P (1989) Apidaecins: antibacterial peptides from honeybees. *EMBO J* 8:2387–2391
- Casteels P, Ampe C, Riviere L, Van Damme J, Elicone C, Fleming M, Jacobs F, Tempst P (1990) Isolation and characterization of abaecin, a major antibacterial response peptide in the honeybee (*Apis mellifera*). *Eur J Biochem* 187:381–386
- Casteels P, Ampe C, Jacobs F, Tempst P (1993) Functional and chemical characterization of hymenoptaecin, an antibacterial polypeptide that is infection-inducible in the honey bee (*Apis mellifera*). *J Biol Chem* 268:7044–7054
- Chang EH, Huang J, Lin Z, Brown AC (2019) Catechin-mediated restructuring of a bacterial toxin inhibits activity. *Biochim Biophys Acta Gen Subj* 1863:191–198
- Colgrave ML, Kotze AC, Huang YH, O’Grady J, Simonsen SM, Craik DJ (2008) Cyclotides: natural, circular plant peptides that possess significant activity against gastrointestinal nematode parasites of sheep. *Biochemistry* 47:5581–5589
- Cornara L, Biagi M, Xiao J, Burlando B (2017) Therapeutic properties of bioactive compounds from different honeybee products. *Front Pharmacol* 8:412
- Cornet B, Bonmatin JM, Hetru C (1995) Refined three-dimensional solution structure of insect defensin A. *Structure* 3:435–448
- Crane E (1999) *The world history of beekeeping and honey hunting*. Duckworth, London
- Daglia M (2012) Polyphenols as antimicrobial agents. *Curr Opin Biotech* 23:174–181
- Dams M, Dams L (1977) Spanish rock art depicting honey gathering during the Mesolithic. *Nature* 268:228–230

- Danihlík J, Aronstein K, Petrivalský M (2015) Antimicrobial peptides: a key component of honey bee innate immunity. *J Api Res* 54:123–136
- Danihlík J, Škrabišová M, Lenobel R, Šebela M, Omar E, Petřivalský M, Crailsheim K, Brodschneider R (2018) Does the pollen diet influence the production and expression of antimicrobial peptides in individual honey bees? *Insects* 9:79
- DeGrandi-Hoffman G, Chen Y (2015) Nutrition, immunity and viral infections in honey bees. *Curr Opin Insect Sci* 10:170–176
- Diamond G, Beckloff N, Weinberg A, Kisich KO (2009) The roles of antimicrobial peptides in innate host defense. *Curr Pharm Des* 15:2377–2392
- Dong J et al (2013) Apigenin alleviates the symptoms of *Staphylococcus aureus* pneumonia by inhibiting the production of alpha-hemolysin. *FEMS Microbiol Lett* 338:124–131
- Dubery IA, Sanabria NM, Huang JC (2012) Nonself perception in plant innate immunity. *Adv Exp Med Biol* 738:79–107
- Ebrahimnesbat F, Behnke S, Kleinhofs A, Apel K (1989) Cultivar-related differences in the distribution of cell-wall-bound thionins in compatible and incompatible interactions between barley and powdery mildew. *Planta* 179:203–210
- Evans J, Wang YD, Shaw KP, Vernon LP (1989) Cellular responses to pyricularia thionin are mediated by Ca²⁺ influx and phospholipase a₂ activation and are inhibited by thionin tyrosine iodination. *Proc Natl Acad Sci* 86:5849–5853
- Evans JD, Aronstein K, Chen YP, Hetru C, Imler JL, Jiang H, Kanost M, Thompson GJ, Zou Z, Hultmark D (2006) Immune pathways and defence mechanisms in honey bees *Apis mellifera*. *Insect Mol Biol* 15:645–656
- Evans JD, Spivak M (2010) Socialized medicine: individual and communal disease barriers in honey bees. *J Invertebr Pathol* 103(Suppl. 1):S62–S72
- Florack DE, Stiekema WJ (1994) Thionins: properties, possible biological roles and mechanisms of action. *Plant Mol Biol* 26:25–37
- Fratini F, Cilia G, Mancini S, Felicioli A (2016a) Royal Jelly: an ancient remedy with remarkable antibacterial properties. *Microbiol Res* 192:130–141
- Fratini F, Cilia G, Turchi B, Felicioli A (2016b) Beeswax: a minireview of its antimicrobial activity and its application in medicine. *Asian Pac J Trop Med* 9:839–843
- Fujita T, Kozuka-Hata H, Ao-Kondo H, Kunieda T, Oyama M, Kubo T (2013) Proteomic analysis of the Royal Jelly and characterization of the functions of its derivation glands in the honeybee. *J Proteome Res* 12:404–411
- Funatogawa K, Hayashi S, Shimomura H, Yoshida T, Hatano T, Ito H, Hirai Y (2004) Antibacterial activity of hydrolyzable tannins derived from medicinal plants against *Helicobacter pylori*. *Microbiol Immunol* 48:251–261
- Garcia-Olmedo F, Molina A, Alamillo JM, Rodriguez-Palenzuela P (1998) Plant defense peptides. *Biopolymers* 47:479–491
- Gätschenberger H, Azzami K, Tautz J, Beier H (2013) Antibacterial immune competence of honey bees (*Apis mellifera*) is adapted to different life stages and environmental risks. *PLoS One* 8:e66415
- Gran L, Sletten K, Skjeldal L (2008) Cyclic peptides from *Oldenlandia affinis* DC. Molecular and biological properties. *Chem Biodivers* 5:2014–2022
- Han B, Fang Y, Feng M, Lu X, Huo X, Meng L, Wu B, Li J (2014) In-depth phosphoproteomic analysis of royal jelly derived from western and eastern honeybee species. *J Proteome Res* 13:5928–5943
- Hashemipour MA, Tavakolineghad Z, Arabzadeh SA, Iranmanesh Z, Nassab SA (2014) Antiviral Activities of Honey, Royal Jelly, and Acyclovir Against HSV-1. *Wounds* 26:47–54
- Hepburn HR, Bernard RTF, Davidson BC, Muller WJ, Lloyd P, Kurstjens SP et al (1991) Synthesis and secretion of beeswax in honeybees. *Apidologie* 22:21–36
- Hisano M et al (2003) Inhibitory effect of catechin against the superantigen staphylococcal enterotoxin B (SEB). *Arch Dermatol Res* 295:183–189
- Huang S, Zhang CP, Wang K, Li GQ, Hu FL (2014) Recent advances in the chemical composition of propolis. *Molecules* 19:19610–19632

- Hughes P, Dennis E, Whitecross M, Llewellyn D, Gage P (2000) The cytotoxic plant protein beta-purothionin, forms ion channels in lipid membranes. *J Biol Chem* 275:823–827
- Ilyasov RA, Gaifullina LR, Saltykova ES et al (2013) Defensins in the honeybee anti-infectious protection. *J Evol Biochem Phys* 49:1–9
- Ireland DC, Colgrave ML, Craik DJ (2006) A novel suite of cyclotides from *Viola odorata*: sequence variation and the implications for structure, function and stability. *Biochem J* 400:1–12
- Jamwal K, Bhattacharya S, Sunil Puri S (2018) Plant growth regulator mediated consequences of secondary metabolites in medicinal plants. *J Appl Res Med Aromat Plants* 9:26–38
- Jia L, Cheruyot Kosgey J, Wang J et al (2020) Antimicrobial and mechanism of antagonistic activity of *Bacillus* sp. A2 against pathogenic fungus and bacteria: the implication on honey's regulatory mechanism on host's microbiota. *Food Sci Nutr* 8:4857–4867
- Jones J, Dangl J (2006) The plant immune system. *Nature* 444:323–329
- Kim BY, Lee KS, Jung B, Choi YS, Kim HK, Yoon HJ, Gui ZZ, Lee J, Jin BR (2019) Honeybee (*Apis cerana*) major royal jelly protein 4 exhibits antimicrobial activity. *J Asia Pac Entomol* 22:175–182
- Komosinska-Vassev K, Olczyk P, Kaźmierczak J, Mencner L, Olczyk K (2015) Bee pollen: chemical composition and therapeutic application. *Evid Based Complementary Altern Med: eCAM* 2015:297425
- Kosalec I, Pepeljnjak S, Bakmaz M, Vladimir-Knezević S (2005) Flavonoid analysis and antimicrobial activity of commercially available propolis products. *Acta Pharma* 55:423–430
- Lan Y, Ye Y, Kozłowska J, Lam JKW, Drake AF, Mason AJ (2010) Structural contributions to the intracellular targeting strategies of antimicrobial peptides. *Biochim Biophys Acta Biomembr* 1798:1934–1943
- Larsen A, Reynaldi F, Guzman-Novoa E (2019) Fundamentals of the honey bee (*Apis mellifera*) immune system. *Rev Mex Cienc Pecu* 10:705–728
- Leandro LF, Mendes CA, Casemiro LA, Vinholis AH, Cunha WR, de Almeida R, Martins CH (2015) Antimicrobial activity of apitoxin, melittin and phospholipase A2 of honey bee (*Apis mellifera*) venom against oral pathogens. *An Acad Bras Cienc* 87:147–155
- Lee JH, Park JH, Lee J (2012) Flavone reduces the production of virulence factors, Staphyloxanthin and α -hemolysin *Staphylococcus aureus*. *Curr Microbiol* 65:726–732
- Li Y, Xiang Q, Zhang Q, Huang Y, Su Z (2012) Overview on the recent study of antimicrobial peptides: origins, functions, relative mechanisms and application. *Peptides* 37:207–215
- Li Z, Mao R, Teng D et al (2017) Antibacterial and immunomodulatory activities of insect defensins-DLP2 and DLP4 against multidrug-resistant *Staphylococcus aureus*. *Sci Rep* 7:12124
- Lin MH, Chang FR, Hua MY, Wu YC, Liu ST (2011) Inhibitory effects of 1,2,3,4,6-penta-O-gallyol-beta-D-glucopyranose on biofilm formation by *Staphylococcus aureus*. *Antimicrob Agents Chemother* 55:1021–1027
- Majewski J, Stec B (2001) X-ray scattering studies of model lipid membrane interacting with purothionins provide support for a previously proposed mechanism of membrane lysis. *Eur Biophys J* 39:1155–1165
- Mandal MD, Mandal S (2011) Honey: its medicinal property and antibacterial activity. *Asian Pac J Trop Biomed* 1:154–160
- Maori E, Garbian Y, Kunik V, Mozes-Koch R, Malka O, Kalev H, Sabath N, Sela I, Shafir S (2019) A transmissible RNA pathway in honey bees. *Cell Rep* 27:1949–1959
- McMenamin AJ, Daughenbaugh KF, Parekh F, Pizzorno MC, Flenniken ML (2018) Honey bee and bumble bee antiviral defense. *Viruses* 10:395
- Melliou E, Chinou I (2005) Chemistry and bioactivity of Royal Jelly from Greece. *J Agric Food Chem* 53:8987–8992
- Memariani H, Memariani M, Moravvej H, Shahidi-Dadras M (2020) Melittin: a venom-derived peptide with promising anti-viral properties. *Eur J Clin Microbiol Infect Dis* 39:5–17
- Mercan N, Guvensen A, Celik A, Katircioglu H (2007) Antimicrobial activity and pollen composition of honey samples collected from different provinces in Turkey. *Nat Prod Res* 21:187–195
- Molan P (1999) Why honey is effective as a medicine. 1. Its use in modern medicine. *Bee World* 80:80–92

- Montesinos E (2007) Antimicrobial peptides and plant disease control. *FEMS Microbiol Lett* 270:1–11
- Moreno M, Giralte E (2015) Three valuable peptides from bee and wasp venoms for therapeutic and biotechnological use: melittin, apamin and mastoparan. *Toxins (Basel)* 7:1126–1150
- Nawrot R, Barylski J, Nowicki G, Broniarczyk J, Buchwald W, Goździcka-Józefiak A (2014) Plant antimicrobial peptides. *Folia Microbiol (Praha)* 59:181–196
- Nolkemper S, Reichling J, Sensch KH, Schnitzler P (2010) Mechanism of herpes simplex virus type 2 suppression by propolis extracts. *Phytomedicine* 17:132–138
- Olchownik-Grabarek E, Sekowski S, Bituicki M, Dobrzynska I, Shlyonsky V, Ionov M, Burzynski P, Roszkowska A, Swiecicka I, Abdulladjanova N, Zamaraeva M (2020) Inhibition of interaction between *Staphylococcus aureus* α -hemolysin and erythrocytes membrane by hydrolysable tannins: structure-related activity study. *Sci Rep* 10:11168
- Osorio e Castro VR, Vernon LP (1989) Hemolytic activity of thionin from *pyrularia pubera* nuts and snake venom toxins of *naja naja* species: *Pyrularia* thionin and snake venom cardiotoxin compete for the same membrane site. *Toxicol* 27:511–517
- Pamplona-Zomenhan LC, Pamplona BC, da Silva CB, Marcucci MC, Mimica LM (2011) Evaluation of the *in vitro* antimicrobial activity of an ethanol extract of Brazilian classified propolis on strains of *Staphylococcus aureus*. *Braz J Microbiol* 42:1259–1264
- Park MJ, Kim BY, Park HG, Deng Y, Yoon HJ, Choi YS, Lee KS, Jin BR (2019) Major royal jelly protein 2 acts as an antimicrobial agent and antioxidant in royal jelly. *J Asia Pac Entomol* 22:684–689
- Pellati F, Prencipe FP, Bertelli D, Benvenuti S (2013) An efficient chemical analysis of phenolic acids and flavonoids in raw propolis by microwave-assisted extraction combined with high-performance liquid chromatography using the fused-core technology. *J Pharm Biomed Anal* 81–82:126–132
- Phan HTT, Yoda T, Chahal B, Morita M, Takagi M, Vestergaard MC (2014) Structure-dependent interactions of polyphenols with a biomimetic membrane system. *Biochim Biophys Acta Biomembr* 1838:2670–2677
- Prabhu S, Dennison SR, Lea B, Snape TJ, Nicholl ID, Radecka I, Harris F (2013) Anionic antimicrobial and anticancer peptides from plants. *CRC Crit Rev Plant Sci* 32:303–320
- Pranting M, Loov C, Burman R, Goransson U, Andersson DI (2010) The cyclotide cycloviolacin O2 from *Viola odorata* has potent bactericidal activity against Gram-negative bacteria. *J Antimicrob Chemother* 65:1964–1971
- Przybyłek I, Karpiński TM (2019) Antibacterial properties of propolis. *Molecules (Basel, Switzerland)* 24:2047
- Quan TH, Benjakul S, Sae-leaw T, Balange AK, Maqsood S (2019) Protein–polyphenol conjugates: antioxidant property, functionalities and their applications. *Trends Food Sci Technol* 91:507–517
- Romanelli A, Moggio L, Montella RC, Campiglia P, Iannaccone M, Capuano F, Pedone C, Capparelli R (2011) Peptides from Royal Jelly: studies on the antimicrobial activity of jelleins, jelleins analogs and synergy with temporins. *J Peptide Sci* 17:348–352
- Roffet-Salque M, Regert M, Evershed R et al (2016) Widespread exploitation of the honeybee by early Neolithic farmers. *Nature* 534:S17–S18
- Sabatini AG, Marcazzan GL, Caboni MF, Bogdanov S, de Almeida-Muriadian LB (2009) Quality and standardisation of Royal Jelly. *J ApiProduct ApiMed Sci* 1:1–6
- Semprini A, Singer J, Braithwaite I, Shortt N, Thayabaran D, McConnell M, Weatherall M, Beasley R (2019) Kanuka honey versus aciclovir for the topical treatment of herpes simplex labialis: a randomised controlled trial. *BMJ Open* 9:e026201
- Schnitzler P, Neuner A, Nolkemper S, Zundel C, Nowack H, Sensch KH, Reichling J (2010) Antiviral activity and mode of action of propolis extracts and selected compounds. *Phytother Res* 24 Suppl 1:S20–S28. Erratum in: *Phytother Res* 24:632
- Sforcin JM (2016) Biological Properties and Therapeutic Applications of Propolis. *Phytother Res* 30:894–905
- Sforcin, JM, Bankova V (2011) Propolis: is there a potential for the development of new drugs? *J Ethnopharmacol* 133:253–260

- Shah S, Stapleton PD, Taylor PW (2008) The polyphenol (–)-epicatechin gallate disrupts the secretion of virulence-related proteins by *Staphylococcus aureus*. *Lett Appl Microbiol* 46:181–185
- Shahzad A, Cohrs RJ (2012) In vitro antiviral activity of honey against varicella zoster virus (VZV): A translational medicine study for potential remedy for shingles. *Transl Biomed* 3:2
- Shimamura Y, Utsumi M, Hirai C, Nakano S, Ito S, Tsuji A, Ishii T, Hosoya T, Kan T, Ohashi N, Masuda S (2015) Binding of catechins to Staphylococcal enterotoxin A. *Molecules* 23:1125
- Song M et al (2016) Apigenin protects mice from pneumococcal pneumonia by inhibiting the cytolytic activity of pneumolysin. *Fitoterapia* 115:31–36
- Stec B (2006) Plant thionins—the structural perspective. *Cell Mol Life Sci* 63:1370–1385
- Sun X, Udenigwe CC (2020) Chemistry and Biofunctional Significance of Bioactive Peptide Interactions with Food and Gut Components. *J Agric Food Chem* 68:12972–12977
- Tam JP, Wang S, Wong KH, Tan WL (2015) Antimicrobial peptides from plants. *Pharmaceuticals (Basel, Switzerland)* 8:711–757
- Tan HT, Rahman RA, Gan SH, Halim AS, Hassan SA, Sulaiman SA et al (2009) The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to manuka honey. *BMC Complement Alternat Med* 9:34
- Tang F et al (2019) Inhibition of alpha-hemolysin expression by resveratrol attenuates *Staphylococcus aureus* virulence. *Microb Pathog* 127:85–90
- Tarahovsky YS (2008) Plant polyphenols in cell-cell interaction and communication. *Plant Signal Behav* 3:609–611
- Trentin DS et al (2013) Tannins possessing bacteriostatic effect impair *Pseudomonas aeruginosa* adhesion and biofilm formation. *PLoS One* 8:e66257
- Tulloch AP (1980) Beeswax-composition and analysis. *Bee World* 61:47–62
- Vasilchenko AS, Smirnov AN, Zavriev SK et al (2017) Novel Thionins from black seed (*Nigella sativa* L.) demonstrate antimicrobial activity. *Int J Pept Res Ther* 23:171–180
- Verhelst R, Schroyen M, Buys N, Niewold TA (2013) *E. coli* heat labile toxin (LT) inactivation by specific polyphenols is aggregation dependent. *Vet Microbiol* 163:319–324
- Wan DC, Morgan SL, Spencley AL et al (2018) Honey bee Royalactin unlocks conserved pluripotency pathway in mammals. *Nat Commun* 9:5078
- Wang CK, Colgrave ML, Gustafson KR, Ireland DC, Goransson U, Craik DJ (2008) Anti-HIV cyclotides from the Chinese medicinal herb *Viola yedoensis*. *J Nat Prod* 71:47–52
- Wang J et al (2015) Morin hydrate attenuates *Staphylococcus aureus* virulence by inhibiting the self-assembly of α -hemolysin. *J Appl Microbiol* 118:753–763
- Wang S, Zeng X, Yang Q, Qiao S (2016) Antimicrobial peptides as potential alternatives to antibiotics in food animal industry. *Int J Mol Sci* 17:603
- Weber F (2020) Antiviral innate immunity: introduction. The reference module in life sciences. <https://doi.org/10.1016/B978-0-12-809633-8.21290-9>
- Wink M (2008) Evolutionary advantage and molecular modes of action of multi-component mixtures used in phytomedicine. *Curr Drug Metab* 9:996–1009
- Wu J, Gao B, Zhu S (2014) The fungal defensin family enlarged. *Pharmaceuticals* 7:866–880
- Xu P, Shi M, Chen XX (2009) Antimicrobial peptide evolution in the Asiatic honey bee *Apis cerana*. *PLoS One* 4:e4239
- Yi HY, Chowdhury M, Huang YD, Yu XQ (2014) Insect antimicrobial peptides and their applications. *Appl Microbiol Biotechnol* 98:5807–5822
- Yi SM, Zhu JL, Fu LL, Li JR (2010) Tea polyphenols inhibit *Pseudomonas aeruginosa* through damage to the cell membrane. *Int J Food Microbiol* 144:111–117
- Zumla A, Lulat A (1989) Honey a remedy rediscovered. *J R Soc Med* 82:384–385

Chapter 16

Delivery Systems of Plant-Derived Antimicrobials



Enas Elmowafy, Eman M. El-Marakby, Haidy A. Gad, and Heba A. Gad

Abstract The use of essential oils as antimicrobial agents is widely applied in medical field, pharmaceutical industry, and food preservation. However, essential oils are hydrophobic, volatile, and unstable; in addition, they undergo degradation by environmental factors, such as light, oxygen, and heat, which represent obstacles for their wide utility. Green industry via incorporating these natural substances in pharmaceutical vehicles is one of the most commonly used technologies to impart exclusive features for the essential oils. Desirably, assortment of lipid and non-lipid-based delivery systems have been employed aiming to improve their stability and antimicrobial activity, provide sustained release pattern, and boost their bioavailability. This chapter offers an overview of various delivery systems that have been utilized to encapsulate some antimicrobial essential oils exploitable in the pharmaceutical field in recent literature. The versatility of nature of the proposed vehicles and harnessed natural and synthetic polymers, as well as advancement in fabrication techniques, are highlighted. Specifically, the stability and fulfillment of antimicrobial effectiveness of encapsulated essential oils are greatly emphasized.

Keywords Essential oils · Antimicrobial · Encapsulation · Delivery systems

Abbreviations

BER	Berberine chloride
CA	Cellulose acetate
CAD	Cinnamaldehyde

E. Elmowafy · E. M. El-Marakby · H. A. Gad (✉)
Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt
e-mail: h.gad@pharma.asu.edu.eg

H. A. Gad
Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt
Department of Pharmacognosy, Faculty of Pharmacy, King Salman International University, South Sinai, Egypt

CA-NCs	Cellulose acetate nanocapsules
CEO	<i>Carum copticum</i> essential oil
CFU	Colony forming units
CLP	<i>Citrus limon</i> var. <i>pompia</i>
CMC	Carboxymethyl cellulose
CNCs	Cellulose nanocrystals
CS	Chitosan
DCL	Drug in cyclodextrin in liposomes
EG-PEVs	Ethylene glycol Penetration Enhancer-containing Vesicles
EOs	Essential oils
GMS	Glyceryl monostearate
HP- β -CD	2-hydroxypropyl- β - cyclodextrin
HSV-1	Herpes simplex virus type 1
LNCs	Lipid nanocapsules
MBC	Minimum bactericidal concentration
MCC	Minimum cidal concentration
MIC	Minimum inhibitory concentration
NaAlg	Sodium alginate
NFs	Nanofibers
NHDF	Normal human dermal fibroblasts
NLCs	Nanostructured lipid carriers
NPs	Nanoparticles
PCL	Poly caprolactone
PDI	poly dispersity index
PEG 400	polyethylene glycol 400
PEVs	Penetration Enhancer-containing Vesicles
PG-PEVs	Propylene glycol Penetration Enhancer-containing Vesicles
PLA	Poly-lactic acid
PLGA	Poly(lactic- <i>co</i> -glycolic acid)
PS	Particle size
PVA	Polyvinyl alcohol
ROS	Reactive oxygen species
SA	Stearic acid
SLNs	Solid lipid nanoparticles
SNEDDS	Self-nanoemulsifying delivery systems
TC	<i>Thymus capitatus</i>
THY	Thymol
TPP	Pentasodium tripolyphosphate
T-TCP	CS micelles loaded with thymol oil
UVB	Ultraviolet radiation
ZTO	Zedoary turmeric oil

1 Introduction

In recent time, it is obvious that there is an increasing interest concerning the utilization of plant-derived natural products. Natural products are characterized by wide-ranging biological activities, in addition to being safe and harmless to the environment; consequently, they attracted many researchers in various areas. Essential oils (EOs) represent a great concern in the pharmaceutical and nutraceutical fields in consequence of their remarkable biological activities including antimicrobial, antioxidant, anti-inflammatory, and anti-mutagenic activities (Lau et al. 2002; Asbahani et al. 2015). The multidrug-resistant bacteria progression is a principal cause to a continuous demand for discovering new drugs and alternative treatments against infections. The exploration of the antimicrobial effect of EOs that are frequently used today in various attributes as cosmetics, health care, traditional medicine, and food industry could be one of the promising solutions for this global problem. Furthermore, numerous studies have emphasized EOs antimicrobial effects even against multi-resistant bacteria (Calo et al. 2015).

The biocidal activities of EOs (bactericidal, virucidal, and fungicidal) could stimulate their application as natural antimicrobials in food, beverage, and pharmaceutical products. However, EOs pose some problems for their wide application, including their instability, volatility, ease of degradation (by light, heat, and oxidation); in addition to the high reactivity and hydrophobicity of the EOs that represent major obstacles towards their incorporation in delivery systems (Solórzano-Santos and Miranda-Novales 2012; Carson et al. 2002). Many researchers investigated the incorporation of EOs in delivery systems to protect them from the environment, increase their stability, augment their antimicrobial effect, and provide sustained release effect.

The EOs loaded delivery systems have been used for their antimicrobial competence against different infections such as dental and wound infections or as a disinfectant for medical tools and surfaces or as preservatives in food industry (Asbahani et al. 2015). Hence, the aim of this chapter is to discuss the investigated EOs based delivery systems with antimicrobial effect that were used as pharmaceutical products to treat infections and to enhance EOs antimicrobial effect in medical field, while EOs incorporated in delivery systems to preserve food and beverage are beyond our scope. In addition, the chapter will provide a highlight on the antimicrobial mechanism of action of EOs.

2 Essential Oils Main Components and Their Mechanism of Action

EOs are secreted in distinctive cells, secretory ducts, cavities, or in glandular hairs that are present in various plant parts as leaves, stems, barks, roots, flowers, and fruits (Calo et al. 2015), from which they are obtained by water or steam distillation, enfleurage, pressing, in addition to supercritical fluid extraction (Adlard 2010).

Chemically, EOs consist of terpene compounds (mono-, sesqui-, and diterpenes), alcohols, acids, esters, epoxides, phenols aldehydes, ketones, amines, and sulfides that may be attributed to their antimicrobial activities (Calo et al. 2015; Solórzano-Santos and Miranda-Novales 2012). Differences in functional group structure and composition of these EOs play a significant role in the function of their antimicrobial activity, which influences bacterial growth. Owing to the great number of diverse functional groups of chemical constituents exist in EOs, it is most probably that their antibacterial activity is not endorsed to only one specific mode of action but that there are several targets in the cell because of interaction between numerous constituents of EOs (Carson et al. 2002; Bassole and Juliani 2012).

The antimicrobial effects of EOs have been investigated against an inclusive variety of microorganisms throughout the years, but their mode(s) of action are still not compressively explored. Several mechanisms have been suggested to clarify the actions of the chemical constituents contained in the EOs (Burt 2004; Cox et al. 2000).

Several researchers have estimated that the antimicrobial action of EOs may be attributed to their capability to penetrate through bacterial membranes to the cell interiors and display inhibitory action on the functional properties of the cell, owing to their lipophilic properties (Burt 2004; Bajpai et al. 2012; Guinoiseau et al. 2010; Fisher and Phillips 2009; Friedly et al. 2009). Phenolic compounds disrupt the cell membrane and ultimately cause leakage of the internal contents of the cell (Bajpai et al. 2012). The mechanisms of action may be related to the capability of phenolic compounds to modify microbial cell permeability, destruct cytoplasmic membranes, interfere with cellular energy (ATP) generation system, and disturb the proton motive force (Bajpai et al. 2012; Burt 2004; Friedly et al. 2009; Li et al. 2011). The interrupted permeability of the cytoplasmic membrane can end in cell death (Li et al. 2011).

The interaction of EOs with microbial cell membranes results in the inhibition of the growth of some Gram-positive and Gram-negative bacteria (Calsamiglia et al. 2007). Gram-positive bacteria such as *Staphylococcus aureus* (*S. aureus*), *Listeria monocytogenes* (*L. monocytogenes*), and *Bacillus cereus* are more susceptible to EOs than Gram-negative bacteria, such as *Escherichia coli* (*E. coli*) and *Salmonella Enteritidis* (Chorianopoulos et al. 2004). In general, it is assumed that EOs systematically should be more active against Gram-positive bacteria owing to the direct interaction of the cell membrane with hydrophobic components of the EOs (Chao et al. 2000; Soković et al. 2010) (Cimanga et al. 2002). On the contrary, based on this evidence, Gram-negative cells should be more resistant to plant EOs owing to their hydrophilic cell wall (Kim et al. 2011). This outer layer aids to inhibit the penetration of hydrophobic compounds (Calsamiglia et al. 2007; Ravichandran et al. 2011). Deans and Ritchie (1987) concluded that both Gram-positive and Gram-negative bacteria were equally sensitive to citrus EOs and their components (Deans and Ritchie 1987). However, Dorman and Deans (2000) investigated that carvacrol and thymol acted in a different manner against Gram-positive and Gram-negative bacteria (Dorman and Deans 2000). Thymol and carvacrol were capable to cause degeneration of the outer membrane of

Gram-negative bacteria, liberating lipopolysaccharides and increasing the cytoplasmic membrane permeability to ATP (Burt 2004). In another study, *Origanum vulgare* L. essential oil suppressed some physiological attributes of the *S. aureus* (Gram-positive bacteria) strains such as coagulase, lipase, and salt tolerance. The oil interfered with the microbial metabolic activity in a dose-dependent manner (Carneiro De Barros et al. 2009). In general, the EOs owning the strongest antibacterial properties comprise a high percentage of phenolic compounds such as carvacrol, eugenol (2-methoxy-4-(2-propenyl) phenol), thymol, perillaldehyde, cinnamaldehyde, and cinnamic acid (Dorman and Deans 2000; Djenane et al. 2011; De Oliveira et al. 2011; Gill and Holley 2004). It seems reasonable that their mechanism of action would therefore be similar to other phenolics mentioned before; this is generally considered the disturbance of the cytoplasmic membrane, disrupting the proton motive force, electron flow active transport, and coagulation of cell contents (Denyer and Hugo 1991; Davidson 1997; Sikkema et al. 1995).

In addition to their antibacterial activities, EOs have been extensively studied because of their antifungal properties (Mahmoudvand et al. 2014; Piras et al. 2013; Tolouee et al. 2010; Gumus 2010; Elaissi et al. 2012; Stević et al. 2014; Ahmadi et al. 2010; Sokovic et al. 2009). In addition to their effect on cell membrane (Tiwari et al. 2009); they also trigger defense mechanisms in the infected plant: produce alkalization of the medium, stimulate oxidative burst and induce defense genes (Chang et al. 2011). Disruption of cytoplasmic membranes and intracellular organelles, detachment of plasma membrane from the cell wall, cytoplasm depletion, and complete disorganization of hyphal compartments; in addition to swelling and deformation of hyphal tips, formation of short branches, and collapse of entire hyphae were the among major changes attributed to antifungal activity (Tolouee et al. 2010).

Therefore, many works analyze plant EOs and extracts, focusing on their phenolic content and their activity against different fungi, like *Aspergillus*, *Penicillium*, *Botrytis*, or *Candida* (Boyraz and Özcan 2006; Rasooli and Abyaneh 2004; Murthy et al. 2009; Rasooli et al. 2006; Martins et al. 2015).

Different EOs had been reported for their antimicrobial activities as EO of thyme (Tohidpour et al. 2010; Hazzit et al. 2009; Rota et al. 2008; Braga et al. 2008; Khadir et al. 2016), basil (Hussain et al. 2008; Bozin et al. 2006), eucalyptus (Gilles et al. 2010), pistachio (Koutsoudaki et al. 2005) (Taran et al. 2010), savory (Oke et al. 2009; Cavar et al. 2008; Vagionas et al. 2007; Skocibusic et al. 2006), oregano (Carneiro De Barros et al. 2009; Winward et al. 2008; Bendahou et al. 2008), black cumin (Ramadan 2007; Piras et al. 2013), coriander (Lo Cantore et al. 2004), peppermint (Gulluce et al. 2007), sumac (Fazeli et al. 2007), clove, cinnamon (Oussalah et al. 2007; Oussalah et al. 2006), guarana (Majhenic et al. 2007), fennel (Lai and Roy 2004), orange, geranium, juniper, rosemary (Schelz et al. 2006), cumin, laurel, marjoram, sage (Ozcan et al. 2006; Kelen and Tepe 2008; Tepe et al. 2005), pepper, turmeric, ginger, and pine (Sacchetti et al. 2005).

3 Essential-Oils-Loaded Delivery Systems

Taking into account their investable role in today's pharmacotherapy, many types of delivery systems, lipid and non-lipid based ones, are utilized to encapsulate active pharmaceutical agents. Versatile natural and synthetic polymers are well-documented to be favorably engaged in the drug delivery field via assorted administration routes, owing to their peculiarities of safety, biodegradability, sustained drug release, and enhanced bioavailability (Elmowafy et al. 2019d; Elmowafy and Soliman 2019; Elmowafy et al. 2019a; Shamarekh et al. 2020b; Shamarekh et al. 2020a; Nasr et al. 2019; Tawfik et al. 2019). In this direction, both types of polymers are also attempted in the fabrication of effective encapsulated EOs vehicles, presenting remarkable pharmacotherapeutic prospects. In the following sections, extensive applicability of various EOs loaded delivery systems and their beneficial antimicrobial capabilities are addressed. Examples of a substantial amount of research studies dealing with different encapsulated EOs and their paramount achievements are encompassed in this chapter (Tables 16.1, 16.2, 16.3, 16.4, and 16.5). In addition, Fig. 16.1 shows schematic illustration of different delivery systems encapsulating antimicrobial essential oils.

3.1 Lipid-Based Delivery Systems

Microemulsions and Nanoemulsions

Colloidal delivery systems based on microemulsions or nanoemulsions are being extensively utilized in the food and pharmaceutical industries to encapsulate, protect, and deliver bioactive EOs. The terms microemulsion is used to describe a thermodynamically stable, optically clear isotropic colloidal dispersion of water and oil stabilized by an interfacial film of a mixture of surfactant and co-surfactant (Schulman et al. 1959; Danielsson and Lindman 1981; Shinoda and Lindman 1987). Microemulsions have gained great interest in the last 40 years due to its unique characteristics. The spontaneous formation from relatively simple ingredients and its ability to deliver both hydrophilic and lipophilic drugs are among the encouraging factors for their immense use in pharmaceutical research field (Fanun 2012). Moreover, the small particle size possessed by the microemulsion renders it many of its unique characters. These small droplet sizes increase the surface area to volume ratio for drug absorption leading to improved bioavailability. Additionally, these small droplet sizes are able to resist gravitational separation and hence enhance the stability of the microemulsion system (Chiappisi et al. 2016). It is worth to mention that the droplet size exhibited by a microemulsion ranges from 10 nm to 100 nm.

The main differences between the microemulsion and the conventional emulsion is that the former exhibit thermodynamic stability, clear, or translucent appearance and ease of preparation, while the emulsions are relatively unstable, cloudy, and require high energy input for their preparation which may increase the cost for

Table 16.1 Representative examples of essential oils encapsulated into microemulsions and nanoemulsions

Essential oil	Delivery system	Activity against	Application	Ref.
Nettle oil	Nanoemulsion	<i>Aspergillus brasiliensis</i> , <i>S. aureus</i> , <i>B. subtilis</i> and <i>E. coli</i>	Antimicrobial	Gharibzahedi and Mohammadnabi (2016)
Vitex negundo oil		<i>E. coli</i> , <i>Enterobacter aerogenes</i> , <i>Enterococcus faecalis</i>	Antimicrobial Larvicidal	Balasubramani et al. (2017)
Citral oil		<i>S. aureus</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , <i>Enterococcus faecalis</i> , <i>S. typhimurium</i> , and <i>L. monocytogenes</i>	Antimicrobial	Lu et al. (2018)
Cinnamon oil and usnic acid	Microemulsion based oral spray	<i>C. albicans</i> and <i>Trichophyton mentagrophytes</i>	Anti-fungal	Kumar et al. (2019)
Clove oil		<i>C. albicans</i>	Oral candidiasis	Monton et al. (2020)
Oregano oil	Pickering emulsions	<i>S. aureus</i> , <i>B. subtilis</i> , <i>E. coli</i> and <i>Saccharomyces cerevisiae</i>	Antimicrobial	Zhou et al. (2018)
Zedoary turmeric oil	Self-nanoemulsifying drug delivery system (SNEDDS)	–	Antimicrobial	Zhao et al. (2010)
Lemongrass oil	Chitosan stabilized nanoemulsion	<i>E. coli</i> , <i>S. aureus</i> , <i>Gardnerella vaginalis</i>	Treatment of cutaneous or pulmonary infective pathologies.	Bonferoni et al. (2017)

industrial production (Lawrence and Rees 2012). The nanoemulsion can be regarded as traditional emulsion with minute PS (McClements 2012). The use of the terms “microemulsions” and “nanoemulsions” is always confusing in the scientific literature. However, they are completely different types of colloidal dispersions: a microemulsion is thermodynamically stable, whereas a nanoemulsion is not (Rao and McClements 2012; Vladisavljević 2019). The reason beyond this confusion is due to the prefixes used to denote them, which would suggest that nanoemulsions contain particles that are smaller than those in microemulsions. Indeed, the opposite is usually the case; the particles in a microemulsion are smaller than those in a nanoemulsion (McClements 2012). The detailed differences and similarities between the two systems are beyond our scope where they were discussed by other researchers (McClements 2012; Gupta et al. 2016).

Table 16.2 Representative examples of essential oils encapsulated into liposomes

Essential oil	Delivery system	Activity against	Application	Ref.
<i>Artemisia arborescens</i> oil	Liposomes	<i>Herpes simplex virus type 1</i>	Anti-herbal	Sinico et al. (2005)
<i>Salvia triloba</i> and <i>Rosmarinus officinalis</i> oils		<i>E. coli</i> , <i>K. pneumoniae</i> , <i>Proteus mirabilis</i> , <i>P. aeruginosa</i> and <i>S. aureus</i>	Anti-microbial	Risaliti et al. (2019)
<i>Eucalyptus camaldulensis</i> oil	Liposomal gel	<i>Microsporum canis</i> , <i>M. gypseum</i> , <i>Trichophyton rubrum</i> and <i>Trichophyton verrucosum</i>	Anti-fungal	Moghimpour et al. (2012)
Tea tree oil	Liposomes	<i>P. aeruginosa</i> , <i>S. aureus</i> and <i>C. albicans</i>	Antimicrobial	Low et al. (2013)
<i>Santolina insularis</i> oil	Penetration enhancer containing vesicles (PEVs)	–	Antibacterial Antiviral	Castangia et al. (2015)
<i>Thymus capitatus</i> oil	Liposomes, glycosomes and PEVs	<i>S. mutans</i> <i>Lactobacillus acidophilus</i> and <i>Streptococcus sanguinis</i>	Mouthwash for the treatment of oral cavity diseases	Manconi et al. (2018)
<i>Thymus capitatus</i> oil / <i>Citrus limon</i> var. <i>pompia</i> extract		<i>S. mutans</i> and <i>C. albicans</i>	Antimicrobial in caries prevention	Pinna et al. (2019)
Clove oil	Liposomes dry powder	–	Antimicrobial Anti-fungal Anti-viral	Sebaaly et al. (2016)

The encapsulation of EOs in the emulsion-based systems (microemulsion and nanoemulsion) represents a promising strategy to increase the physical stability of the bioactive substances, protect them from the environment (oxidation, light, and high temperature), decrease their volatility, prolong EOs release, and enhance their bioactivity (Bilia et al. 2014). Table 16.1 summarizes the different emulsion-based systems encapsulating EOs.

For example, Gharibzahedi and Mohammadnabi (2016) extracted the nettle EO from the aerial part of the dried nettle leaves and incorporated it in nanoemulsions to enhance its stability and efficacy as antimicrobial. Various nanoemulsions were prepared using canola oil and Tween (40, 60, and 80) as oil and surfactant, respectively, with different surfactant to oil ratios. As revealed, the minimum inhibitory concentration (MIC) for nettle oil-loaded nanoemulsion against four target microorganisms of *Aspergillus brasiliensis*, *S. aureus*, *B. subtilis* (*B. subtilis*), and *E. coli*

Table 16.3 Representative examples of essential oils encapsulated into lipid nanoparticles

Essential oils	Delivery system	Activity against	Application	Ref.
<i>Artemisia arborescens</i> oil	SLNs	<i>Herpes simplex virus 1</i>	Herpes (viral infections)	Lai et al. (2007)
<i>Nigella sativa</i> oil		–	Dermal and cosmetic	Al-Haj et al. (2010)
Clove oil		<i>C. albicans</i>	Oral candidiasis (fungal infections)	Garg and Singh (2011)
Eugenol (clove oil)	SLNs in hydrogel	(depend on previous study)	Epidermal targeting (fungal infections)	Garg and Singh (2014)
<i>Zataria multiflora</i> oil	SLNs	<i>Aspergillus ochraceus</i> , <i>Aspergillus niger</i> , <i>Aspergillus flavus</i> , <i>Alternaria solani</i> , <i>Rhizoctonia solani</i> , and <i>Rhizopus stolonifer</i> .	Fungal infections	Nasseri et al. (2016)
<i>Copaiba</i> oil		<i>C. krusei</i> , <i>C. parapsilosis</i> , <i>Trichophyton rubrum</i> and <i>Microsporum canis</i>	Skin fungal infections	Svetlichny et al. (2015)
<i>Copaiba</i> oil		<i>C. parapsilosis</i>	Skin fungal infections	Svetlichny et al. (2017)
<i>Rosmarinus officinalis</i> , <i>Lavandula x intermedia</i> “Sumian”, <i>Origanum vulgare</i> subsp. <i>hirtum</i> oils	NLCs	<i>C. albicans</i> and <i>C. krusei</i>	Skin fungal infections	Carbone et al. (2019)
<i>Eugenia caryophyllata</i>	SLNs	<i>Salmonella typhi</i> , <i>P. aeruginosa</i> , <i>S. aureus</i> and <i>C. albicans</i> .	Reduction of microbial resistance	Fazly Bazzaz et al. (2018)
Eucalyptus or rosemary oils	SLNs and NLCs	<i>S. aureus</i> and <i>Streptococcus pyogenes</i> .	Wound healing	Saporito et al. (2018)
Peppermint oil	NLCs	<i>E. coli</i> , <i>S. typhimurium</i> , <i>P. aeruginosa</i> , <i>S. aureus</i> , <i>S. epidermidis</i> , <i>Bacillus anthracis</i> , <i>Staphylococcus pneumoniae</i> , and <i>L. monocytogenes</i>	Wound healing	Ghodrati et al. (2019)
Rosemary oil		<i>S. epidermidis</i> , <i>S. aureus</i> , <i>L. monocytogenes</i> , <i>E. coli</i> and <i>P. aeruginosa</i>	Wound healing	Khezri et al. (2019)
<i>Mentha pulegium</i>		<i>S. epidermidis</i> , <i>S. aureus</i> , <i>L. monocytogenes</i> , <i>E. coli</i> and <i>P. aeruginosa</i>	Wound healing	Khezri et al. (2020)

(continued)

Table 16.3 (continued)

Essential oils	Delivery system	Activity against	Application	Ref.
Oregano, clove, cinnamon oils	LNCs	<i>Acinetobacter baumannii</i> , <i>K. pneumoniae</i> , <i>E. coli</i> and <i>P. aeruginosa</i> .	Reduction of microbial resistance	Valcourt et al. (2016)
Eucalyptus and orange oil		<i>C. albicans</i>	Dermal fungal infections	Hussein et al. (2020)

Abbreviations: SLNs solid lipid nanoparticles, NLCs nanostructured lipid carriers, LNCs lipid nanocapsules

were 0.25–1.25, 1.5–8.25, 0.625–5.0, and 8.5–17.5 µg/ mL, respectively. The highest and lowest resistance to antimicrobial nettle oil-loaded nanoemulsions was for Gram-negative bacterium of *E. coli* and yeast *Aspergillus brasiliensis*, respectively. The authors assumed that the lower antimicrobial potential of the nettle oil-loaded nanoemulsion compared to the pure nettle oil was due to its small percent (1.25%) in the final formula. However, the transparent nanoemulsion prepared with polysorbates (Tween® 40 and Tween® 60) showed good stability and high potentiality to incorporate the antimicrobial oil making it a good candidate for beverages and drug applications (Gharibzahedi and Mohammadni 2016).

In a subsequent study, Gharibzahedi (2017) attempted to optimize the major variables of high-intensity ultrasonic homogenization process for developing new nettle oil-loaded nanoemulsion. The oily phase was composed of canola oil and nettle oil, while the aqueous phase was formed of the nonionic surfactant Tween® 40 and the purified polysaccharide fraction of jujube fruits as a stabilizing agent dispersed in a buffer acetate solution. The formulation exhibited no change in particle size (PS) when stored at 4 °C and 25 °C for 45 days indicating its stability. Similar to his previous study, pure nettle oil had a higher antimicrobial activity than nettle oil nanoemulsion (Gharibzahedi 2017).

In another study, dried leaves of *Vitex negundo* L. were hydro-distilled producing a yellowish color EO (0.5% v/w). The EO nanoemulsion was prepared by using 5% EO and 5% of polysorbate 20 as a surfactant and investigated for its bactericidal, larvicidal, and antioxidant activity. Three strains (*E. coli*, *Enterobacter aerogenes*, and *Enterococcus faecalis*) were used to determine the MIC for the pure isolated oil and the oil-loaded nanoemulsion. The oil-loaded nanoemulsion showed lower MIC than the pure oil for all the tested strains. Furthermore, the larvicidal mortality was higher for the nanoemulsion than for the pure oil because of high surface area and stability of the prepared nanoemulsion. These findings encourage the promising application of *Vitex negundo* L. oil-loaded nanoemulsion as antimicrobial and repellents (Balasubramani et al. 2017).

Parallel to the above sequence, citral in water nanoemulsions was prepared using a blend of Span® 85 and Brij® 97 as surfactants and ethylene glycol as a co-solvent. The interesting point in this study was mixing different ratios of Span® 85 and Brij® 97 to obtain proper HLB values necessary to maintain the equilibrium between the oil and water phases. The citral nanoemulsions showed different

Table 16.4 Representative examples of essential oils encapsulated into non-lipid based delivery systems

Essential oil	Delivery system	Activity against	Application	Ref.
Thyme oil	Hydrogels	<i>S. epidermidis</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> and <i>C. albicans</i>	Wound healing	Boccalon et al. (2020)
Clove oil, Thyme oil	Orabase gel	<i>Candida</i> species	Oral candidiasis	Labib and Aldawsari (2015)
Citronella oil	Chitosan microcapsules	–		Hsieh et al. (2006)
Coriander oil		Food pathogens	Pharmaceutical industries	Duman and Kaya (2016)
Holy basil oil	Gelatin microcapsules	<i>E. coli</i> , <i>S. aureus</i> and <i>S. typhimurium</i>	Oral delivery	Ngamekaue and Chitprasert (2019)
<i>Carum copticum</i> oil		Food pathogens	Pharmaceutical industries	Esmaeili and Asgari (2015)
<i>Cinnamomum zeylanicum</i> oil	β -cyclodextrin modified chitosan nanoparticles	–	Pharmaceutical industries	Matshetshe et al. (2018)
Lemongrass oil	Cellulose acetate nanocapsules	<i>E. coli</i> and <i>S. aureus</i>	Medical and pharmaceutical applications for preventing microbial resistance.	Liakos et al. (2016)
Peppermint, cinnamon and lemongrass oils		<i>S. aureus</i> , <i>P. aeruginosa</i> , <i>E. coli</i> and <i>C. albicans</i>	Medical, pharmaceutical recipients and in household products for treating or preventing microbial colonization and biofilm development.	Liakos et al. (2018)
Tea tree oil	Pluronic P123 and F127 mixed micelles	<i>E. coli</i> and <i>S. aureus</i>	Antimicrobial & wound healing properties	Ganguly et al. (2020)
Thymol oil	Chitosan micelles	<i>L. monocytogenes</i> and <i>S. aureus</i>	Topical disinfectant	Wang et al. (2019)

antimicrobial activities against *S. aureus* and *L. monocytogenes*, *E. coli*, *Pseudomonas aeruginosa* (*P. aeruginosa*), *Enterococcus faecalis*, *Salmonella typhimurium* (*S. typhimurium*) (Lu et al. 2018).

More recently, Kumur et al. (2019) prepared a nanoemulsion containing a mixture of cinnamon oil and usnic acid. The prepared nanoemulsion comprised cinnamon oil as an EO, Tween® 20 as a surfactant, ethanol as co-surfactant, usnic acid as

Table 16.5 Representative examples of essential oils encapsulated into casting films and nanofiber mats for wound and burns care and management

Essential oil	Delivery system	Activity against	Ref.
Thyme oil	Film	<i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i> and <i>S. aureus</i>	Altıok et al. (2010)
Eucalyptus oil	Nanoemulsion-impregnated film	<i>S. aureus</i>	Sugumar et al. (2015)
Clove and Melaleuca oils	Film	<i>S. aureus</i> , <i>E. coli</i> , and <i>C. albicans</i>	Pereira Dos Santos et al. (2019)
Black pepper and Ginger oils		<i>Bacillus cereus</i> , <i>S. aureus</i> , <i>E. coli</i> and <i>S. typhimurium</i>	Amalraj et al. (2020)
Chamomile blue, cinnamon, lavender, tea tree, peppermint, eucalyptus, lemon and lemongrass oils		<i>E. coli</i> and <i>C. albicans</i>	Liakos et al. (2014)
Thyme oil		<i>P. aeruginosa</i> , <i>E. coli</i> , <i>S. aureus</i> and <i>B. subtilis</i>	Kavoosi et al. (2013)
<i>Zataria multiflora</i> oil		<i>P. aeruginosa</i> , <i>E. coli</i> , <i>S. aureus</i> and <i>B. subtilis</i>	Kavoosi et al. (2017)
<i>Hypericum perforatum</i> oil		<i>E. coli</i> and <i>S. aureus</i>	Güneş and Tihminlioğlu (2017)
<i>Zataria multiflora</i> oil	Nanofiber mats	<i>P. aeruginosa</i> , <i>S. aureus</i> and <i>C. albicans</i>	Ardekani et al. (2019)
Lavender oil		<i>S. aureus</i>	Hajiali et al. (2016)
Thymol (thyme oil)		<i>E. coli</i> and <i>S. aureus</i>	Karami et al. (2013)
Peppermint oil		<i>E. coli</i> and <i>S. aureus</i>	Unalan et al. (2019b)
Clove oil		<i>E. coli</i> and <i>S. aureus</i>	Unalan et al. (2019a)
<i>Citrus sinensis</i> oil		<i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i> , and <i>S. aureus</i>	Abdollahi et al. (2020)
Lavender oil		<i>S. aureus</i> and <i>K. pneumoniae</i>	Balasubramanian and Kodam (2014)
Cinnamon, lemongrass and peppermint oils		<i>E. coli</i>	Liakos et al. (2015)
Lavender oil		<i>E. coli</i> and <i>S. aureus</i>	Sofi et al. (2019)
Thyme oil		<i>E. coli</i> and <i>S. aureus</i>	Liu et al. (2019)
Clove oil	<i>E. coli</i> and <i>S. aureus</i>	Qin et al. (2020)	
Cabreuva oil	Nanoparticles imbedded into nanofiber mats	<i>C. albicans</i> , <i>S. aureus</i> , <i>S. epidermidis</i> , <i>E. coli</i> , <i>S. aureus</i>	Lamarra et al. (2020)

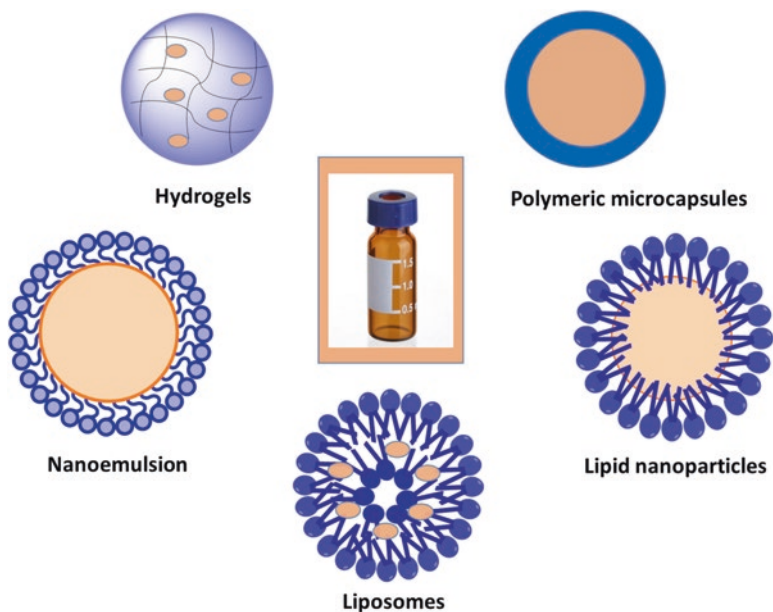


Fig. 16.1 Representative delivery systems used for encapsulating EOs

drug, and deionized water as continuous phase. The antifungal efficacy of the prepared nanoemulsion against *Candida albicans* (*C. albicans*) and *Trichophyton mentagrophytes* was performed on two animal models: cutaneous candidiasis on rats and dermatophytosis on guinea pigs, respectively. Only one animal out of six was detected positive fungal culture for those treated with cinnamon EO and usnic acid nanoemulsion. However, the number of positive animals that showed positive cultures were four and six animals for drug solution and control group animals, respectively. The formulation-treated group showed significant reduction in colony-forming units (CFU) in comparison to untreated control group and drug solution indicating the feasibility of nanoemulsion formulation for topical preparation (Kumar et al. 2019).

It is evident from the literature that most of EOs loaded nanoemulsion exhibited enhanced antimicrobial activity compared to pure oil. This can be attributed to increased surface area of nanoemulsion droplets and the fusion of the emulsifier droplets with the phospholipid bilayer of the cell membrane of bacteria. Besides, the electrostatic interaction between the positively charged nanoemulsions droplets and the negatively charged bacterial cell walls can concentrate the EOs at the site of action. Additionally, the release of EO can be sustained leading to prolonged activity.

In another context, microemulsion was prepared for the treatment of acne vulgaris using a combination of cinnamaldehyde (CAD) and berberine chloride (BER). CAD is an EO isolated from the Cinnamomum species such as *Cinnamomum cassia* L. (J. Presl) and *Cinnamomum zeylanicum* L. (Darchini). Berberine is an

isoquinoline alkaloid that exists in *Berberis aristata* DC., *Berberis vulgaris* L., and *Coptis chinensis* Franch. Both entities are well known for their antibacterial action against different strains of microorganisms. The oily mixture containing clove oil, CAD, and BER was mixed with the surfactant mixture (polysorbate 80 and polyethylene glycol 400 [PEG 400]), then titrated with double distilled water until microemulsion formation. The resultant microemulsion had an average PS of 67.81 ± 4.21 nm. The anti-acne activity was studied by measuring the percent change in ear thickness of Wistar rats. All the tested formulae containing different concentrations of the bioactive materials exhibited a significant change in the ear thickness compared to the control group. As a conclusion from this study, although the high concentration (1% w/w) of CAD developed irritation on the hairless back of Wistar rats, formula with 0.75% w/w CAD loaded microemulsion can be an effective alternative prescription for the treatment of acne vulgaris (Gull et al. 2020).

In another important study, clove oil microemulsion oral spray was developed for the treatment of oral candidiasis. Clove oil is a volatile oil that is steam distilled from the flower buds of *Syzygium aromaticum* (L.) Merr. & L. M. Perry (Myrtaceae). Eugenol is the major component of the clove oil. It demonstrates antibacterial, antiviral, antifungal, antiparasitic, insect-repelling, antioxidant, anti-inflammatory, and cytotoxic activity. The oily phase was a blend of clove oil, peppermint oil, and spearmint oil mixed with Tween® 80 and PEG 400 as surfactant and co-surfactant respectively. The clove oil microemulsion exhibited a PS measured in nanometers (26.3–86.1 nm). The highest stability at 4, 25, and 40 °C was encountered with formulations containing no PEG 400. The anti-*Candida albicans* activity in all the formulations was stable throughout the 90-day storage period indicating its ability to effectively treat oral candidiasis (Monton et al. 2020).

As mentioned earlier, the conventional emulsion suffers from stability problems as coalescence and Oswald ripening. Recently, solid particle-stabilized emulsions (Pickering emulsions) have gained much more attention because of its great resistance to coalescence. Additionally, being free from surfactants, Pickering emulsions are good candidate for cosmetic and pharmaceutical applications where surfactants may show some adverse effects (Binks 2002; Aveyard et al. 2003; Chevalier and Bolzinger 2013). Many types of particles, either inorganic or organic, proved to be efficient stabilizer for Pickering emulsions as calcium carbonate and barium sulfate, clays (montmorillonite and laponite), carbon black, latex, magnetic particles, carbon nanotubes, block copolymer micelles. Adsorption of solid particles at the oil/water interface requires the partial wetting of the solid by water and oil. Pickering emulsions can be considered advantageous for encapsulating EOs where their high volatility and reactivity are main challenges. Wen et al. (2014) used cellulose nanocrystals (CNCs) prepared from corncob cellulose to stabilize D-limonene Pickering emulsions. D-Limonene (4-isopropenyl-1-methylcyclohexene) is a main constituent of citrus fruits with a well-defined bactericide, antioxidant, and chemopreventative activity. The D-limonene oil Pickering emulsions were successfully prepared by sonicating d-limonene and CNCs aqueous dispersion. The PS of the produced emulsion was greatly affected by the concentration of CNCs which reached 4.2 μm at 0.2% w/w. Regarding the stability of the prepared emulsion, it

showed good stability which was affected by the pH, ionic strength, and salt concentration of the surrounding media (Wen et al. 2014).

Another similar work was published where oregano essential oil Pickering emulsion was prepared using CNCs as a stabilizer. Oregano EO from *Origanum vulgare* L. clearly demonstrated antioxidant and antimicrobial activities. Similarly, the PS decreased as the CNCs concentration increased or the oil/water ratio decreased. The antimicrobial activity was studied against four different microorganisms including the Gram-positive *S. aureus*, *B. subtilis*, the Gram-negative *E. coli*, and the yeast *Saccharomyces cerevisiae*. Although the oregano essential oil Pickering emulsion exerted strong inhibitory effect against all the tested microorganisms, this activity was lower than that of pure oil. This can be attributed to the repulsion effect between the negatively charged surfaces of microorganisms and the negatively charged emulsion droplets (Zhou et al. 2018). As a conclusion, the Pickering emulsion stabilized by CNCs could be a promising alternative for the delivery of the antimicrobial EOs in the food and pharmaceutical industry overcoming the challenges associated with the essential oil volatility and reactivity and the stability issues of the traditional emulsions delivery systems.

Another area of growing interest is the use of self-nano and -micro emulsifying systems for the oral delivery of lipophilic drugs. Self-nanoemulsifying delivery systems (SNEDDS) are isotropic concentrate of oil, surfactant, co-surfactant, and drug, which can be easily dispersed in the aqueous environment of the gastrointestinal tract to form a fine oil-in-water emulsion with a droplet size less than 100 nm under gentle agitation provided by the peristaltic movement. The attraction of SNEDDS lies in being able to solubilize lipophilic drugs; hence, its bioavailability can be improved, it is thermodynamically stable, and it can be directly filled into soft or hard gelatin capsules for oral delivery. These benefits make SNEDDS as a good strategy for the oral delivery of lipophilic anti-microbial EOs (Constantinides 1995; Chakraborty et al. 2009; Date and Nagarsenker 2007).

In this concern, Zhao et al. (2010) developed SNEDDS for the oral delivery of Zedoary turmeric oil (ZTO), EO derived from the dry rhizome of *Curcuma zedoaria* Rosc. with antibacterial, hepatoprotection, tumor suppression, and anti-oxidative activity, to overcome the problems associated with its stability and bioavailability. The PS of the resultant emulsion ranged from 38 nm to 180 nm. The pharmacokinetic parameters showed that the C_{\max} and $AUC_{(0-24)}$ of SNEDDS increased by 2.5-fold and 1.7-fold, respectively, compared to the pure ZTO. As proposed by the authors, after oral administration, ZTO SNEDDS could disperse in the gastrointestinal tract forming nanoemulsion with large surface area for drug absorption. The oil in the solubilized form and the high concentration of the surfactant used could enhance its permeability across the cell membranes. This study demonstrated the potentiality of SNEDDS for the oral delivery of EOs where ZTO itself could serve as a partial lipid phase with the dual advantages of increasing drug loading as well as minimizing the amount of the inert oils required (Zhao et al. 2010).

The importance of SNEDDS arises from its ability to deliver the antimicrobial essential oils orally as a liquid dosage form and enhancing the bioavailability of this

hydrophobic entity because of the small particle size formed upon dispersion in the gastric fluids.

In another attempt to achieve more nanoemulsion stability, Bonferoni et al. (2017) explored the successful use of chitosan oleate amphiphilic stabilizer in the preparation of lemongrass oil (*Cymbopogon citratus* DC. Stapf. EO) o/w nanoemulsion (Bonferoni et al. 2017). It is worth to mention that chitosan oleate, a derivative formed by ionic interaction between chitosan (CS) and oleic acid, both of which are characterized by their antimicrobial effect, a feature that can assist EO antimicrobial activity. Results revealed the formation of small monodispersed nanoemulsion with good stability characteristic that was attributed to the formation of CS shell around the oil as established by the positive zeta potential. In addition, the presence of CS shell on the oil surface elucidates the mucoadhesive property of the nanoemulsion. Lemongrass oil nanoemulsion was biocompatible with different epithelial human cell lines with enhanced antibacterial and antifungal activity against many strains, which are involved in ocular and vaginal infections, in addition to the prospect of its use in the treatment of mucosal and/or skin lesions topically.

The above-mentioned emulsion-based systems can be successful delivery systems for antimicrobial EOs. The EO can constitute the oily phase of the emulsion alone or mixed with other oils thus achieving high loading. The oil, surfactant, and co-surfactant types and concentration are among the factors that significantly affect the physicochemical properties (PS, zeta potential, etc.) of the formulation. Unfortunately, until now, there is no study that could correlate between the physical properties of the formula and its antimicrobial effect.

Liposomes

Liposomes are one of the most common colloidal drug delivery systems that gained immense popularity in the cosmetics and the pharmaceutical fields. They are spherical self-assembled vesicles of lipid bilayer (mainly one or more naturally occurring phospholipid bilayers and cholesterol) enclosing an aqueous compartment (Drulis-Kawa and Dorotkiewicz-Jach 2010). Liposomes can be categorized according to their size (small, large, and giant vesicles), number of bilayers (uni-, oligo-, and multi-lamellar) and phospholipid charge (neutral, anionic, or cationic) (Patil and Jadhav 2014). The popularity of liposomes arises from its ability to entrap both hydrophilic and lipophilic entities, their biodegradability and biocompatibility, modulation of drug release, improved bioavailability and targeted delivery (Maja et al. 2020; Slingerland et al. 2012; Hathout et al. 2019; Hathout et al. 2017).

In an interesting study, *Artemisia arborescens* L. EO was encapsulated in different liposomal (multilamellar and unilamellar) formulations. The effect of liposomes composition on the antiviral activity of *A. arborescens*. EO was studied against *herpes simplex virus type 1* (HSV-1). Surprisingly, the multilamellar vesicles exhibited higher antiviral activity compared to the sonicated unilamellar vesicles and pure *Artemisia arborescens* L. EO due to a higher leakage of the EO components from the smallest and unilamellar vesicles (Sinico et al. 2005).

The EOs of two aromatic perennial Lamiaceae herbs named *Salvia triloba* L. and *Rosmarinus officinalis* L. were also loaded in liposomes and evaluated for their antioxidant, anti-inflammatory, and antibacterial activities. The antimicrobial activity of *Salvia triloba* L. liposomes was tested against four Gram-negative bacteria (*E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *P. aeruginosa*) and one Gram-positive bacterium (*S. aureus*). Significant inhibitory effect was shown against *Klebsiella pneumoniae* (*K. pneumoniae*) where the liposomal formulation had higher antimicrobial activity than the unformulated oils [117]. In the same context, liposomal gel formulation loaded with *Eucalyptus camaldulensis* Dehn. EO was developed with adequate stability that can improve its antifungal activity (Moghimpour et al. 2012).

In the same encounter, Low et al. (2013) encapsulated both tea tree oil and silver ion in controlled release liposomes. When silver ion and tea tree oil are used in combination, lower amounts of each agent are required to achieve enhanced antimicrobial activity. The results showed enhanced antimicrobial activity against *P. aeruginosa*, *S. aureus*, and *C. albicans* when treating the microorganisms with liposomes encapsulating the combined agents (Low et al. 2013).

In another important study conducted by Hammoud et al. (2019), a series of EO compounds (estragole, eucalyptol, isoeugenol, pulegone, terpineol, and thymol) were encapsulated in lipoid S100-liposomes using the ethanol injection method. The effect of chemical structure, Henry's law constant, and aqueous solubility of essential oil components on their liposomal encapsulation was studied. The addition of the phenolic EO components, isoeugenol and thymol, induced an enlargement of vesicles in comparison to blank liposomes. The oils bearing hydroxyl groups as isoeugenol, terpineol, and thymol were highly loaded in liposomes (> 18%) compared to other oils (estragole, eucalyptol, and pulegone) where the loading ratios didn't exceed 5%. This might be explained by the interaction of hydroxyl groups of the oils with the membrane components of liposomes (phospholipids, cholesterol). The PS for isoeugenol and thymol loaded liposomes were significantly greater than that of blank liposome due to aggregate formation. Different release patterns were observed from different EOs vesicles that were not correlated to their chemical structures, their aqueous solubility values, and their log P values. After 10 months storage at 4 °C, the prepared batches exhibited adequate stability regarding the PS and the amount of oil retained. These promising results encouraged the authors to include cyclodextrin in liposomes for further enhancement of the oil loading and liposomes stability (Hammoud et al. 2019).

Not only liposomes were utilized in the encapsulation of EOs but also the newly developed liposomal generations like glycosomes and Penetration Enhancer-containing Vesicles (PEVs) were attempted. In light, *Thymus capitatus* L. Hoff. EO was incorporated in liposomes, glycosomes, and PEVs. The vesicles were prepared using soy lecithin and water to produce liposomes or increasing amounts of glycerol or propylene glycol in water (12.5, 25, 50% v/v) to produce glycosomes and PEVs, respectively. The inclusion of glycerol or propylene glycol enhanced the vesicles stability where conventional liposomes always encounter stability problems. The antimicrobial activity of the vesicles against the cariogenic *Streptococcus*

mutans (*S. mutans*) and *Lactobacillus acidophilus* was very similar to *Thymus capitatus* L. Hoff. EO indicating the potential utility of these vesicles as mouthwash (Manconi et al. 2018). Similarly, *Santolina insularis* Genn. EO known for its antibacterial and antiviral properties was encapsulated in liposomes. Propylene glycol (PG-PEVs) or ethylene glycol (EG-PEVs) was added to the aqueous phase to prepare PEVs aiming at enhancing the stability of the conventional liposomes. The confocal laser scanning microscopy study revealed that liposomes were attached to the skin surface, where they can fuse with the skin lipids, thus releasing their content, while PEVs diffused deeper in the viable epidermis where the loaded drug can slowly be released. Thus, the author recommended the use of PEVs as a promising carrier for the topical delivery of the antimicrobial EOs (Castangia et al. 2015).

Although *S. mutans* represents the main pathogen associated with dental caries, it has been investigated that *C. albicans* usually appear with *S. mutans* in the plaque biofilm. The strong relation between the two organisms exacerbates the virulence of the biofilm with a subsequent increase in disease progression (Falsetta et al. 2014), which necessitates the use of an antimicrobial agent with a potential activity against them. In this regard, vesicular nanocarriers as liposomes, glycosomes, and PG-PEVs encapsulating *Thymus capitatus* L. Hoff. (TC) EO and *Citrus limon* L. Osbeck var. *pompia* (CLP) extract as raw extracts were prepared. The aim was to protect them from degradation, decrease its volatility, and enhance its therapeutic effect against *S. mutans* and *C. albicans*. Cytotoxicity test results revealed the safety of the raw extracts and the nanovesicles against human gingival fibroblasts. The highest antimicrobial effect of TC was recorded against *S. mutans* and *C. albicans*, which is attributed to the high percent of the carvacrol in TC resulting in lethal effect against *Candida*. Carvacrol is characterized by its hydrophobicity that permits its partitioning into the cell membrane allowing its instability with subsequent fungal death. Despite CLP had the same effect on decreasing *S. mutans*, it showed only fungistatic effect against *Candida*. Moreover, the phospholipid vesicles showed similar behavior to the raw extracts indicating the important role of the lipid component of the vesicles in disrupting the bacterial cells (Pinna et al. 2019).

Recently, an increasing proportion of new studies addressed the benefits associated with employing cyclodextrin in encapsulating volatile and EOs. Cyclodextrins are ring-shaped, water-soluble; macrocycle carbohydrate polymers formed of α -(1-4)-linked d-glucopyranose units. This magic molecule has been used to increase the physical and chemical stability of EOs and volatile compounds, increase the solubility of lipophilic molecules, control the release of the bioactive material and transform the liquid and oily material into powder form (Marques 2010). In the light of the above, the concept of drug in cyclodextrin in liposomes (DCL) was first adopted by McCormack and Gregoriadis where the drug/cyclodextrin inclusion complexes were encapsulated into liposomes (McCormack and Gregoriadis 1994). Furthermore, cyclodextrin plays a great role as cryoprotectant when lyophilization is required to increase the shelf life of the prepared liposomal formulation. For example, the inclusion complexes of Eugenol or clove oil and 2-hydroxypropyl- β -cyclodextrin (HP- β -CD) were prepared and then encapsulated into liposomes. The eugenol and clove oil in cyclodextrin inclusion complexes loaded in liposomes

showed smaller PS and higher encapsulation efficiencies relative to eugenol and clove oil liposomes without cyclodextrin. Besides the mean PS, and zeta potential values of cyclodextrin containing formulae remained nearly unchanged during the storage period. Regarding the lyophilization process, the freeze-dried liposomes containing HP- β -CD/Eugenol inclusion complex had mean size, poly dispersity index (PDI), and zeta potential values similar to those obtained before lyophilization while the lyophilization of the eugenol loaded liposomes revealed a significant increase in both PS and PDI values. These results revealed the potentiality of cyclodextrin to maintain the stability of both the EOs and liposomes (Sebaaly et al. 2016).

In another similar study, Gharib et al. (2019) prepared different conventional liposomes and DCL vesicles containing estragole by ethanol injection method using hydrogenated phospholipid in presence of cholesterol. It was found that the inclusion of cyclodextrin increased encapsulation efficiencies and the oil loading into liposomes. The presence of HP- β -CD or estragole did not affect the liposomal size. Estragole DCL size, PDI, and zeta potential values remained unchanged before and after freeze-drying (Gharib et al. 2019).

Interestingly, different EOs including estragole, eucalyptol, isoeugenol, pulegone, terpineol, and thymol were encapsulated in Drug-in-hydroxypropyl- β -cyclodextrin-in-lipoid S100/cholesterol liposomes using ethanol injection method. It was found that phenylpropenes oils (estragole and isoeugenol) were encapsulated to a greater extent (Entrapment efficiencies >85%), than the monoterpenes eucalyptol, pulegone, terpineol, and thymol (Entrapment efficiencies <62%) where the presence of a propenyl tail in the structure of an EO component could increase the liposomes membrane fluidity and enhance its retention in DCL vesicles. In addition, a linear correlation was found between the loading ratios of monoterpenes into DCLs and their hydrophobicity character expressed as log P value. All the selected EO components showed a slow release pattern from DCLs, indicating that they are efficiently retained, and thus their volatility are reduced. The author suggested that the designed systems entrapping different antimicrobial EO may be suitable for preserving food, cosmetics, and pharmaceutical products during storage (Hammoud et al. 2020).

Lipid Nanoparticles

Solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs) and lipid nanocapsules (LNCs) are lipid-based carriers that gained great interest in the last years owing to their advantageous physicochemical properties. SLNs are formed by substituting liquid lipid in (o/w) nano-emulsions by solid lipids such as beeswax, carnuba wax, triglycerides, cetyl alcohol, and cholesterol butyrate, which solidify at room temperature upon dispersion with water and stabilized by surfactants (Müller et al. 2002). SLNs were developed to combine the advantages of liposomes and emulsion. SLNs are biocompatible and biodegradable; they are able to protect the encapsulated drug from degradation and can also achieve controlled release. In addition, they are able to incorporate different molecules with different sizes and

hydrophilicity (Gad et al. 2019; Müller et al. 2002). However, the major limitations of SLNs are the low encapsulation efficiency and the burst drug release for hydrophilic drugs (Das and Chaudhury 2011). NLCs were developed to overcome the drawbacks of SLNs. NLCs are formed by blending liquid lipids (oils) and solid lipids, which increase the lipid matrix ability to accommodate more drug. Finally, LNCs are a hybrid structure of both polymeric nanocapsules and liposomes. They are formed from an oily core of medium chain triglycerides that is stabilized by a membrane formed of mixture of lecithin and PEGylated surfactant. LNCs have been widely investigated due to its low energy and safe method of preparation avoiding the use of toxic solvents, in addition to the advantages of lipid-based carriers (Huynh et al. 2009).

Lia et al. (2007) developed solid lipid nanoparticles (SLNs), using Compritol® 888 ATO lipid, and *Artemisia arborescens* EO as a topical formulation, possessing antiherpetic activity (Lai et al. 2007). The authors conducted a quantitative tetrazolium-based colorimetric method to assess the *in vitro* antiviral effect of free and encapsulated EO against HSV-1. Besides, *in vitro* skin permeation study was performed using newborn pig's skin. The efficacy of the proposed highly stable system to encapsulate EO (87% and 92%), which was ascribed by the authors to the high EO lipophilicity was demonstrated. Furthermore, *Artemisia arborescens* EO loaded SLNs exhibited paramount cutaneous accumulation of the encapsulated EO compared to the free EO that easily diffused through the skin. Significant reduction in the viral cytopathic effect of HSV-1 infected Vero cells (> 30%) was also reported, indicating promising antiviral potential. The described system presents an interesting delivery system to be loaded with antiviral EO, necessitating further *in vivo* studies.

Due to above mentioned features of the lipid-based nanoparticles, they can be considered as potential alternatives for encapsulating different essential oils with high loading and adequate stability. Considering the antimicrobial activity of *Nigella sativa* L. EO (black seed oil) (Aboul-Ela 2002; Salem and Hossain 2000), Al-Haj et al. (2010) prepared *Nigella sativa* EO-loaded SLNs for dermal and cosmetic applications (Al-Haj et al. 2010). SLNs, made of hydrogenated palm oil Softisan® 154 as lipid, were developed using high-pressure homogenization technique. The *in vitro* results demonstrated the formation of nano-sized lipid particles of low crystallinity, conferring high stability over a period of three months. However, the authors did not perform the antimicrobial activity of the prepared formulations.

Garg and Singh (2011) investigated the *in vitro* and *in vivo* antifungal efficacy of eugenol, phenolic component of clove oil, and eugenol-loaded SLNs (single and binary using solid stearic acid alone or mixed with liquid caprylic triglyceride, respectively) oral formulation against *C. albicans* (Garg and Singh 2011). The physicochemical characterization revealed that adding the liquid lipid to the proposed SLNs yielded smaller nano-sized lipid particles (< 100 nm), encapsulating larger EO amounts (> 95%) with higher biphasic EO release and stability (over a period of three months). In the *in vitro* antifungal test, both free and encapsulated eugenol caused 100% inhibition of the growth of *C. albicans*. Besides, testing *in vivo* anti-*Candida* efficacy in a model of oral candidiasis in immunosuppressed

rats using dexamethasone exhibited improved antifungal effect of encapsulated EO, manifested by significantly lower CFU values, owing to enhanced stability, extended release, and heightened permeation in oral epithelial cells.

The same authors continued their investigation on eugenol formulating it in SLNs, made of stearic acid or Compritol® 888 ATO lipids and their corresponding carbopol hydrogel for the treatment of topical fungal infections (Garg and Singh 2014). The authors studied the rheological and mechanical properties (hardness, adhesiveness, cohesiveness, and resilience) of the hydrogel before and after incorporation of SLNs. To delineate the epidermal targeting and its mechanism, an *ex vivo* permeation study using human cadaver skin, *in vitro* and *ex vivo* occlusion studies were also carried out. Loading SLNs into carbopol hydrogel effectively preserved their non-aggregating nano-size, shape and stability (over 180 days) with extended EO release. Besides, the loaded hydrogel exhibited acceptable pseudo-plasticity, thixotropy, and mechanical properties. Moreover, enhanced epidermal retention was revealed compared to almond oil eugenol solution or its inclusion complex-in-hydrogel ($62.65 \pm 4.35 \mu\text{g}/\text{cm}^2$, $52.86 \pm 3.76 \mu\text{g}/\text{cm}^2$, $9.77 \pm 1.16 \mu\text{g}/\text{cm}^2$, and $3.45 \pm 0.6 \mu\text{g}/\text{cm}^2$ for stearic acid SLNs hydrogel, Compritol® 888 ATO SLNs-hydrogel, inclusion complex-in-hydrogel, and almond oil eugenol solution, respectively). The authors also reported the improved skin hydration and ascribed this high occlusivity to the formation of SLNs film on the surface, a depot from which eugenol could be released.

Not only the antifungal effect of encapsulated EO was studied against *C. albicans* but also their efficacy against other fungal pathogens was extensively evaluated. In this context, Nasser et al. (2016) evaluated the *in vitro* antifungal activity of *Zataria multiflora* Boiss. EO encapsulated in glyceryl monostearate (GMS) and Precirol® ATO 5 based SLNs, against fungal pathogens; *Aspergillus ochraceus*, *Aspergillus niger*, *Aspergillus flavus*, *Alternaria solani*, *Rhizoctonia solani*, and *Rhizopus stolonifer* using a contact assay (Nasser et al. 2016). *In vitro* results indicated the formation of homogenous spherical NPs and high EO encapsulation (~80%). Higher inhibition of hyphal growth (average of 79%) and remarkably declined MIC values was reported compared to unencapsulated EO highlighting their antifungal effect under *in vitro* conditions.

In another study, Svetlichny et al. (2015) utilized cetyl palmitate-based SLNs to load copaiba oil from *Copaifera martii* Hayne for optimal treatment of skin infections caused by yeasts and dermatophytes (dermatophytosis) (Svetlichny et al. 2015). The authors incorporated also allantoin, commonly used in dermatology for its pharmacological effect, and evaluated its influence on the antifungal activity of the proposed systems. The antifungal effect of SLNs was performed against *Candida krusei* (*C. krusei*), *Candida parapsilosis* (*C. parapsilosis*), *Trichophyton rubrum*, and *Microsporum canis* using broth micro-dilution assay. The *in vitro* mycological analysis revealed that SLNs possessed promising antifungal effect compared to free EO or free allantoin. Interestingly, those SLNs containing allantoin showed higher fungicidal and fungistatic effectiveness compared with allantoin-free ones in terms of lower MIC values. In particular, the most susceptible MO was found to be

C. parapsilosis (MIC values were 125 mg/mL and 7.8 mg/mL for allantoin SLNs and allantoin free SLNs, respectively).

Taking into consideration these encouraging results, the same research team continued their investigation and studied, in depth, the impact of their formulations in controlling the multi-drug resistant *C. parapsilosis* and their antifungal mechanism (Svetlichny et al. 2017). For this, sorbitol protection assay and ergosterol effect assay were carried out to assess the antifungal activity *in vitro*. Testing *C. parapsilosis* susceptibility demonstrated the absence of both alteration of MIC in sorbitol presence and affinity between SLNs and ergosterol. The authors hypothesized that the prepared SLNs had an unspecific action mechanism on both fungal cell wall and membrane.

Combining mediterranean EOs (*Rosmarinus officinalis*, *Lavandula x intermedia* “Sumian”, *Origanum vulgare* subsp. *hirtum*) and antifungal drug (clotrimazole) in SLNs topical formulation has been utilized by Carbone et al. (2019). The research group performed *in vitro* characterization, release, stability, and cell viability studies. The *in vitro* antifungal activity of the combined system against three *Candida* species *C. albicans*, *C. krusei*, and *C. parapsilosis*, was also tested. The stable EO-loaded NPs showed prolonged release of clotrimazole. Biosafety results indicated anti-proliferative potential of both *Lavandula* and *Rosmarinus* EOs. *In vitro* antifungal susceptibility test demonstrated that among the tested fungi, the highest sensitivity and resistance were observed with *C. parapsilosis* and *C. krusei*, respectively.

EOs that have been extensively investigated for their antibacterial effects and their encapsulation show great promises in the treatment of bacterial infections. Fazly Bazzaz et al. (2018) evaluated the antibacterial and antifungal effects of *Eugenia caryophyllata* L. loaded in SLNs using Precirol, stearic acid (SA), and glyceryl monostearate (GMS) lipids (Fazly Bazzaz et al. 2018). The antimicrobial activity against human pathogens, i.e., *Salmonella typhi*, *P. aeruginosa*, *S. aureus*, and *C. albicans*, was demonstrated by using MIC, minimum cidal concentration (MCC), and “time-kill” methods. The formation of nano-sized, spherical, and stable lipid particles, encapsulating satisfactory EO amounts (~ 70%) was demonstrated. EO-loaded SLNs displayed 2- to 20-fold reduction in MIC/MCC values compared to those of unencapsulated EO, in particular, in SLNs made of SA and Precirol: GMS. However, the authors also reported the lower activity of formulations against Gram-positive bacteria. Time-kill studies, performed to assess rate of microbial killing, revealed stronger bactericidal activity of SLNs against Gram-negative bacteria (*Salmonella typhi* and *P. aeruginosa*) and fungi than against Gram-positive ones, in particular, those prepared using SA. Higher efficacy observed with those containing SA were due to smaller size and better size distribution, highlighting the significant influence of physicochemical attributes on the antimicrobial activity.

In the field of wound healing, EO-loaded lipid NPs were extensively utilized as a lucrative antibacterial option, combating microbial wound colonization that caused wound recovery delay and opening opportunities for antibiotic-free wound healing. In this regard, Saporito et al. (2018) developed both SLNs and NLCs containing either eucalyptus or rosemary EO (Saporito et al. 2018). The proposed

systems were prepared using cocoa butter, olive oil, or sesame oil and pullulan as solid, liquid lipids, and viscosifying agent, respectively. Stable, bioadhesive, and flexible NLCs were fabricated and showed an excellent cyto-compatibility. An *in vitro* augmented proliferation with antimicrobial potential against Gram-positive bacterial strains (*S. aureus* and *Streptococcus pyogenes*) and wound repair activity via re-epithelization and stratum corneum formation was also observed. As reported by authors, these promising therapeutic values were due to the presence of synergistic effect of olive oil with eucalyptus EO.

The incorporation of NLCs into gels for ease of topical application was attempted by several authors. Ghodrati et al. (2019) developed xanthan gel containing peppermint oil loaded NLCs, made of Precirol® ATO 5 as solid lipid and Miglyol 812® as liquid lipid (Ghodrati et al. 2019). *In vitro* characterization, antibacterial activity using microdilution assay and *in vivo* infected wound healing in mice model were conducted. Histopathological study, granulation tissue total bacterial count analysis, and the wound immuno-fluorescent staining for fibroblast evaluation of gene expression profile were also carried out. Peppermint EO was highly encapsulated inside lipid matrices (~90%), showing noticeable antibacterial efficacy, in free and encapsulated forms, against *Staphylococcus epidermidis* (*S. epidermidis*), *S. aureus*, and *L. monocytogenes*, *E. coli*, and *P. aeruginosa*, while *Bacillus anthracis*, *S. typhimurium*, and *Streptococcus pneumoniae* were demonstrated to be resistant. Concerning *in vivo* studies, the prepared NPs exhibited enhancement of wound healing with capability of reducing wound area, edema, and total bacterial count, while hastening neovascularization, fibroblast infiltration, and re-epithelization.

Similarly, Khezri et al. (2019) prepared topical gels containing rosemary-loaded NLCs for the treatment of infectious wounds. NLCs were prepared using Precirol® and Miglyol® as solid and liquid lipids, respectively (Khezri et al. 2019). NLCs and corresponding gels showed promises in reducing bacterial strains counts (*S. epidermidis*, *S. aureus*, *L. monocytogenes*, *E. coli*, and *P. aeruginosa*) and provoking wound repair via reduction of tissue bacterial colonization rate and augmentation of neovascularization, re-epithelization, wound contraction ratio, and serum concentrations of inflammatory cytokines.

The same authors utilized another EO; *Mentha pulegium* L. essential oil for the treatment of infectious wounds via fabricating NLCs and their corresponding gels (Khezri et al. 2020). Likewise, considerable effect of rosemary-loaded NLCs and corresponding gels, EO-loaded systems showed increased antibacterial efficacy against *S. epidermidis*, *S. aureus*, *L. monocytogenes*, *E. coli*, and *P. aeruginosa* and triggered wound healing by heightening proliferation. Furthermore, the previous systems showed the ability of shortening inflammation via upregulation of gene expression of TGF- β , IL-10, and b-FGF.

Combining EO with antibacterial agents, aiming at combating Gram-negative resistant bacteria, was also investigated by Valcourt and his colleagues (Valcourt et al. 2016). In this study, three EO constituents carvacrol (oregano oil), eugenol (clove oil), and cinnamaldehyde (cinnamon oil) and doxycycline were loaded in LNCs, made of polyoxyl-15-hydroxystearate, hydrogenated lecithin, and triglycerides. Their *in vitro* interactions were evaluated using a checkerboard method and a

“time-kill” assay. The tested Gram-negative strains were *Acinetobacter baumannii*, *K. pneumoniae*, *E. coli*, and *P. aeruginosa*. The authors reported the successful development of small-sized LNCs, highly incorporating EO components (20% loading). Interestingly, EO and doxycycline showed bactericidal and bacteriostatic actions, respectively, as manifested by equality of MIC and minimum bactericidal concentration (MBC) values for EO and higher MBC than MIC values for the antibiotic (40–320 times). The synergistic bactericidal effect of the proposed system was also demonstrated.

More recently, Hussein et al. (2020) studied the *in vitro* fungal effects of two EOs: orange and eucalyptus against *C. albicans* by encapsulating them in lipid nanocapsules (LNCs) (Hussein et al. 2020). Interestingly, the authors not only used the proposed EO for their pharmacological effects but also for their role in the preparation of LNCs, outperforming medium chain triglyceride (Miglyol® 812) that are the main components of LNCs. For such purpose, the feasibility conditions of each EO was demonstrated by constructing ternary phase diagrams, characterization, stability (over 6 months), and *in vitro* antimycotic activity were performed. The antifungal efficacy of the used EO was found to be preserved in the lipid particles; with eucalyptus, EO showing higher antifungal activity compared to orange EO (respective inhibition zones were 30.56 ± 1.2 mm and 26 ± 1 mm).

3.2 Non-Lipid-Based Delivery Systems

Gels

Drug delivery based on hydrophilic polymers has been widely investigated due to their ability to retain large amount of water, good flexibility, and viscoelastic properties, which render them favorable candidates for many pharmaceutical applications (Bhattarai et al. 2010; Elmowafy et al. 2019b). One of the main uses of the hydrophilic polymers is the preparation of hydrogels. Hydrogels have been used as a delivery system for different routes such as topical, ophthalmic, and oral and transdermal delivery (Millon and Wan 2006). Hydrogels are widely used in local delivery to the skin and the mucosal membrane due to their high viscosity and mucoadhesive properties, which enhance their retention at the site of application (Soliman et al. 2018; Gad et al. 2008). In an attempt to replace synthetic antimicrobials, Boccalona et al. (2020) reported the encapsulation of two well-known active ingredients with antimicrobial effect (thyme oil and silver NPs) into composite hydrogels. Composite hydrogels were formed of polyvinyl alcohol (PVA), poly(ethylene)oxide, borax, and sodium alginate blend supported on layered double hydroxides that act as filler, which can be molded and shaped to be used as patch for wound healing. The prepared composite hydrogel showed short gelling time, good plasticity, coherent swelling characteristics that indicate its ability to absorb wound exudates, in addition to sustained release behavior of the encapsulated thyme EO and silver NPs. Antimicrobial tests revealed a good antimicrobial activity of the

prepared system against Gram-positive (*S. epidermidis*, *S. aureus*), Gram-negative (*P. aeruginosa*) bacteria and yeast (*C. albicans*). The obtained results revealed the validity of the thyme oil as antimicrobial, a substitution for synthetic one, with the suitability of the prepared system to prevent wound contamination. However, authors recommended further *in vivo* studies (Boccalon et al. 2020).

Orabase® gel with benzocaine has been widely used for relief of pain resulted from oral aphthous stomatitis. In this concern, Labib and Aldawsari (2015) investigated the incorporation of clove oil, thyme oil, and their phenolic compounds (eugenol and thymol, respectively) into Orabase® gel for the management of oral candidiasis (Labib and Aldawsari 2015). All the investigated oils and their active ingredients demonstrated inhibitory effect against *C. albicans* as reflected by the measured MIC values. The prepared gels showed total phenolic content close to that of the crude ingredients with good loading capacity of the EOs without phase separation. The oil-loaded gels possessed neutral pH values, which could ensure reduced oral irritation, good viscosity, and promising mucoadhesive properties, which ensured oral cavity retention, in addition to moderate spreadability, allowing ease of application. The oil-loaded Orabase® gels (especially that of thymol oil and thyme, reaching 98% and 80% cumulative erosion, respectively, at the end of the experiment) showed better erosion than unloaded one. Slow diffusion and dissolution of the EOs from the base resulted in slow release profile with slight burst effect in simulated *in vivo* conditions. All prepared bases showed inhibitory effect against *C. albicans*, where the maximum antifungal activity was observed with thymol oil, which recommended its use in oral candidiasis.

Polymeric Microcapsules

Microencapsulation of active pharmaceutical ingredients is a widely used technique to improve their stability and release behavior. Polymeric microcapsules are formed from liquid core surrounded by a biodegradable polymer, such as chitosan, gelatin, and PVA (Sawalha et al. 2011). In this regard, citronella oil, which helps in mood elevation, decreasing depression, and restless symptoms, in addition to its sensitizing, sterilizing, and bug repelling effects, was successfully encapsulated into CS microcapsules by Hsieh et al. (2006). The study demonstrated the significant effect of preparation conditions of the modified orifice method and the thermal pretreatment of microcapsules on their physicochemical properties, viz. PS, oil encapsulation efficiency, and oil release rate. By varying CS concentration, the EO encapsulation efficiency could be adjusted, reaching 98.2% with 0.5% of CS. PS of the microcapsules was significantly decreased by increasing the emulsification speed. In addition, the thermal pretreatment of chitosan microcapsules (at 80 °C), could successfully control the EO release due to chitosan wall membrane shrinkages with subsequent change in pore size between CS molecules (Hsieh et al. 2006).

Using the same system, coriander EO, isolated from *Coriandrum sativum* L., was loaded into crayfish (*Astacus leptodactylus* Esch.) based CS microcapsules and was investigated by Duman and Kaya (2016) for its antioxidant and antimicrobial

effects (Duman and Kaya 2016). CS was obtained from *A. leptodactylus* using spray-drying technique. Results of the study showed that the prepared microcapsules had PS between 400 nm and 7 μ m with optimum swelling behavior at pH 2.2. The *in vitro* release study revealed a bi-phasic release pattern, which is sustained for 72 hours. The antimicrobial and antioxidant activities of EO-loaded microcapsules exhibited superior results than the oil and pure CS, which favors its use in the food and pharmaceutical industries.

In a recent study, Ngamekaue and Chitprasert (2019) investigated the microencapsulation of holy basil essential oil into gelatin microcapsules that was coated with composite emulsion formed from beeswax (1, 2, and 3%) and carboxymethyl cellulose (CMC) (Ngamekaue and Chitprasert 2019). The aim was to increase the oil stability and enhance its antioxidant and antimicrobial effects suitable for oral administration. The prepared microcapsules showed nearly spherical shape except the 3% beeswax-CMC coated microcapsules that showed irregularities, which is attributed to highly viscous coating solution. Results of surface oil content assay, which reflects the amount of non-encapsulated oil, revealed the enhanced stability of beeswax-CMC coated microcapsules (3 \times 1 \times 2%) in comparison to uncoated ones for 3 months. Only, the 2% beeswax-CMC coated microcapsule showed antioxidant activity. However, all microcapsules showed good antibacterial activities over 3 months. *In vitro* release results in simulated intestinal fluid demonstrated the ability of the 2% beeswax-CMC-coated microcapsule to control and delay the EO release in alkaline pH. Thus, the use of beeswax-CMC-coated microcapsule could efficiently delay the EO release until the distal region of the small intestine, the site of bacterial infection action, which is the distal region of the small intestine.

Polymeric Nanoparticles

Polymeric nanoparticles (NPs) have attracted great interest as delivery system for encapsulating wide range of drugs including proteins and peptides due to their ability to control drug release and target organs and tissue (Hamdi et al. 2020; Elmowafy et al. 2019d). Polymeric nanoparticles include nanospheres and nanocapsules depending on the method of preparation. Dispersion of the drug within the polymeric matrix yield nanospheres, while in nanocapsules, the drug is entrapped in the core surrounded by the polymeric membrane (Soppimath et al. 2001).

One of the earliest trials of encapsulating EO into polymeric NPs was based on the encapsulation of carvacrol using simple two steps method, which involved the formation of oil-in water emulsion followed by ionic gelation of CS with pentasodium tripolyphosphate (TPP). The oil-loaded NPs were spherical, small-sized, positively charged with EO encapsulation efficiency and loading capacity ranged from 14–31% and 3–21%, respectively. The presence of EO resulted in decreasing surface charge. CS-NPs enabled carvacrol-pH-dependent release (highest amount in acetate buffer with pH 3 > in phosphate buffer with pH 11 > in phosphate buffer with pH 7), with sustained release for 30 days. CS-NPs enhanced EO antimicrobial

effect against *S. aureus*, *Bacillus cereus*, and *E. coli* with an MIC of 0.257 mg/ml (Keawchaoon and Yoksan 2011).

Another study investigated the ability of CS-NPs to encapsulate oregano oil, which is extracted from *Origanum vulgare*, using TPP as a cross-linking agent (Hosseini et al. 2013). Oregano oil is characterized by its antimicrobial and antioxidant properties, which are mainly attributed to its main two phenols, namely carvacrol and thymol (Rota et al. 2004). The fabricated oil-loaded NPs had PS less than 400 nm, with encapsulation efficiency and loading capacity ranging from 21–47% and 3–8%, respectively. The oil-loaded NPs showed an initial burst effect with further slower release, which ensures controlled release (Hosseini et al. 2013). The authors recommend future studies regarding the use of this drug delivery system with other oils.

With the aim of improving the loading of EOs into NPs, Esmaeili and Asgari (2015) successfully encapsulated *Carum copticum* L. essential oil (CEO) into CS-NPs cross-linked with TPP, which could maintain its antioxidant activity and improve its antimicrobial activity (Esmaeili and Asgari 2015). The CEO-loaded NPs were spherical in shape with PS less than 80 nm. In addition, they exhibited an initial burst release, followed by pH-dependent sustained release behavior release rate (acidic pH > basic or neutral pH) that could enable its application in pharmaceutical uses. The CEO was able to retain its antioxidant and antimicrobial properties after encapsulation as investigated by the DPPH and agar disk diffusion methods, respectively. In conclusion, CEO encapsulation into NPs could improve its quality.

More recently, Matshetshe et al. (2018) applied synthesized binary system of β -cyclodextrin modified CS NPs using ionic gelation technique to improve *Cinnamomum zeylanicum* L. (Darchini) essential oil stability and reduce its volatility via encapsulation technique (Matshetshe et al. 2018). The process resulted in positively charged spherical particles with high EO encapsulation efficiency (> 50%), which were able to control and sustain EO release over 120 h when compared to single system of EO-loaded CS-NPs. The proposed system could enhance the therapeutic effects of EO (including its antimicrobial, antioxidant, antispasmodic, antiseptic, analgesics, and anti-carcinogenic effect), prolong its shelf life thus, and improve patient compliance.

Other synthetic polymers have been also utilized in the encapsulation of EOs, owing to their biodegradability and non-toxicity. In this context, Liakos et al. (2016) combined cellulose acetate (CA) polymer with lemongrass oil to form nanocapsules, where the oil takes part in the formation of nanocapsules as a surfactant and stabilizer, which is attributed to its structure (Liakos et al. 2016). Results revealed the formation of small spherical stable nanocapsules. Analysis of the formed nanocapsules confirmed the presence of lemongrass oil nanopores within the CA matrix, in addition to the formation of stable chemical bond between CA and EO. Bioadhesion and antimicrobial studies demonstrated the ability of EO-loaded nanocapsules to adhere to mucous membranes and to inhibit *E. coli* and *S. aureus* growth at low concentrations.

Furthermore, Liakos et al. (2018) extended their research to prepare cellulose acetate nanocapsules (CA-NCs) loaded with one of three oils (peppermint,

cinnamon, and lemongrass) (Liakos et al. 2018). Cinnamon CA-NCs were smaller in size than peppermint and lemongrass CA-NCs, which is attributed to the steric hindrance effect of the single molecule cinnamaldehyde that exists in cinnamon EO. Controversy, the other two oils are characterized by the existence of more than one molecule, such as aldehyde and ketone molecules, that are bonded to CA resulting in larger CA-NCs. The order of the oil-loading capacity CA-NCs was lemongrass > peppermint > cinnamon. All CA-NCs were characterized by low *in vitro* cytotoxicity against cultured diploid human cells, in addition to, good antimicrobial activity against *S. aureus*, *P. aeruginosa*, *E. coli*, and *C. albicans*, which favors the use of EO-loaded CA-NCs in medical and pharmaceutical applications for preventing microbial resistance.

Micelles

Micelles are colloidal dispersions that are formed from the self-assembly of amphiphilic molecules (such as surfactants and amphiphilic copolymers) in water above their critical micelle concentration (Elezaby et al. 2017). They are characterized by a hydrophobic core that can entrap hydrophobic drugs and stabilized by the hydrophilic tail (Narang et al. 2007; Elmowafy et al. 2019c). In this regard, the large differences in the hydrophilic–lipophilic balance values of poloxamers (Pluronic®) surfactants, in addition to their safety profile and their ability to solubilize lipophilic drugs, enable their use in the preparation of micelles for drug delivery applications (Paoli et al. 2010; Elmowafy et al. 2019c). In this regard, Ganguly et al. (2020) investigated the effect of tea tree EO addition as an excipient on the morphology and the structure of the Pluronic P123® and Pluronic F127® mixed micelles in antimicrobial and wound healing applications. Results revealed the transition of mixed micelles shape from spherical-to-worm-like micelles to vesicular structural transitions due to the addition of tea tree oil. The antimicrobial activity of tea tree oil against *E. coli* was maintained in pure and mixed micelles. In contrast, its antibacterial activity against Gram-positive *S. aureus* bacteria was maintained only with tea tree solubilized P123 solution (less active with micelles formed from F127 solution and 50:50 mixture of P123 and F127). In addition, results revealed the superior effect of pluronic P123 as carrier for antimicrobial agent, whereas pluronic F127 based liquid crystalline hydrogel showed superiority over P123 as a topical drug delivery matrix because of its better wound-healing abilities based on rheological properties (Ganguly et al. 2020).

During this struggle to overcome bacterial biofilm associated with high bacterial resistance to conventional antimicrobials, Wang et al. (2019) fabricated modified CS micelles loaded with thymol oil (T-TCP) to achieve light controllable release of the antimicrobial agent to the biofilm. The micelles were prepared via self-assembly by an amphiphilic copolymer consisted of toluidine blue O grafted chitosan and poly(propylene sulfide). Toluidine blue O, a well-known reactive oxygen species (ROS) generator, is widely used as a light-activated antimicrobial photosensitizer. The ability of T-TCP to bind the bacterial biofilm was assigned to the electrostatic

interaction between the CS cationic groups and the anionic biofilm surface, resulting in variation biofilm membrane permeability and its barrier function. The T-TCP micelles biofilm binding capacity was confirmed using fluorescence detection. The EO-loaded micelles were spherical, positively charged of 45.3 mV, due to the amine groups of CS on their surface, with a thymol-loading content and encapsulation efficiency of 9.7% (w/w) and 30.8%, respectively. Irradiation of fluorescein-loaded TCP for 5 min resulted in 70% of EO release after 30 h, which are three times higher than the percent released without irradiation (20%). Upon irradiation of the T-TCP micelles, a high concentration of ROS was produced, which resulted in two effects, the first one is causing micelles disassembly (as confirmed by scanning electron microscopy that stimulate thymol release) and the second one is the disturbance of the biofilm that is allocated to its inherent bactericidal effect. Antibiofilm activity revealed the successful eradication of *L. monocytogenes* and *S. aureus* using EO-loaded T-TCP after 24 h treatment, in contrast to thymol oil alone, which showed weak effect. The synergistic antimicrobial effect generated from the investigated system could augment its future use a topical disinfectant (Wang et al. 2019)

Casting Films and Nanofiber Mats

Casting films and nanofibers (NFs) mats have been widely investigated as promising advanced dressings for wound and burns care and management owing to their beneficial properties. They protect wound and burns from the external environment which reduces pain and bacterial infections, enhances wound healing and skin repairing. The ideal material for fabricating casting films and NFs fabricated should be biodegradable, biocompatible, with antimicrobial effect. In addition impregnation of these delivery systems with EOs could augment their healing power due to their antibacterial and anti-inflammatory activities. Natural polymers have been extensively employed in the fabrication of casting films. Among natural polymers, CS has been extensively utilized, by many researchers, in the field of wound care management via the formation of robust films with noticeable mechanical and physical properties. In particular, CS films fortified with EO have been well-documented, possessing significant antimicrobial activities for pharmaceutical and food packaging applications (Shojaee-Aliabadi et al. 2014; Li et al. 2019). CS was found to interact with EO, mainly by hydrogen bonding (Mayachiew et al. 2010), however, the weak nature of hydrogen bonds affected greatly EO retention and release characteristics, necessitating adding other beneficial substances, such as emulsifiers for efficient EO dispersion and homogenous film solution formation and other polymers for film properties modulation (i.e., composite films) (Hromiš et al. 2015; Perdones et al. 2016; Xu et al. 2018).

In this context, Altiok et al. (2010) developed CS films loaded with thyme oil by dropping ethanolic solution of EO into CS film forming solution and evaluated their antimicrobial and antioxidant activities (Altiok et al. 2010). The growth inhibition effects of the prepared films were tested against both Gram-negative (*E. coli*, *K. pneumoniae*, *P. aeruginosa*) and Gram-positive (*S. aureus*) microorganisms. EO

concentration of 1.2% (v/v) was found to be the minimum concentration with the highest antioxidant and antibacterial activities. Another study conducted by Sugumar and his team (2015) investigated CS films enriched with eucalyptus oil (*Eucalyptus globulus*) nanoemulsion for their physicochemical and antibacterial characteristics (Sugumar et al. 2015). The authors, first, optimized preparation conditions parameters to form stable nanoemulsion, containing 1, 3, 5% EO of 9.4 nm using Tween 80® and then impregnating it into CS film-forming solution. The nanoemulsion-impregnated CS film exhibited higher antibacterial efficacy against *S. aureus* than CS film (7–15 mm inhibition zones versus 7 mm respectively). The results indicated the bactericidal potential of 5% emulsion-impregnated films.

In another related recent study, CS films were fabricated by Pereira dos Santos et al. (2019), incorporating clove and melaleuca essential oil emulsions (≈ 100 nm) (Pereira Dos Santos et al. 2019). The antimicrobial assay was conducted against Gram-positive bacteria (*S. aureus*), Gram-negative bacteria (*E. coli*), and fungi (*C. albicans*). *In vitro* antimicrobial results of the robust films demonstrated that clove EO offered higher inhibition against the tested microorganisms than that of melaleuca essential oil.

In order to improve CS retention and release properties of essential oil from CS films, other additives, such as gum acacia and PVA, had been combined with CS, forming composite films. More recently, Amalraj and his research group (2020) fabricated composite CS films as efficient matrices in pharmaceutical field and food industry (Amalraj et al. 2020). Composite films, made of PVA, gum Arabic and CS were designed for black pepper and ginger EOs incorporation. Homogenization was performed to ensure EO dispersion in film-forming solution. The loading of the two oils in the fabricated films reduced the swelling and the water solubility of the films and increased their thickness, compactness, resistance to breakage, and flexibility that give good properties for their use in food packaging and wound-dressing applications. Oil release from the films was biphasic with an initial burst effect followed by slow release for 48 h. Better retention and release rates were observed with black pepper incorporated composite films compared to ginger incorporated films. Interestingly, the fabricated films were capable of inhibiting growth of the four strains used in this work (*Bacillus cereus*, *S. aureus*, *E. coli*, and *S. typhimurium*), showing greater inhibition zones for black pepper incorporated films than that of ginger incorporated ones (16.82–20.43 mm and 14.59–17.83 mm inhibition zones, respectively, for the tested strains).

Likewise, outstanding antimicrobial efficacy of EO incorporated CS films, other natural polymers-based films have been fabricated and thoroughly investigated. For instance, composite films of sodium alginate (NaAlg) matrix were attempted by Liakos et al. (2014). Nine EOs were incorporated inside the matrices, which are elicriso italic, chamomile blue, cinnamon, lavender, tea tree, peppermint, eucalyptus, lemongrass, and lemon oils. A surfactant, Igepal® CO-520, was added to facilitate the dispersion of EOs in the NaAlg matrix. Evidenced antibacterial (against *E. coli*) and antifungal (against *C. albicans*) effects were realized, depending on the type and concentration of the investigated EO. The obtained results demonstrated the capability of all studied EOs to inhibit *C. albicans* growth except chamomile

blue EO. In addition, out of the investigated EOs, only cinnamon, lemongrass, and peppermint oils showed antibacterial activity against *E. coli*.

The promising characteristic of gelatin motivated the researchers to develop hydrogel cross-linked films based on gelatin alone or in combination with other polymers. Kavooosi and his research team (2013) prepared cross-linked gelatin films containing thymol EO using glutaraldehyde and glycerol as cross-linking agent and plasticizer, respectively (Kavooosi et al. 2013). The authors reported excellent thymol concentration dependent antioxidant and antibacterial capacities of the investigated films against *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa*. Both disk diffusion and viable colony counting assays indicated that films incorporated with 8% thymol showed inhibition zones > 1 mm and high percentages of bacterial growth reduction, approaching 100%, respectively. Such obtained results evidenced greater antibacterial activity against Gram-positive bacteria than that against Gram-negative bacteria.

In continuation of the previous study and based on strong chemical interaction between gelatin and PVA, Kavooosi et al. (2017) added PVA to gelatin film forming solution, forming gelatin/PVA cross-linked films containing *Zataria multiflora* Boiss essential oil (Kavooosi et al. 2017). Potent antioxidant and antibacterial capacities, tested on the four aforementioned bacteria, were revealed for EO-incorporated gelatin/PVA composite films compared to gelatin and PVA counterparts. According to the reported results of antibacterial assay, MIC and MBC were the least for films prepared using gelatin/PVA blend, where *B. subtilis* was the most susceptible bacteria, while *P. aeruginosa* was the most resistant bacteria.

These previously reported studies highlighted the potential of using EO incorporated casting films for wound-healing applications. However, the safety of the prepared films and further wound healing studies for practical applicability were not performed in such works. To evaluate the *in vitro* biocompatibility and proliferation of EO-enriched films, Güneş and Tihminlioğlu (2017) prepared CS films containing *Hypericum perforatum* L. essential oil, using Tween® 80 for EO dispersion (Güneş and Tihminlioğlu 2017). In addition to the investigation of antibacterial capacity against *E. coli* and *S. aureus*, *in vitro* biocompatibility and proliferation studies were performed in NIH/3 T3 mouse fibroblasts cell lines. Furthermore, elucidation of attached cells on CS films and their visualization was conducted using fluorescence and scanning electron microscopes, respectively. *E. coli* showed higher sensitivity to the prepared film than *S. aureus* as manifested by EO concentration-dependent inhibition zones (with respective values of 24.7–29 and 142–197 mm). Nearly 100% cell viability was obtained for EO-loaded films at concentrations ranging from 0.25 to 1.5%) at all incubation periods (24, 48, and 72 hours). Besides, successful cell–CS interaction, and hence cell attachment and proliferation were confirmed.

Interestingly, the aforementioned parameters and *in vivo* wound healing assay were focused on by many researchers in their electrospun NF-based research articles as will be manipulated in the following section. Electrospinning process is a recent technique for drug encapsulation into nanocarriers, which provides a benefit of the absence of heat that helps in protecting the encapsulated oil from degradation. In addition, electrospun nanocarriers are characterized by high surface area and

high safety in comparison to nanocarriers produced by other techniques (Wen et al. 2017). Both natural, synthetic polymers and their combinations have been utilized in the fabrication of electrospun NFs. In particular, CS-based NF mats have been proposed by numerous researchers, however, other synthetic polymers were combined with CS to improve its poor electrospinnability and assist NF fabrication with better mechanical strength.

In light, Ardekani et al. (2019) fabricated chitosan/poly(vinyl alcohol)/gelatin (CS/PVA/Gel) solutions NF mats fortified with *Zataria multiflora* Boiss EO and evaluated their mechanical, antimicrobial, and biocompatible properties (Ardekani et al. 2019). The GC-MS results revealed that thymol was the main component of the investigated oil. According to the antimicrobial assay, EO possessed similar MIC and MBC values of 2–4 $\mu\text{L}/\text{mL}$ for *P. aeruginosa* and *S. aureus*. While MIC and minimum fungicidal concentration of EO for *C. albicans* were found to be 0.062 and 0.5 $\mu\text{L}/\text{mL}$, respectively, highlighting bactericidal, fungistatic, and fungicidal potential of the investigated EO. Incorporating EO into NF mats presented considerable blocking of bacterial and fungal growth, showing complete reduction of *C. albicans* colonies at all studied EO concentrations (2, 5, and 10%) and bacterial colonies at all used EO concentrations except 2% (\hat{c} 90% reduction rate). As for cytotoxicity, mouse fibroblast (L929) cells exhibited excellent tolerance to the prepared EO-loaded NF mats, irrespective of EO concentration (% cell viability range: 91–106%).

In a related study, Mouro et al. (2019) utilized emulsion electrospinning (needle-free nanospider) for the development of eugenol incorporated NF mats, combining poly-caprolactone (PCL) and PVA with CS (Mouro et al. 2019). According to the results of *in vitro* studies, controlled EO release for up to 120 hours, potent bactericidal and bacteriostatic activities against *S. aureus* and *P. aeruginosa*, showing high percentages of bacterial reduction (\hat{c} 80%), and prominent viability of normal human dermal fibroblasts (NHDF) over a period of 7 days were realized.

Similarly, for ease of electrospinning, alginate natural polymer was combined with polyethylene oxide and Pluronic F127® to form lavender EO enriched dual-function NF mats (Hajiali et al. 2016). The proposed system was evaluated for both anti-inflammatory and antimicrobial capacities, pro-inflammatory cytokine expression, and cytotoxicity using HFF-1 cells. Furthermore, *in vivo* study on midrange ultraviolet radiation (UVB) induced skin inflammation was carried out using commercially available alginate-based dressing (Tegaderm™) for a comparative purpose. EO incorporated NF mats were capable of blocking growth of *S. aureus* bacteria with an inhibition zone of 2.17 mm and *in vitro* and *in vivo* production of pro-inflammatory cytokines. *In vitro* biocompatibility confirmed non-cytotoxicity of the prepared constructs. *In vivo* results evidenced greater inhibitory potential of the prepared NF mats on cytokine production than that of Tegaderm™ following UVB exposure in the first 6 hours. However, alginate NF dressings are not mechanically strong and need further study to clarify their potential for use in treating deep burns and severe wounds.

On the account of their noticeable electrospinnability, synthetic polymers-based NF mats have been developed as efficient robust constructs with satisfactory better

mechanical properties compared with natural polymers (Bhattarai et al. 2018; Tiwari et al. 2018). The commonly used synthetic polymers in electrospinning are poly caprolactone (PCL), poly-lactic acid (PLA), poly(lactic-co-glycolic acid) (PLGA), polyurethane, etc. EO has been enclosed in an assortment of synthetic polymers-based NFs for wound-healing application.

For instance, Karami and his research group (Karami et al. 2013) prepared thymol-loaded electrospun NF mats based on PCL, PLA, and their 50/50 blend (Karami et al. 2013). Antibacterial and *in vivo* wound healing, using the commercially available wound ulcer dressing Comfeel® Plus, and gauze bandages as positive and negative controls, respectively, in addition to histological evaluations were performed. As for antimicrobial assay, 50/50 PCL/PLA hybrid NF mats encasing thymol possessed greater inhibition zone against *S. aureus* than that against *E. coli* with respective values of 10.4 and 7.8 mm. The *in vivo* results demonstrated considerable wound repair effect as manifested by marked higher percentage of wound-closure at 14 days post-treatment compared with those of the investigated Comfeel® Plus, and gauze bandages controls (respective values of 92.4, 87, and 68%).

In another study, Unalan et al. (2019a, 2019b) utilized PCL in the fabrication of electrospun NF mats enclosing peppermint essential oil with micrometric dimensions ($1.0 \pm 0.2 \mu\text{m}$) for wound-healing applications (Unalan et al. 2019b). Antibacterial and biocompatibility investigations were carried out. The obtained results revealed marked EO concentration-dependent reduction in viability of both *S. aureus* and *E. coli* bacteria, indicating potent antimicrobial activity. Besides, safety of the prepared constructs was proven as manifested by 100% viability of normal human dermal fibroblast (NHDF) cells.

In continuation of this previous study, Unalan et al. (2019a, 2019b) proposed NF mats, based on blending gelatin with PCL, having smaller diameters in nano-scale (305 nm) (Unalan et al. 2019a). Clove EO was incorporated in the composite NF mats using glacial acetic acid as a “benign” (non-toxic) solvent. In addition to the antimicrobial and cell viability assays performed in the previous work, *in vitro* wound healing study was conducted using NHDF cells to assess the migration of cells into the wound site and the rate of wound closure. As expected, the proposed systems possessed potent antibacterial activity against *S. aureus* and *E. coli* and non-cytotoxicity against normal human dermal fibroblast (NHDF) cells. As for *in vitro* wound-healing analysis, EO demonstrated concentration-dependent inhibition of NHDF cell migration and proliferation and increment of wound-closure rate, reaching 86% at the highest investigated EO dose.

Recently, Abdollahi et al. (2020) presented novel PCL-based NF mats, impregnated with nanogel containing *Citrus sinensis* L. Osbeck EO for wound-healing application (Abdollahi et al. 2020). As ascribed by the authors, nanogel, prepared by adding carbomer 940 to EO loaded nanoemulsion, was utilized to ensure ease of topical applicability and block entry of pathogens into the wound area. Interestingly, the proposed newly developed system exhibited nearly 0% growth of four human bacterial strains (*E. coli*, *Klebsiella pneumonia*, *P. aeruginosa*, and *S. aureus*).

PCL-NF loaded with carvacrol, thymol, tyrosol, and squalene and formulated into patches were also evaluated for their anti-inflammatory properties. The study

demonstrated the superiority of thymol-loaded patches in reducing the inflammatory response in comparison to tyrosol patches and the combination patch containing tyrosol and thymol. The obtained results proposed the possible use of thymol-loaded patches as a dressing with both bactericidal and anti-inflammatory properties for wound healing (García-Salinas et al. 2020).

Some researchers have introduced EO-enriched NF mats based on other synthetic polymers, finding their application in wound repair and skin injury. For instance, polyacrylonitrile (PAN) has been addressed in the preparation of NF mats containing lavender (Balasubramanian and Kodam 2014). NaCl solution was added to polymer solution, in order to enhance its electrospinnability. The results of *in vitro* antibacterial activity and cytotoxicity, using mouse fibroblast NIH/3 T3, demonstrated sustained bactericidal effect against *S. aureus* and *Klebsiella pneumoniae* over 30 days (inhibition zones of 14–15 mm) and 90–100% cell viability over 48 hours, respectively. However, the brittle nature of polyacrylonitrile NF seemed to negatively affect the application of NF to the wound area.

Cellulose acetate-based NF mats have been also attempted by Liakos et al. (2015) for the incorporation of three EOs: cinnamon, lemongrass, and peppermint (Liakos et al. 2015). The prepared NF mats showed considerable blocking of *E. coli* growth; however, they were not effective in inhibiting fungal growth which was ascribed by the authors to the larger diameter of *C. albicans* than that of *E. coli*, hindering their penetration into NF meshes. High viability of immortalized fibroblasts (NIH/3 T3-cells) and normal human keratinocytes (HaCaT cells), irrespective of EO type and concentration, was also evidenced.

In a recent work conducted by Sofi et al. (2019), polyurethane polymer was used in the fabrication of composite NF mats enclosing lavender and silver NPs for wound care management (Sofi et al. 2019). As expected, a synergistic bactericidal attributes of both lavender and silver NPs, used at concentrations 15 and 5% respectively, was demonstrated against *E. coli* and *S. aureus* (respective inhibition zones are 16.2 and 5.9 mm). Besides, high viability, up to the limiting concentration of 15% EO/5% silver NPs, proliferation and attachment of chicken embryo fibroblasts on NF mats were revealed, providing better cellular support and facilitating fibroblasts growth.

Despite the prominent competency of NF mats as a practical alternative to the conventional wound-dressing materials, they suffered from improper wound application, and poor accommodation of wounds having irregular shape, causing negative consequences of secondary wound injury. To tackle these limitations, direct deposition of NF mats onto the wound area could be achieved via *in situ* electrospinning, conducted using portable electrospinning devices (Yan et al. 2016; Yan et al. 2019; Dong et al. 2020).

In this regard, Liu et al. (2019) proposed zein NF mats enclosing thyme EO using *in situ* electrospinning technique (Liu et al. 2019). They achieved proper *in situ* deposition onto the wound area resulted in significant inhibition of growth of the investigated bacterial strains *E. coli* and *S. aureus*, preventing wound infection, and promoting wound healing. Interestingly, the prepared NF demonstrated superhydrophilicity, ensuring the capability of meshes to absorb wound exudates. Furthermore,

complete wound healing was achieved after 11 days. The same authors continued their research work encasing clove EO in zein NF mats and evaluated the prepared meshes in terms of antibacterial and *in vivo* wound healing experiments (Qin et al. 2020). The authors examined also fibroblasts biocompatibility of as-spun NF mats. The obtained results, as anticipated, confirmed beneficial effects of the proposed system in terms of bactericidal effect, super hydrophilicity, promotion of wound healing, and non-cytotoxicity (>90% fibroblasts viability).

In another study, Lamarra et al. (2020) investigated the encapsulation of cabreuva oil into CS-crosslinked NPs embedded into PVA-based NF formed by electrospinning process (Lamarra et al. 2020). The obtained system provided a controlled release pattern of the encapsulated oil with antimicrobial activity against *C. albicans*, *S. aureus*, *S. epidermidis*, *E. coli*, *S. aureus*). Therefore, cabreuva oil-loaded NF could serve as a controlled release matrix or as a tissue-engineering scaffold.

4 Conclusions and Perspectives

At the present, availing the antimicrobial performance of EOs is boosting the pharmaceutical community to their vehiculation, to overcome their poor physico-chemical properties and industrial technology transfer, producing natural safe remedies. However, prior to such green industry, the researchers should take into consideration that fabrication of formulated EO remedies contains several inactive ingredients that might influence physico-chemical properties and safety of EOs. Hence, proper investigation of several issues related to long-term stability of the natural products and their toxicological profile and adverse profile on animals and human should be carried out. In addition, clinical efficacies and pharmacotherapeutic treatments need to be verified. Considering these issues, EOs marketed products would soon follow to treat a variety of topical and systemic diseases.

References

- Abdollahi A, Zarenezhad E, Osanloo M, Ghaznavi G, Khalili Pour M (2020) Promising antibacterial activity of a mat of polycaprolactone nanofibers impregnated with a green nanogel. *Nanomed Res J* 5:192–201
- Aboul-Ela EI (2002) Cytogenetic studies on *Nigella sativa* seeds extract and thymoquinone on mouse cells infected with schistosomiasis using karyotyping. *Mutat Res* 516:11–17
- Adlard ER (2010) Handbook of essential oils. Science, technology and applications. *Chromatographia* 72:1021–1021
- Ahmadi F, Sadeghi S, Modarresi M, Abiri R, Mikaeli A (2010) Chemical composition, in vitro anti-microbial, antifungal and antioxidant activities of the essential oil and methanolic extract of *Hymenocater longiflorus* Benth., of Iran. *Food Chem Toxicol* 48:1137–1144
- Al-Haj NA, Shamsudin M, Alipiah N, Zamri H, Ahmad B, Siddig I, Rasedee A (2010) Characterization of *Nigella sativa* L. essential oil-loaded solid lipid nanoparticles. *Am J Pharmacol Toxicol* 5:52–57

- Altıok D, Altıok E, Tihminlioglu F (2010) Physical, antibacterial and antioxidant properties of chitosan films incorporated with thyme oil for potential wound healing applications. *J Mater Sci Mater Med* 21:2227–2236
- Amalraj A, Haponiuk JT, Thomas S, Gopi S (2020) Preparation, characterization and antimicrobial activity of polyvinyl alcohol/gum arabic/chitosan composite films incorporated with black pepper essential oil and ginger essential oil. *Int J Biol Macromol* 151:366–375
- Ardekani NT, Khorram M, Zomorodian K, Yazdanpanah S, Veisi H, Veisi H (2019) Evaluation of electrospun poly (vinyl alcohol)-based nanofiber mats incorporated with *Zataria multiflora* essential oil as potential wound dressing. *Int J Biol Macromol* 125:743–750
- Asbahani AE, Miladi K, Badri W, Sala M, Addi EHA, Casabianca H, Mousadik AE, Hartmann D, Jilale A, Renaud FNR, Elaissari A (2015) Essential oils: from extraction to encapsulation. *Int J Pharm* 483:220–243
- Aveyard R, Binks BP, Clint JH (2003) Emulsions stabilised solely by colloidal particles. *Adv Colloid Interf Sci* 100-102:503–546
- Bajpai VK, Baek K-H, Kang SC (2012) Control of Salmonella in foods by using essential oils: a review. *Food Res Int* 45:722–734
- Balasubramani S, Rajendhiran T, Moola AK, Diana RKB (2017) Development of nanoemulsion from *Vitex negundo* L. essential oil and their efficacy of antioxidant, antimicrobial and larvicidal activities (*Aedes aegypti* L.). *Environ Sci Pollut Res Int* 24:15125–15133
- Balasubramanian K, Kodam KM (2014) Encapsulation of therapeutic lavender oil in an electrolyte assisted polyacrylonitrile nanofibres for antibacterial applications. *RSC Adv* 4:54892–54901
- Bassole IHN, Juliani HR (2012) Essential oils in combination and their antimicrobial properties. *Molecules* 17:3989–4006
- Bendahou M, Muselli A, Grignon-Dubois M, Benyoucef M, Desjobert J-M, Bernardini A-F, Costa J (2008) Antimicrobial activity and chemical composition of *Origanum glandulosum* Desf. essential oil and extract obtained by microwave extraction: comparison with hydrodistillation. *Food Chem* 106:132–139
- Bhattarai DP, Aguilar LE, Park CH, Kim CS (2018) A review on properties of natural and synthetic based electrospun fibrous materials for bone tissue engineering. *Membranes* 8:62
- Bhattarai N, Gunn J, Zhang M (2010) Chitosan-based hydrogels for controlled, localized drug delivery. *Adv Drug Deliv Rev* 62:83–99
- Bilia AR, Guccione C, Isacchi B, Righeschi C, Firenzuoli F, Bergonzi MC (2014) Essential oils loaded in nanosystems: a developing strategy for a successful therapeutic approach. *Evid Based Compl Alternat Med* 2014:651593
- Binks BP (2002) Particles as surfactants—similarities and differences. *Curr Opin Colloid Interface Sci* 7:21–41
- Boccalon E, Pica M, Romani A, Casciola M, Sterflinger K, Pietrella D, Nocchetti M (2020) Facile preparation of organic-inorganic hydrogels containing silver or essential oil with antimicrobial effects. *Appl Clay Sci* 190:105567
- Bonferoni MC, Sandri G, Rossi S, Usai D, Liakos I, Garzoni A, Fiamma M, Zanetti S, Athanassiou A, Caramella C, Ferrari F (2017) A novel ionic amphiphilic chitosan derivative as a stabilizer of nanoemulsions: improvement of antimicrobial activity of *Cymbopogon citratus* essential oil. *Colloids Surf B: Biointerfaces* 152:385–392
- Boyras N, Özcan M (2006) Inhibition of phytopathogenic fungi by essential oil, hydrosol, ground material and extract of summer savory (*Satureja hortensis* L.) growing wild in Turkey. *Int J Food Microbiol* 107:238–242
- Bozin B, Mimica-Dukic N, Simin N, Anackov G (2006) Characterization of the volatile composition of essential oils of some Lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils. *J Agric Food Chem* 54:1822–1828
- Braga PC, Culici M, Alfieri M, Dal Sasso M (2008) Thymol inhibits *Candida albicans* biofilm formation and mature biofilm. *Int J Antimicrob Agents* 31:472–477
- Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods—a review. *Int J Food Microbiol* 94:223–253

- Calo JR, Crandall PG, O'bryan CA, Ricke SC (2015) Essential oils as antimicrobials in food systems – a review. *Food Control* 54:111–119
- Calsamiglia S, Busquet M, Cardozo PW, Castillejos L, Ferret A (2007) Invited review: essential oils as modifiers of rumen microbial fermentation. *J Dairy Sci* 90:2580–2595
- Carbone C, Teixeira MDC, Sousa MDC, Martins-Gomes C, Silva AM, Souto EMB, Musumeci T (2019) Clotrimazole-loaded mediterranean essential oils NLC: a synergic treatment of *Candida* skin infections. *Pharmaceutics* 11:231
- Carneiro De Barros J, Lúcia Da Conceição M, Gomes Neto NJ, Vieira Da Costa AC, Siqueira Júnior JP, Basílio Junior ID, Leite De Souza E (2009) Interference of *Origanum vulgare* L. essential oil on the growth and some physiological characteristics of *Staphylococcus aureus* strains isolated from foods. *LWT – Food Sci Technol* 42:1139–1143
- Carson CF, Mee BJ, Riley TV (2002) Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. *Antimicrob Agents Chemother* 46:1914–1920
- Castangia I, Manca ML, Caddeo C, Maxia A, Murgia S, Pons R, Demurtas D, Pando D, Falconieri D, Peris JE, Fadda AM, Manconi M (2015) Faceted phospholipid vesicles tailored for the delivery of *Santolina insularis* essential oil to the skin. *Colloids Surf B: Biointerfaces* 132:185–193
- Cavar S, Maksimovic M, Solic ME, Jerkovic-Mujkic A, Besta R (2008) Chemical composition and antioxidant and antimicrobial activity of two *Satureja* essential oils. *Food Chem* 111:648–653
- Chakraborty S, Shukla D, Mishra B, Singh S (2009) Lipid – an emerging platform for oral delivery of drugs with poor bioavailability. *Eur J Pharm Biopharm* 73:1–15
- Chang X, Heene E, Qiao F, Nick P (2011) The phytoalexin resveratrol regulates the initiation of hypersensitive cell death in *Vitis* cell. *PLoS One* 6:e26405
- Chao SC, Young DG, Oberg CJ (2000) Screening for inhibitory activity of essential oils on selected bacteria, fungi and viruses. *J Essent Oil Res* 12:639–649
- Chevalier Y, Bolzinger M-A (2013) Emulsions stabilized with solid nanoparticles: pickering emulsions. *Colloids Surf A Physicochem Eng Asp* 439:23–34
- Chiappisi L, Noirez L, Gradzielski M (2016) A journey through the phase diagram of a pharmaceutically relevant microemulsion system. *J Colloid Interface Sci* 473:52–59
- Chorianopoulos N, Kalpoutzakis E, Aligiannis N, Mitaku S, Nychas G-J, Haroutounian SA (2004) Essential oils of *Satureja*, *Origanum*, and *thymus* species: chemical composition and antibacterial activities against foodborne pathogens. *J Agric Food Chem* 52:8261–8267
- Cimanga K, Kambu K, Tona L, Apers S, De Bruyne T, Hermans N, Totté J, Pieters L, Vlietinck AJ (2002) Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. *J Ethnopharmacol* 79:213–220
- Constantinides PP (1995) Lipid microemulsions for improving drug dissolution and oral absorption: physical and biopharmaceutical aspects. *Pharm Res* 12:1561–1572
- Cox SD, Mann CM, Markham JL, Bell HC, Gustafson JE, Warmington JR, Wyllie SG (2000) The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). *J Appl Microbiol* 88:170–175
- Danielsson I, Lindman B (1981) The definition of microemulsion. *Colloids Surf* 3:391–392
- Das S, Chaudhury A (2011) Recent advances in lipid nanoparticle formulations with solid matrix for oral drug delivery. *AAPS PharmSciTech* 12:62–76
- Date AA, Nagarsenker MS (2007) Design and evaluation of self-nanoemulsifying drug delivery systems (SNEDDS) for cefpodoxime proxetil. *Int J Pharm* 329:166–172
- Davidson PM (1997) Chemical preservatives and natural antimicrobial compounds. In: Doyle MP, Beuchat LR, Montville TJ (eds) *Food microbiology: fundamentals and frontiers*. ASM, Washington, pp 520–556
- De Oliveira TLC, De Araújo SR, Ramos EM, Das Graças Cardoso M, Alves E, Piccoli RH (2011) Antimicrobial activity of *Satureja montana* L. essential oil against *Clostridium perfringens* type A inoculated in mortadella-type sausages formulated with different levels of sodium nitrite. *Int J Food Microbiol* 144:546–555

- Deans SG, Ritchie G (1987) Antibacterial properties of plant essential oils. *Int J Food Microbiol* 5:165–180
- Denyer SP, Hugo WB (1991) Mechanisms of antibacterial action—a summary. In: Denyer SP, Hugo WB (eds) *Mechanisms of action of chemical biocides*. Blackwell, Oxford, pp 331–334
- Djenane D, Yangüela J, Montañés L, Djerbal M, Roncalés P (2011) Antimicrobial activity of *Pistacia lentiscus* and *Satureja montana* essential oils against *listeria monocytogenes* CECT 935 using laboratory media: efficacy and synergistic potential in minced beef. *Food Control* 22:1046–1053
- Dong W-H, Liu J-X, Mou X-J, Liu G-S, Huang X-W, Yan X, Ning X, Russell SJ, Long Y-Z (2020) Performance of polyvinyl pyrrolidone-isatis root antibacterial wound dressings produced in situ by handheld electrospinner. *Colloids Surf B: Biointerfaces* 188:110766
- Dorman HJ, Deans SG (2000) Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J Appl Microbiol* 88:308–316
- Drulis-Kawa Z, Dorotkiewicz-Jach A (2010) Liposomes as delivery systems for antibiotics. *Int J Pharm* 387:187–198
- Duman F, Kaya M (2016) Crayfish chitosan for microencapsulation of coriander (*Coriandrum sativum* L.) essential oil. *Int J Biol Macromol* 92:125–133
- Elaissi A, Rouis Z, Salem NA, Mabrouk S, Ben Salem Y, Salah KB, Aouni M, Farhat F, Chemli R, Harzallah-Skhirri F, Khouja ML (2012) Chemical composition of 8 eucalyptus species' essential oils and the evaluation of their antibacterial, antifungal and antiviral activities. *BMC Complement Altern Med* 12:81
- Elezaby RS, Gad HA, Metwally AA, Geneidi AS, Awad GA (2017) Self-assembled amphiphilic core-shell nanocarriers in line with the modern strategies for brain delivery. *J Control Release* 261:43–61
- Elmowafy E, Abdal-Hay A, Skouras A, Tiboni M, Casettari L, Guarino V (2019a) Polyhydroxyalkanoate (PHA): applications in drug delivery and tissue engineering. *Expert Rev Med Devices* 16:467–482
- Elmowafy E, Cespi M, Bonacucina G, Soliman ME (2019b) In situ composite ion-triggered gellan gum gel incorporating amino methacrylate copolymer microparticles: a therapeutic modality for buccal applicability. *Pharm Dev Technol* 24:1258–1271
- Elmowafy E, Gad H, Biondo F, Casettari L, Soliman ME (2019c) Exploring optimized methoxy poly (ethylene glycol)-block-poly (ϵ -caprolactone) crystalline cored micelles in anti-glaucoma pharmacotherapy. *Int J Pharm* 566:573–584
- Elmowafy E, Soliman ME (2019) Losartan-chitosan/dextran sulfate microplex as a carrier to lung therapeutics: dry powder inhalation, aerodynamic profile and pulmonary tolerability. *Int J Biol Macromol* 136:220–229
- Elmowafy EM, Tiboni M, Soliman ME (2019d) Biocompatibility, biodegradation and biomedical applications of poly (lactic acid)/poly (lactic-co-glycolic acid) micro and nanoparticles. *J Pharm Inves* 49:347–380
- Esmaeili A, Asgari A (2015) In vitro release and biological activities of *Carum copticum* essential oil (CEO) loaded chitosan nanoparticles. *Int J Biol Macromol* 81:283–290
- Falsetta ML, Klein MI, Colonne PM, Scott-Anne K, Gregoire S, Pai CH, Gonzalez-Begne M, Watson G, Krysan DJ, Bowen WH, Koo H (2014) Symbiotic relationship between *Streptococcus mutans* and *Candida albicans* synergizes virulence of plaque biofilms in vivo. *Infect Immun* 82:1968–1981
- Fanun M (2012) Microemulsions as delivery systems. *Curr Opin Colloid Interface Sci* 17:306–313
- Fazeli MR, Amin G, Attari MMA, Ashtiani H, Jamalifar H, Samadi N (2007) Antimicrobial activities of Iranian sumac and avishan-e shirazi (*Zataria multiflora*) against some food-borne bacteria. *Food Control* 18:646–649
- Fazly Bazzaz B, Khameneh B, Namazi N, Iranshahi M, Davoodi D, Golmohammadzadeh S (2018) Solid lipid nanoparticles carrying *Eugenia caryophyllata* essential oil: the novel nanoparticulate systems with broad-spectrum antimicrobial activity. *Lett App Microbiol* 66:506–513

- Fisher K, Phillips C (2009) The mechanism of action of a citrus oil blend against enterococcus faecium and enterococcus faecalis. *J Appl Microbiol* 106:1343–1349
- Friedly EC, Crandall PG, Ricke SC, Roman M, O'bryan C, Chalova VI (2009) In vitro Antilisterial effects of citrus oil fractions in combination with organic acids. *J Food Sci* 74:M67–M72
- Gad HA, Abd El-Rahman FA, Hamdy GM (2019) Chamomile oil loaded solid lipid nanoparticles: a naturally formulated remedy to enhance the wound healing. *J Drug Deliv Sci Technol* 50:329–338
- Gad HA, El-Nabarawi MA, Abd El-Hady SS (2008) Formulation and evaluation of secnidazole or doxycycline dento-oral gels. *Drug Dev Ind Pharm* 34:1356–1367
- Ganguly R, Kumar S, Tripathi A, Basu M, Verma G, Sarma HD, Chaudhari DP, Aswal VK, Melo JS (2020) Structural and therapeutic properties of Pluronic® P123/F127 micellar systems and their modulation by salt and essential oil. *J Mol Liq* 310:113231
- Garcia-Salinas S, Evangelopoulos M, Gámez-Herrera E, Arruebo M, Irusta S, Taraballi F, Mendoza G, Tasciotti E (2020) Electrospun anti-inflammatory patch loaded with essential oils for wound healing. *Int J Pharm* 577:119067
- Garg A, Singh S (2011) Enhancement in antifungal activity of eugenol in immunosuppressed rats through lipid nanocarriers. *Colloids Surf B: Biointerfaces* 87:280–288
- Garg A, Singh S (2014) Targeting of eugenol-loaded solid lipid nanoparticles to the epidermal layer of human skin. *Nanomedicine* 9:1223–1238
- Gharib R, Haydar S, Charcosset C, Fourmentin S, Greige-Gerges H (2019) First study on the release of a natural antimicrobial agent, estragole, from freeze-dried delivery systems based on cyclodextrins and liposomes. *J Drug Deliv Sci Technol* 52:794–802
- Gharibzahedi SMT (2017) Ultrasound-mediated nettle oil nanoemulsions stabilized by purified jujube polysaccharide: process optimization, microbial evaluation and physicochemical storage stability. *J Mol Liq* 234:240–248
- Gharibzahedi SMT, Mohammadnabi S (2016) Characterizing the novel surfactant-stabilized nanoemulsions of stinging nettle essential oil: thermal behaviour, storage stability, antimicrobial activity and bioaccessibility. *J Mol Liq* 224:1332–1340
- Ghodrati M, Farahpour MR, Hamishehkar H (2019) Encapsulation of Peppermint essential oil in nanostructured lipid carriers: in-vitro antibacterial activity and accelerative effect on infected wound healing. *Colloids Surf A Physicochem Eng Asp* 564:161–169
- Gill AO, Holley RA (2004) Mechanisms of bactericidal action of cinnamaldehyde against *Listeria monocytogenes* and of eugenol against *L. monocytogenes* and *Lactobacillus sakei*. *App Environ Microbiol* 70:5750–5755
- Gilles M, Zhao J, An M, Agboola S (2010) Chemical composition and antimicrobial properties of essential oils of three Australian Eucalyptus species. *Food Chem* 119:731–737
- Guinoiseau E, Luciani A, Rossi PG, Quilichini Y, Ternengo S, Bradesi P, Berti L (2010) Cellular effects induced by *Inula graveolens* and *Santolina corsica* essential oils on *Staphylococcus aureus*. *Eur J Clin Microbiol Infect Dis* 29:873–879
- Gull A, Ahmed S, Ahmad FJ, Nagaich U, Chandra A (2020) Hydrogel thickened microemulsion; a local cargo for the co- delivery of cinnamaldehyde and berberine to treat acne vulgaris. *J Drug Deliv Sci Technol* 58:101835
- Gulluce M, Sahin F, Sokmen M, Ozer H, Daferera D, Sokmen A, Polissiou M, Adiguzel A, Ozkan H (2007) Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp. *longifolia*. *Food Chem* 103:1449–1456
- Gumus T (2010) Determination of the changes of antifungal properties of *Satureja hortensis*, *Thymus vulgaris* and *Thymbra spicata* exposed to gamma irradiation. *Radiat Phys Chem* 79:109–114
- Güneş S, Tihminhoğlu F (2017) Hypericum perforatum incorporated chitosan films as potential bioactive wound dressing material. *Int J Biol Macromol* 102:933–943
- Gupta A, Eral HB, Hatton TA, Doyle PS (2016) Nanoemulsions: formation, properties and applications. *Soft Matter* 12:2826–2841

- Hajjali H, Summa M, Russo D, Armirotti A, Brunetti V, Bertorelli R, Athanassiou A, Mele E (2016) Alginate–lavender nanofibers with antibacterial and anti-inflammatory activity to effectively promote burn healing. *J Mater Chem B* 4:1686–1695
- Hamdi M, Abdel-Bar HM, Elmowafy E, Al-Jamal KT, Awad GA (2020) An integrated vitamin E-coated polymer hybrid nanoplatform: a lucrative option for an enhanced in vitro macrophage retention for an anti-hepatitis B therapeutic prospect. *PLoS One* 15:e0227231
- Hammoud Z, Gharib R, Fourmentin S, Elaissari A, Greige-Gerges H (2019) New findings on the incorporation of essential oil components into liposomes composed of lipoid S100 and cholesterol. *Int J Pharm* 561:161–170
- Hammoud Z, Gharib R, Fourmentin S, Elaissari A, Greige-Gerges H (2020) Drug-in-hydroxypropyl- β -cyclodextrin-in-lipoid S100/cholesterol liposomes: effect of the characteristics of essential oil components on their encapsulation and release. *Int J Pharm* 579:119151
- Hathout RM, Gad HA, Abdel-Hafez SM, Nasser N, Khalil N, Ateyya T, Amr A, Yasser N, Nasr S, Metwally AA (2019) Gelatinized core liposomes: a new Trojan horse for the development of a novel timolol maleate glaucoma medication. *Int J Pharm* 556:192–199
- Hathout RM, Gad HA, Metwally AA (2017) Gelatinized-core liposomes: toward a more robust carrier for hydrophilic molecules. *J Biomed Mater Res A* 105:3086
- Hazzit M, Baaliouamer A, Veríssimo AR, Faleiro ML, Miguel MG (2009) Chemical composition and biological activities of Algerian Thymus oils. *Food Chem* 116:714–721
- Hosseini SF, Zandi M, Rezaei M, Farahmandghavi F (2013) Two-step method for encapsulation of oregano essential oil in chitosan nanoparticles: preparation, characterization and in vitro release study. *Carbohydr Polym* 95:50–56
- Hromiš NM, Lazić VL, Markov SL, Vaštag ŽG, Popović SZ, Šuput DZ, Džinić NR, Velićanski AS, Popović LM (2015) Optimization of chitosan biofilm properties by addition of caraway essential oil and beeswax. *J Food Eng* 158:86–93
- Hsieh WC, Chang CP, Gao YL (2006) Controlled release properties of chitosan encapsulated volatile citronella oil microcapsules by thermal treatments. *Colloids Surf B Biointerfaces* 53:209–214
- Hussain AI, Anwar F, Hussain Sherazi ST, Przybylski R (2008) Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chem* 108:986–995
- Hussein A, Abdel-Mottaleb MM, El-Assal M, Sammour O (2020) Novel biocompatible essential oil-based lipid nanocapsules with antifungal properties. *J Drug Deliv Sci Technol* 56:101605
- Huynh NT, Passirani C, Saulnier P, Benoit JP (2009) Lipid nanocapsules: a new platform for nanomedicine. *Int J Pharm* 379:201–209
- Karami Z, Rezaeian I, Zahedi P, Abdollahi M (2013) Preparation and performance evaluations of electrospun poly (ϵ -caprolactone), poly (lactic acid), and their hybrid (50/50) nanofibrous mats containing thymol as an herbal drug for effective wound healing. *J Appl Polym Sci* 129:756–766
- Kavoosi G, Bordbar Z, Dadfar SM, Dadfar SMM (2017) Preparation and characterization of a novel gelatin–poly (vinyl alcohol) hydrogel film loaded with *Zataria multiflora* essential oil for antibacterial–antioxidant wound-dressing applications. *J Appl Polym Sci* 134:45351
- Kavoosi G, Dadfar SMM, Purfard AM (2013) Mechanical, physical, antioxidant, and antimicrobial properties of gelatin films incorporated with thymol for potential use as nano wound dressing. *J Food Sci* 78:E244–EE50
- Keawchaon L, Yoksan R (2011) Preparation, characterization and in vitro release study of carvacrol-loaded chitosan nanoparticles. *Colloids Surf B: Biointerfaces* 84:163–171
- Kelen M, Tepe B (2008) Chemical composition, antioxidant and antimicrobial properties of the essential oils of three salvia species from Turkish flora. *Bioresour Technol* 99:4096–4104
- Khadir A, Sobeh M, Gad HA, Benbelaid F, Bendahou M, Peixoto H, Sporer F, Ashour ML, Wink M (2016) Chemical composition and biological activity of the essential oil from *Thymus lanco-latus*. *Zeitschrift fur Naturforschung C, J Biosci* 71:155–163

- Khezri K, Farahpour MR, Mounesi Rad S (2019) Accelerated infected wound healing by topical application of encapsulated Rosemary essential oil into nanostructured lipid carriers. *Artif Cell Nanomed Biotechnol* 47:980–988
- Khezri K, Farahpour MR, Rad SM (2020) Efficacy of *Mentha pulegium* essential oil encapsulated into nanostructured lipid carriers as an in vitro antibacterial and infected wound healing agent. *Colloids Surf A Physicochem Eng Asp* 589:124414
- Kim SY, Kang DH, Kim JK, Ha YG, Hwang JY, Kim T, Lee SH (2011) Antimicrobial activity of plant extracts against *Salmonella Typhimurium*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on fresh lettuce. *J Food Sci* 76:M41–M46
- Koutsoudaki C, Krsek M, Rodger A (2005) Chemical composition and antibacterial activity of the essential oil and the gum of *Pistacia lentiscus* Var. *chia*. *J Agric Food Chem* 53:7681–7685
- Kumar P, Ramteke PW, Pandey AC, Pandey H (2019) Evaluation of antifungal activity of blended cinnamon oil and usnic acid nanoemulsion using candidiasis and dermatophytosis models. *Biocatal Agric Biotechnol* 18:101062
- Labib GS, Aldawsari H (2015) Innovation of natural essential oil-loaded Orabase for local treatment of oral candidiasis. *Drug Des Dev Ther* 9:3349–3359
- Lai F, Sinico C, De Logu A, Zaru M, Müller RH, Fadda AM (2007) SLN as a topical delivery system for *Artemisia arborescens* essential oil: in vitro antiviral activity and skin permeation study. *Int J Nanomedicine* 2:419
- Lai PK, Roy J (2004) Antimicrobial and chemopreventive properties of herbs and spices. *Curr Med Chem* 11:1451–1460
- Lamarra J, Calienni M, Rivero S, Pinotti A (2020) Electrospun nanofibers of poly (vinyl alcohol) and chitosan-based emulsions functionalized with cabreuva essential oil. *Int J Biol Macromol* 60:307–318
- Lau GKK, Nanji A, Hou J, Fong DYT, Au WS, Yuen ST, Lin M, Kung HF, Lam SK (2002) Thymosin- α 1 and famciclovir combination therapy activates T-cell response in patients with chronic hepatitis B virus infection in immune-tolerant phase. *J Viral Hepat* 9:280–287
- Lawrence MJ, Rees GD (2012) Microemulsion-based media as novel drug delivery systems. *Adv Drug Deliv Rev* 64:175–193
- Li M, Muthaiyan A, O'bryan CA, Gustafson JE, Li Y, Crandall PG, Ricke SC (2011) Use of natural antimicrobials from a food safety perspective for control of *Staphylococcus aureus*. *Curr Pharm Biotechnol* 12:1240–1254
- Li Z, Lin S, An S, Liu L, Hu Y, Wan L (2019) Preparation, characterization and anti-aflatoxigenic activity of chitosan packaging films incorporated with turmeric essential oil. *Int J Biol Macromol* 131:420–434
- Liakos I, Rizzello L, Hajiali H, Brunetti V, Carzino R, Pompa P, Athanassiou A, Mele E (2015) Fibrous wound dressings encapsulating essential oils as natural antimicrobial agents. *J Mater Chem B* 3:1583–1589
- Liakos I, Rizzello L, Scurr DJ, Pompa PP, Bayer IS, Athanassiou A (2014) All-natural composite wound dressing films of essential oils encapsulated in sodium alginate with antimicrobial properties. *Int J Pharm* 463:137–145
- Liakos IL, D'autilia F, Garzoni A, Bonferoni C, Scarpellini A, Brunetti V, Carzino R, Bianchini P, Pompa PP, Athanassiou A (2016) All natural cellulose acetate—Lemongrass essential oil antimicrobial nanocapsules. *Int J Pharm* 510:508–515
- Liakos IL, Iordache F, Carzino R, Scarpellini A, Oneto M, Bianchini P, Grumezescu AM, Holban AM (2018) Cellulose acetate - essential oil nanocapsules with antimicrobial activity for biomedical applications. *Colloids Surf B: Biointerfaces* 172:471–479
- Liu J-X, Dong W-H, Mou X-J, Liu G-S, Huang X-W, Yan X, Zhou C-F, Jiang S, Long Y-Z (2019) In situ electrospun zein/thyme essential oil-based membranes as an effective antibacterial wound dressing. *ACS Appl Bio Mater* 3:302–307
- Lo Cantore P, Iacobellis NS, De Marco A, Capasso F, Senatore F (2004) Antibacterial activity of *Coriandrum sativum* L. and *Foeniculum vulgare* Miller Var. *vulgare* (Miller) essential oils. *J Agric Food Chem* 52:7862–7866

- Low WL, Martin C, Hill DJ, Kenward MA (2013) Antimicrobial efficacy of liposome-encapsulated silver ions and tea tree oil against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. *Lett Appl Microbiol* 57:33–39
- Lu W-C, Huang D-W, Wang C-CR, Yeh C-H, Tsai J-C, Huang Y-T, Li P-H (2018) Preparation, characterization, and antimicrobial activity of nanoemulsions incorporating citral essential oil. *J Food Drug Anal* 26:82–89
- Mahmoudvand H, Sepahvand A, Jahanbakhsh S, Ezatpour B, Ayatollahi Mousavi SA (2014) Evaluation of antifungal activities of the essential oil and various extracts of *Nigella sativa* and its main component, thymoquinone against pathogenic dermatophyte strains. *J Med Mycol* 24:e155–ee61
- Maja L, Željko K, Mateja P (2020) Sustainable technologies for liposome preparation. *J Supercrit Fluids* 165:104984
- Majhenic L, Skerget M, Knez Z (2007) Antioxidant and antimicrobial activity of guarana seed extracts. *Food Chem* 104:1258–1268
- Manconi M, Petretto G, D'hallewin G, Escribano E, Milia E, Pinna R, Palmieri A, Firoznejhad M, Peris JE, Usach I, Fadda AM, Caddeo C, Manca ML (2018) Thymus essential oil extraction, characterization and incorporation in phospholipid vesicles for the antioxidant/antibacterial treatment of oral cavity diseases. *Colloids Surf B: Biointerfaces* 171:115–122
- Marques HMC (2010) A review on cyclodextrin encapsulation of essential oils and volatiles. *Flavour Fragr J* 25:313–326
- Martins N, Barros L, Henriques M, Silva S, Ferreira ICFR (2015) Activity of phenolic compounds from plant origin against *Candida* species. *Ind Crop Prod* 74:648–670
- Matshetshe KI, Parani S, Manki SM, Oluwafemi OS (2018) Preparation, characterization and in vitro release study of β -cyclodextrin/chitosan nanoparticles loaded *Cinnamomum zeylanicum* essential oil. *Int J Biol Macromol* 118:676–682
- Mayachiew P, Devahastin S, Mackey BM, Niranjan K (2010) Effects of drying methods and conditions on antimicrobial activity of edible chitosan films enriched with galangal extract. *Food Res Int* 43:125–132
- Mcclements DJ (2012) Nanoemulsions versus microemulsions: terminology, differences, and similarities. *Soft Matter* 8:1719–1729
- Mccormack B, Gregoriadis G (1994) Drugs-in-cyclodextrins-in liposomes: a novel concept in drug delivery. *Int J Pharm* 112:249–258
- Millon LE, Wan WK (2006) The polyvinyl alcohol–bacterial cellulose system as a new nanocomposite for biomedical applications. *J Biomed Mater Res B Appl Biomater* 9:245–253
- Moghimpour E, Aghel N, Zarei Mahmoudabadi A, Ramezani Z, Handali S (2012) Preparation and characterization of liposomes containing essential oil of *Eucalyptus camaldulensis* leaf. *Jundishapur J Nat Pharm Prod* 7:117–122
- Monton C, Settharaksa S, Suksaeree J, Chusut T (2020) The preparation, characterization, and stability evaluation of a microemulsion-based oral spray containing clove oil for the treatment of oral candidiasis. *J Drug Deliv Sci Technol* 57:101735
- Mouro C, Simões M, Gouveia IC (2019) Emulsion electrospun fiber mats of PCL/PVA/chitosan and eugenol for wound dressing applications. *Adv Polym Technol* 2019:9859506
- Müller RH, Radtke M, Wissing SA (2002) Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. *Adv Drug Deliv Rev* 54:S131–SS55
- Murthy PS, Ramalakshmi K, Srinivas P (2009) Fungitoxic activity of Indian borage (*Plectranthus amboinicus*) volatiles. *Food Chem* 114:1014–1018
- Narang AS, Delmarre D, Gao D (2007) Stable drug encapsulation in micelles and microemulsions. *Int J Pharm* 345:9–25
- Nasr M, Elmowafy E, Soliman ME (2019) The evolution of dendrimers to composite dendrimers: a review of the state of the art. In: Alexandru MG (ed) *Nanoparticles in pharmacotherapy*. Elsevier, William Andrew Publishing, pp 217–249

- Nasseri M, Golmohammadzadeh S, Arouiee H, Jaafari MR, Neamati H (2016) Antifungal activity of *Zataria multiflora* essential oil-loaded solid lipid nanoparticles in-vitro condition. *Iran J Basic Med Sci* 19:1231
- Ngamekaue N, Chitprasert P (2019) Effects of beeswax-carboxymethyl cellulose composite coating on shelf-life stability and intestinal delivery of holy basil essential oil-loaded gelatin microcapsules. *Int J Biol Macromol* 135:1088–1097
- Oke F, Aslim B, Ozturk S, Altundag S (2009) Essential oil composition, antimicrobial and antioxidant activities of *Satureja cuneifolia* Ten. *Food Chem* 112:874–879
- Oussalah M, Caillet S, Saucier L, Lacroix M (2006) Antimicrobial effects of selected plant essential oils on the growth of a *Pseudomonas putida* strain isolated from meat. *Meat Sci* 73:236–244
- Oussalah M, Caillet S, Saucier L, Lacroix M (2007) Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli* O157:H7, *Salmonella* Typhimurium, *Staphylococcus aureus* and *Listeria monocytogenes*. *Food Control* 18:414–420
- Ozcan MM, Sagdic O, Ozkan G (2006) Inhibitory effects of spice essential oils on the growth of bacillus species. *J Med Food* 9:418–421
- Paoli EE, Kruse DE, Seo JW, Zhang H, Kheiruloomoom A, Watson KD, Chiu P, Stahlberg H, Ferrara KW (2010) An optical and microPET assessment of thermally-sensitive liposome biodistribution in the Met-1 tumor model: importance of formulation. *J Control Release* 143:13–22
- Patil YP, Jadhav S (2014) Novel methods for liposome preparation. *Chem Phys Lipids* 177:8–18
- Perdonez Á, Chiralt A, Vargas M (2016) Properties of film-forming dispersions and films based on chitosan containing basil or thyme essential oil. *Food Hydrocoll* 57:271–279
- Pereira Dos Santos E, Nicácio PHM, Coelho Barbosa F, Nunes Da Silva H, Andrade ALS, Lia Fook MV, De Lima Silva SM, Farias Leite I (2019) Chitosan/essential oils formulations for potential use as wound dressing: physical and antimicrobial properties. *Materials* 12:2223
- Pinna R, Filigheddu E, Juliano C, Palmieri A, Manconi M, D'hallewin G, Petretto G, Maioli M, Caddeo C, Manca ML, Solinas G, Bortone A, Campanella V, Milia E (2019) Antimicrobial effect of *Thymus capitatus* and *Citrus limon* var. *pompia* as raw extracts and nanovesicles. *Pharmaceutics* 11:234
- Piras A, Rosa A, Marongiu B, Porcedda S, Falconieri D, Dessì MA, Ozelik B, Koca U (2013) Chemical composition and in vitro bioactivity of the volatile and fixed oils of *Nigella sativa* L. extracted by supercritical carbon dioxide. *Ind Crop Prod* 46:317–323
- Qin M, Mou XJ, Dong WH, Liu JX, Liu H, Dai Z, Huang XW, Wang N, Yan X (2020) In situ electrospinning wound healing films composed of Zein and clove essential oil. *Macromol Mater Eng* 305:1900790
- Ramadan MF (2007) Nutritional value, functional properties and nutraceutical applications of black cumin (*Nigella sativa* L.): an overview. *Int J Food Microbiol Sci Technol* 42:1208–1218
- Rao J, McClements DJ (2012) Food-grade microemulsions and nanoemulsions: role of oil phase composition on formation and stability. *Food Hydrocoll* 29:326–334
- Rasooli I, Abyaneh MR (2004) Inhibitory effects of Thyme oils on growth and aflatoxin production by *aspergillus parasiticus*. *Food Control* 15:479–483
- Rasooli I, Rezaei MB, Allameh A (2006) Growth inhibition and morphological alterations of *Aspergillus niger* by essential oils from *Thymus eriocalyx* and *Thymus x-porlock*. *Food Control* 17:359–364
- Ravichandran M, Hettiarachchy NS, Ganesh V, Ricke SC, Singh S (2011) Enhancement of antimicrobial activities of naturally occurring phenolic compounds by nanoscale delivery against *Listeria monocytogenes*, *Escherichia coli* o157:h7 and *salmonella typhimurium* in broth and chicken meat system. *J Food Saf* 31:462–471
- Risaliti L, Kehagia A, Daoulzi E, Lazari D, Bergonzi MC, Vergkizi-Nikolakaki S, Hadjipavlou-Litina D, Bilia AR (2019) Liposomes loaded with *Salvia triloba* and *Rosmarinus officinalis* essential oils: in vitro assessment of antioxidant, antiinflammatory and antibacterial activities. *J Drug Deliv Sci Technol* 51:493–498
- Rota C, Carramiñana JJ, Burillo J, Herrera A (2004) In vitro antimicrobial activity of essential oils from aromatic plants against selected foodborne pathogens. *J Food Prot* 67:1252–1256

- Rota MC, Herrera A, Martínez RM, Sotomayor JA, Jordán MJ (2008) Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. *Food Control* 19:681–687
- Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini S, Radice M, Bruni R (2005) Comparative evaluation of 11 essential oils of different origin as functional antioxidants, anti-radicals and antimicrobials in foods. *Food Chem* 91:621–632
- Salem ML, Hossain MS (2000) Protective effect of black seed oil from *Nigella sativa* against murine cytomegalovirus infection. *Int J Immunopharmacol* 22:729–740
- Saporito F, Sandri G, Bonferoni MC, Rossi S, Boselli C, Cornaglia AI, Mannucci B, Grisoli P, Vigani B, Ferrari F (2018) Essential oil-loaded lipid nanoparticles for wound healing. *Int J Nanomedicine* 13:175
- Sawalha H, Schroën K, Boom R (2011) Biodegradable polymeric microcapsules: preparation and properties. *Chem Eng J* 169:1–10
- Schelz Z, Molnar J, Hohmann J (2006) Antimicrobial and antiplasmid activities of essential oils. *Fitoterapia* 77:279–285
- Schulman JH, Stoekenius W, Prince LM (1959) Mechanism of formation and structure of micro emulsions by electron microscopy. *J Phys Chem* 63:1677–1680
- Sebaaly C, Charcosset C, Stainmesse S, Fessi H, Greige-Gerges H (2016) Clove essential oil-in-cyclodextrin-in-liposomes in the aqueous and lyophilized states: from laboratory to large scale using a membrane contactor. *Carbohydr Polym* 138:75–85
- Shamarekh KS, Gad HA, Soliman ME & Sasmour OA (2020a) Development and evaluation of protamine-coated PLGA nanoparticles for nose-to-brain delivery of tacrine: in-vitro and in-vivo assessment. *J Drug Deliv Sci Technol* 2020:101724
- Shamarekh KS, Gad HA, Soliman ME, Sasmour OA (2020b) Towards the production of mono-disperse gelatin nanoparticles by modified one step desolvation technique. *J Pharm Inves* 50:189–200
- Shinoda K, Lindman B (1987) Organized surfactant systems: microemulsions. *Langmuir* 3:135–149
- Shojaee-Aliabadi S, Hosseini H, Mohammadifar MA, Mohammadi A, Ghasemlou M, Hosseini SM, Khaksar R (2014) Characterization of κ -carrageenan films incorporated plant essential oils with improved antimicrobial activity. *Carbohydr Polym* 101:582–591
- Sikkema J, De Bont JA, Poolman B (1995) Mechanisms of membrane toxicity of hydrocarbons. *Microbiol Rev* 59:201–222
- Sinico C, De Logu A, Lai F, Valenti D, Manconi M, Loy G, Bonsignore L, Fadda AM (2005) Liposomal incorporation of *Artemisia arborescens* L. essential oil and in vitro antiviral activity. *Eur J Pharm Biopharm* 59:161–168
- Skocibusic M, Bezic N, Dunkic V (2006) Phytochemical composition and antimicrobial activities of the essential oils from *Satureja subspicata* Vis. growing in Croatia. *Food Chem* 96:20–28
- Slingerland M, Guchelaar H-J, Gelderblom H (2012) Liposomal drug formulations in cancer therapy: 15 years along the road. *Drug Discov Today* 17:160–166
- Sofi HS, Akram T, Tamboli AH, Majeed A, Shabir N, Sheikh FA (2019) Novel lavender oil and silver nanoparticles simultaneously loaded onto polyurethane nanofibers for wound-healing applications. *Int J Pharm* 569:118590
- Soković M, Glamočlija J, Marin PD, Brkić D, Van Griensven LJ (2010) Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model. *Molecules* 15:7532–7546
- Sokovic MD, Vukojevic J, Marin PD, Brkic DD, Vajs V, Van Griensven LJ (2009) Chemical composition of essential oils of thymus and *Mentha* species and their antifungal activities. *Molecules* 14:238–249
- Soliman ME, Elmowafy E, Casertari L, Alexander C (2018) Star-shaped poly (oligoethylene glycol) copolymer-based gels: thermo-responsive behaviour and bioapplicability for risedronate intranasal delivery. *Int J Pharm* 543:224–233

- Solórzano-Santos F, Miranda-Novales MG (2012) Essential oils from aromatic herbs as antimicrobial agents. *Curr Opin Biotechnol* 23:136–141
- Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE (2001) Biodegradable polymeric nanoparticles as drug delivery devices. *J Control Release* 70:1–20
- Stević T, Berić T, Šavikin K, Soković M, Godevac D, Dimkić I, Stanković S (2014) Antifungal activity of selected essential oils against fungi isolated from medicinal plant. *Ind Crop Prod* 55:116–122
- Sugumar S, Mukherjee A, Chandrasekaran N (2015) Eucalyptus oil nanoemulsion-impregnated chitosan film: antibacterial effects against a clinical pathogen, *Staphylococcus aureus*, in vitro. *Int J Nanomedicine* 10:67
- Svetlichny G, Küllkamp-Guerreiro I, Cunha S, Silva F, Bueno K, Pohlmann A, Fuentefria A, Guterres S (2015) Solid lipid nanoparticles containing copaiba oil and allantoin: development and role of nanoencapsulation on the antifungal activity. *Pharmazie* 70:155–164
- Svetlichny G, Küllkamp-Guerreiro I, Dalla Lana D, Bianchin M, Pohlmann A, Fuentefria A, Guterres S (2017) Assessing the performance of copaiba oil and allantoin nanoparticles on multidrug-resistant *Candida parapsilosis*. *J Drug Deliv Sci Technol* 40:59–65
- Taran M, Sharifi M, Azizi E, Khanahmadi M (2010) Antimicrobial activity of the leaves of *Pistacia khinjuk*. *J Med Plants* 1:81–85
- Tawfik MS, Abdel-Ghaffar KA, Gamal AY, El-Demerdash FH, Gad HA (2019) Lycopene solid lipid microparticles with enhanced effect on gingival crevicular fluid protein carbonyl as a biomarker of oxidative stress in patients with chronic periodontitis. *J Liposome Res* 29:375–382
- Tepe B, Daferera D, Sokmen A, Sokmen M, Polissiou M (2005) Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* miller (Lamiaceae). *Food Chem* 90:333–340
- Tiwari AP, Joshi MK, Park CH, Kim CS (2018) Nano-nets covered composite nanofibers with enhanced biocompatibility and mechanical properties for bone tissue engineering. *J Nanosci Nanotechnol* 18:529–537
- Tiwari BK, Valdramidis VP, O'donnell CP, Muthukumarappan K, Bourke P & Cullen PJ (2009) Application of natural antimicrobials for food preservation. *J Agric Food Chem* 57:5987–6000
- Tohidpour A, Sattari M, Omidbaigi R, Yadegar A, Nazemi J (2010) Antibacterial effect of essential oils from two medicinal plants against methicillin-resistant *Staphylococcus aureus* (MRSA). *Phytomedicine* 17:142–145
- Tolouee M, Alinezhad S, Saberi R, Eslamifar A, Zad SJ, Jaimand K, Taeb J, Rezaee M-B, Kawachi M, Shams-Ghahfarokhi M, Razzaghi-Abyaneh M (2010) Effect of *Matricaria chamomilla* L. flower essential oil on the growth and ultrastructure of *Aspergillus niger* van Tieghem. *Int J Food Microbiol* 139:127–133
- Unalan I, Endlein SJ, Slavik B, Buettner A, Goldmann WH, Detsch R, Boccaccini AR (2019a) Evaluation of electrospun poly(ϵ -Caprolactone)/gelatin nanofiber mats containing clove essential oil for antibacterial wound dressing. *Pharmaceutics* 11:570
- Unalan I, Slavik B, Buettner A, Goldmann WH, Frank G, Boccaccini AR (2019b) Physical and antibacterial properties of peppermint essential oil loaded poly(ϵ -caprolactone) (PCL) electrospun fiber mats for wound healing. *Front Bioeng Biotechnol* 7:346
- Vagionas K, Graikou K, Ngassapa O, Runyoro D, Chinou I (2007) Composition and antimicrobial activity of the essential oils of three *Satureja* species growing in Tanzania. *Food Chem* 103:319–324
- Valcourt C, Saulnier P, Umerska A, Zanelli M, Montagu A, Rossines E, Joly-Guillou M-L (2016) Synergistic interactions between doxycycline and terpenic components of essential oils encapsulated within lipid nanocapsules against gram negative bacteria. *Int J Pharm* 498:23–31
- Vladislavljević GT (2019) Preparation of microemulsions and nanoemulsions by membrane emulsification. *Colloids Surf A Physicochem Eng Asp* 579:123709
- Wang Z, Bai H, Lu C, Hou C, Qiu Y, Zhang P, Duan J, Mu H (2019) Light controllable chitosan micelles with ROS generation and essential oil release for the treatment of bacterial biofilm. *Carbohydr Polym* 205:533–539

- Wen C, Yuan Q, Liang H, Vriesekoop F (2014) Preparation and stabilization of d-limonene Pickering emulsions by cellulose nanocrystals. *Carbohydr Polym* 112:695–700
- Wen P, Zong M-H, Linhardt RJ, Feng K, Wu H (2017) Electrospinning: a novel nano-encapsulation approach for bioactive compounds. *Trends Food Sci Technol* 70:56–68
- Winward GP, Avery LM, Stephenson T, Jefferson B (2008) Essential oils for the disinfection of grey water. *Water Res* 42:2260–2268
- Xu T, Gao C, Yang Y, Shen X, Huang M, Liu S, Tang X (2018) Retention and release properties of cinnamon essential oil in antimicrobial films based on chitosan and gum arabic. *Food Hydrocoll* 84:84–92
- Yan X, Yu M, Ramakrishna S, Russell SJ, Long Y-Z (2019) Advances in portable electrospinning devices for in situ delivery of personalized wound care. *Nanoscale* 11:19166–19178
- Yan X, Yu M, Zhang L-H, Jia X-S, Li J-T, Duan X-P, Qin C-C, Dong R-H, Long Y-Z (2016) A portable electrospinning apparatus based on a small solar cell and a hand generator: design, performance and application. *Nanoscale* 8:209–213
- Zhao Y, Wang C, Chow AHL, Ren K, Gong T, Zhang Z, Zheng Y (2010) Self-nanoemulsifying drug delivery system (SNEDDS) for oral delivery of Zedoary essential oil: formulation and bioavailability studies. *Int J Pharm* 383:170–177
- Zhou Y, Sun S, Bei W, Zahi MR, Yuan Q, Liang H (2018) Preparation and antimicrobial activity of oregano essential oil Pickering emulsion stabilized by cellulose nanocrystals. *Int J Biol Macromol* 112:7–13

Index

A

- Abacin, 381, 382, 388
Acalypha monostachya, 248, 261
Acetates, 187
Acinetobacter baumannii, 24, 36, 38, 39, 61, 62, 66, 257, 384, 420
Agar dilution method, 7
Agaricus bisporus S-II extracts, 329
Agaricus spp., 366
Aldehydes, 191, 192, 202, 206, 227, 400
Aliivibrio fischeri, 104
Alkaloids, 24, 120, 185, 243, 265, 267, 285, 286, 303, 324, 362, 377
Amanita spp., 363, 367
Amazon, 32–38, 48
Amino acids, 187
Aminoglycosides, 188
Amphenicols, 188
Amphotericin B, 189
Analgesics, 189, 191
Anionic antimicrobial peptides (AAMP), 379
Anogenital cancer, 137, 144
Antibacterial activity, 81, 119–130
 ethanol-obtained extracts, 81
 EVOO, 84
 HPLC analysis, 83
 hydroxytyrosol, 84
 maslinic and oleanic acid, 83
 microdilution assay, 82
 nutraceuticals, 83
 oleuropein, 84
 PEOVW, 83
Antibiotics, 184–188, 190–193
Antibodies, 187
Anti-dermatophytic compounds, 202, 207
Antifungal activity, 77–81, 119–130, 206–207
 fluorescent dye exclusion method, 80
 HPLC-DAD analysis, 78
 interim solution, 78
 MIC values, 79, 80
 natural products, 77
 Planktonic cultures, 80
Antifungals, 281–311
 phytopathogenic fungi, 307, 308
Antimicrobial activities, 21–25, 31–48, 58–63, 66, 75–86, 121, 126, 129, 325–340
 Cinnamomum species, 61–62
 microbial target sites, 8
Antimicrobial agents, 241–267
Antimicrobial drugs, 184–186, 188, 193
Antimicrobial photodynamic therapy (APDT), 303
Antimicrobial resistance (AMR), 54, 60, 62, 66, 185, 186, 242, 374, 376, 377
 GDP, 248
 Global Plan of Action, 246
 IACG report, 246
 Interagency Task Force, 243
 RAND corporation, 246
Antimicrobials, 184, 185, 187, 190, 192, 193, 397–431
Antimycobacterial activity, 36
Antimycotic agents, 77
Antioxidant effects, 76, 77, 190
Antiparasitic, 191
Antiprotozoal, 186
Antiviral, 186
Apis mellifera, 374

- Argentine plants
 antifungal activities, 287–294
 photoactivity, 303, 304
Phytolacca tetramera, 297, 299, 300
Polygonum, 301, 302
 synergistic effects, 305, 306
Zuccagnia puctata, 286, 294–296
- Armillaria* spp., 363, 366
- Aromatic, 93, 191, 192
- Arrabidaea brachypoda*, 190
- Arsphenamine, 188
- Artemisinin, 10
- Aspergillosis, 226–231
- Aspergillus fumigatus*
 minimal inhibitory concentration, 228–229
- Aspergillus* spp., 201, 226, 243, 245
- Azoles, 186
- B**
- Bacillus cereus*, 190
- Bacillus subtilis*, 192
- Bacteria, 125, 127, 129, 130, 185–192, 357, 358, 361, 363–365
- Bacterial activity, 187
- Bacterial infection, 184, 188, 192
- Bactericidal, 187, 192
- Bacteriostatic, 187
- Basidiomycota* fungi, 324
- Basidiomycota mushrooms
 antibacterial activity, 332–334
 antifungal activity, 335–336
 antimicrobial activity, 326, 340
 antifungal peptide, 343
 antimicrobial compounds, 342
 chlorinated orcinol derivatives, 343
 chloroform extract, 344
 culture filtrate, 338
 culture liquid, 338
 enokipodins, 342
 ethanol extracts, 341
 ethyl acetate extract, 341
 fruiting bodies, 327–331
 Gram+ and Gram- bacteria, 338
 Korean cauliflower medicinal mushroom, 343
 methanol extract, 341
 methanolic extracts, 342
 mushroom species, 326
 mycelial biomass, 331, 336
 polysaccharides extracts, 330
 submerged cultivation, 337
 triterpenoids fraction, 341
 water and methanol extracts, 339
 wood-degrading basidiomycetes, 338
 wood-rotting and litter-decomposing, 339
- bioactive compounds, 325
- cultivation conditions, 344–347
 antibacterial activity, 345, 346
 antifungal activity, 344
 exopolysaccharides, 346
 fruiting bodies, 345
 MEB culture medium, 346
 Pontecorvo's medium, 346
 potential therapeutic properties, 325
- Bee bread, 388
- Beehives, 374
 antimicrobial substances, 376–380
 plant immune response, 376
 secondary metabolites (SMs) (*see* Plant secondary metabolites (SMs))
 defense mechanisms, 375
- Beekeeping, 374
- Bee pollen, 375, 383, 388
- Bee venom (Aпитoxin), 389
- Behavioural cooperation, 382
- Berberine
 antibacterial activity of, 125–127
 antifungal activity of, 122
 antifungal properties, 123
 antiobesity and antidiyslipidemia efficacy, 121
 antioxidant activity, 121
 antiproliferative agent, 121
 berberine-rich herbals, 120
 catalase and glutathion peroxidase (GPx) activity, 121
 cell cycle progression, 121
 chemical structure of, 121
 immunotherapy, 121
 low-density lipoprotein (LDL) receptor expression, 121
 mechanisms of action, 122, 123
 synergistic effect of, 128, 129
- β -caryophyllene, 210
- Beta-lactam antibiotics, 128
- Beta-lactamase, 5, 186
- Beta-lactams, 188
- Bidens pilosa*, 245, 248, 255, 256
- Bioactive compounds, 32, 33
- Bioactive therapeutic, 185
- Bioactivity, 31–48, 202, 210, 227
- Biocidal, 188
- Biodiversity, 32–34, 38, 48
- Biofilm, 60, 66, 218, 219, 222
- Biological activities, 190
- Biological control, 324, 338

- Biomedicine, 192
Bioreactors, 190
Biosurfactants, 283
Bolivian plants, 250
Botanical, 32, 34, 37
Brazilian Amazon, 32, 33, 35–38
- C**
- Candida albicans*, 189–191, 218, 283
Candida glabrata, 189, 190, 283
Candida spp., 218, 243, 245
Candida tropicalis, 189
Candidiasis, 78, 81
Carbapenems, 186
Carvacrol, 192
Cassia bakeriana Craib., 189
Cassia fistula L., 189
Cell, 187, 191
Cell membrane, 5, 79, 81, 85
Cell morphology, 191
Cephalosporins, 188
Cervical cancer, 144–149, 158, 159, 161, 171
Chaga mushroom, 367
Chemical structure, 187
Chemistry, 34
Chemotherapy, 184
Chloramphenicol, 188
Chloroform, 191
Chromosomes, 186
Chronic infections, 188
Cinnamaldehyde, 58–60, 62, 64–66
Cinnamon
 antibacterial activity, 60–63
 antibacterial agents, 66
 antibiofilm activity, 66
 antifungal activity, 64–65
 antimicrobial activity, 59
 antiparasitic activity, 65
 antiviral activity, 65
 cinnamic aldehyde, 58
 Cinnamomum cassia, 56
 Cinnamomum zeylanicum, 56
 coumarin, 57
 eugenol, 58
 inhibitory activity, 63
 mechanism of action, 59
 oil formulations, 60
Cistus creticus L., 100–107
 antifungal activity, 103
 antimicrobial activity, 103
 antiviral activity
 clinical study, 105, 106
 in vitro effects, 104
 in vivo effects, 105
 aqueous extracts, 104
 chemical constituents
 essential oil, 102
 HPLC-DAD-ESI-MS/MS
 methodology, 102
 labdane-type diterpenes, 101
 polyphenols, 102
 ethnopharmacology, 101
 food supplementation, 107
 hemorrhagic dengue fever, 105
 history, 101
 in situ studies, 103
 Mediterranean regions, 107
 SARS-CoV-2, 106–107
Clostridium botulinum, 111
Cloves, 191
Conjugation, 186
Cretan dittany, 97
Cryptococcus neoformans
 minimal inhibitory concentrations of, 225
Cryptococcus spp., 223, 224
Cyclotides, 379
Cytoplasm, 191
- D**
- Delivery systems, 397–431
de novo synthesis, 4
Dermatophytes, 201–206, 208–217
Dermatophytosis, 200
Dichloromethane, 189, 190
Diffusion method, 7
5,6-Dihydro-9,10-dimethoxybenzo(g)-1,3-
 benzodioxolo(5,6-a)
 quinolizinium, 120
Dilution methods, 7
Dimeric flavonoids, 191
Diseases, 32–34, 36, 44–46
Disease-causing pathogens, 241–267
Diversity, 326, 331
DNA fragmentation, 123
Dracocephalum forrestii, 190
Drug discovery, 16, 27
Drug-resistant, 186
Drugs, 184–188, 190, 192, 193
- E**
- Echinocandins, 186
Educational programs, 184
Encapsulation, 404, 413, 415–418, 420, 422,
 423, 425, 427
Endocarditis, 188
Enterobacteria, 186
Enterococci, 186

- Ergosterol, 207, 208, 222, 226, 230, 231
- Erhuang powder, 158
- Escherichia coli*, 190, 192, 246, 253–260, 263–266, 272
- Essential oils (EOs), 55, 186, 191–192, 399
- anti-dermatophytic compounds, 202, 207
 - antifungal mechanisms of action, 207, 209, 210
 - antifungal properties, 401
 - antimicrobial activities, 401
 - antimicrobial effects, 400
 - Aspergillus fumigatus*, 226
 - bioactive components, 201
 - biocidal activities, 399
 - components, 219, 222
 - conventional antifungal drugs, 210, 226
 - Eucalyptus citriodora* EO, 211
 - lipid-based delivery systems, 402–420
 - cinnamaldehyde (CAD), 409
 - emulsion-based systems, 404
 - lipid nanoparticles, 405–406 (*see also* Lipid nanoparticles)
 - liposomes, 404, 412–415
 - microemulsions, 402, 403
 - nanoemulsions, 403, 404, 406, 407
 - SNEDDS, 411
 - ZTO SNEDDS, 411
 - loaded delivery systems, 399
 - mechanisms of action, 222
 - microbial cell membranes, 400
 - minimal inhibitory concentration of, 203–206
 - non-lipid-based delivery systems, 407, 420–431
 - casting films, 408, 425, 426
 - hydrogels, 420
 - micelles, 424, 425
 - microencapsulation, 421
 - NF mats (*see* Nanofibers (NFs) mats)
 - Orabase® gel, 421
 - polymeric NPs, 422–424
 - Otacanthus azureus* EO, 210
 - terpene compounds, 400
- Ethers, 191
- Ethnopharmacology, 92
- Ethyl acetate, 189
- European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations, 7
- European Food Safety Authority (EFSA) claims, 44
- Extended-spectrum β -lactamase-producing (ESBL), 384
- Extraction, 40–44, 48
- Extracts, 31–48, 185, 189–190
- F**
- Fennel, 192
- Fermentation, 331, 342, 344, 346, 347
- Flavonoid, 191
- Fleming, Alexander, 184, 185
- Fluconazole, 190, 202, 209, 210, 219, 221–223, 226, 231
- Fluoroquinolones, 188
- Foeniculum vulgare*, 192
- Fomes fomentarius*, 331
- Food-borne bacteria, 83
- Forest, 32, 34, 36, 37
- Fractional inhibitory concentration index (FICI), 7, 230
- Fractions, 189, 190
- Fungal infections, 81, 199–231
- Fungi, 185, 186, 189, 192
- Fungicide, 191
- Fusarium oxysporum*, 284
- Fusarium* spp., 284
- G**
- Ganoderma* spp., 328
- Gentamicin, 190
- Glutathione reductase (GR) activity, 125
- Glycopeptides, 188
- Gonorrhea, 188
- Gram-negative, 190, 192
- Gram-pathogenic, 190
- Gram-positive, 192
- Gram-positive/Gram-negative bacterial species, 85
- Greek mountain tea, *see Sideritis* (Lamiaceae)
- H**
- Health, 185, 186, 188, 192
- Health professionals, 186
- Heat shock proteins (HSPs), 123
- Helicobacter pylori*, 111
- Herpes simplex virus (HSV), 136
- Hexany, 189
- High-performance liquid chromatography electro-spray ionization mass spectrometry (HPLC/ESI-MS), 127
- Honey bee
 - beehive defense resources, 383–389
 - beeswax, 389
 - bee venom, 389
 - honey, 384, 385
 - pollen, 388
 - propolis, 385, 386
 - royal jelly (RJ), 387, 388

- defense mechanisms, 380–383
 - antimicrobial peptides (AMPs), 381, 382
 - immune response, 380
 - social immunity, 382
- Honey-bee defensin 1, 381, 382, 384
- Honey-bee defensin 2, 382
- Honey-bee immunity, 380–383
- Human benefits, 27
- Human papilloma virus (HPV), 136
 - allopathic procedures, 145
 - anti-HPV formulations, 161–170
 - causes, 144
 - cervical intraepithelial dysplasias, 145
 - integrated treatment, 159, 160
 - marine-based anti-HPV formulations, 158
 - phytochemicals, 160–162
 - plant-based anti-HPV
 - formulations, 146–158
 - present review, 144
 - TCM formulations, 147–157, 171
 - traditional Chinese medicine (TCM), 145, 146
- Human pathogens, 286–302
- Hydnum repandum*, 330
- Hydroxyl, 191
- Hymenoptaecin
- Hydroxytyrosol, 75–86
 - chemical structure, 77
- Hymenoptaecin, 381, 382, 387

- I**
- Infectious diseases, 185, 188–190
- Insecticide, 191
- International Organization for Standardization (ISO), 202
- Ionization, 190
- Isolation techniques, 120
- Isoquinoline alkaloid, 122, 129

- J**
- Jelleins, 387

- K**
- Ketones, 191
- Klebsiella pneumoniae*, 243, 245, 246, 256–258, 260, 265

- L**
- Lactarius* spp., 363, 367
- Latin American medicinal plants, 248
 - Acalypha monostachya*, 261
 - Amphipterygium adstringens*, 261, 263
 - Baccharis boliviensis*, 248
 - EtOH extracts, 254, 255
 - Flavobacterium columnare*, 267
 - Helicobacter pylori* agent, 262
 - hydroalcoholic extract, 260
 - Lippia organoides*, 256, 257
 - MeOH extract, 255, 260
 - MIC/MBC values, 260
 - Minthostachys mollis*, 258
 - Parastrephia lepidophylla*, 248
 - Peruvian species, 258
 - Piper regnellii*, 251, 252
 - Plasmodium falciparum*, 261
 - Pseudomonas solanacearum*, 264
 - Rauwolfia* (or *Rauwolfia*) *tetraphylla*, 264
 - Schinus terebinthifolia*, 252
 - Siparuna guianensis*, 264, 265
 - Ziziphus joazeiro*, 253
- Lavandula* spp., 221
- Laxative, 189
- Lichens, 185
- Licosamines, 188
- Lipid nanoparticles
 - lipid nanocapsules (LNCs), 415, 416, 420
 - nanostructured lipid carriers (NLCs), 415, 416, 419
 - solid lipid nanoparticles (SLNs), 416, 417
- Lipodepsipeptides, 188
- Liquid chromatography–mass spectrometry (LC-MS/MS), 126
- Lowest Unoccupied Molecular Orbital (LUMO), 206

- M**
- Macrolides, 188
- Major royal jelly proteins (MRJPs), 387
- Major royal jelly proteins I precursor (MRJP1), 384
- Malt extract broth (MEB), 346
- Mass spectrometry, 189, 190
- Medicinal plants, 185, 188, 189, 193
 - Amazon, 33, 34
 - antimicrobial activity, 34–38
 - Amazon rainforest, 36
 - antifungal activity, 37
 - antileishmanial activity, 37
 - Atlantic Forest plants, 36
 - essential oils, 36
 - Fabaceae, 36
 - Paenibacillus* species, 36
 - phytotoxicity activities, 37
 - plants, 38
 - species and plant fractions, 35

- Medicinal plants (*cont.*)
- Brazilian Amazon, 33
 - conventional techniques, 40–43
 - ethanol, 41
 - hydroalcoholic extraction, 40
 - phenolics, 40
 - Soxhlet extraction, 42
 - Soxhlet method, 41–42
 - homemade, 32
 - human pathogens, 38, 39
 - nanoencapsulation
 - bioactive compounds, 45, 46
 - phenolic compounds and antioxidants, 46
 - nonconventional technologies, 43
 - Medicine, 185, 186, 188, 189, 192, 193
 - Melaleuca alternifolia*, 221
 - Melittin, 389
 - Membranes, 121–124, 126, 129, 130
 - Meningococcal meningitis, 188
 - Mentha piperita*, 192
 - Methicillin, 186
 - Methicillin-resistant *S. aureus* (MRSA), 39, 384
 - Methoxyl, 191
 - Microbial infections, 9–10
 - Microbial membrane, 191
 - Microorganisms, 36–38, 48, 184–187, 189–193
 - Microsporium gypseum*, 284
 - Minimum fungicidal concentration (MFC), 79, 304
 - Minimum inhibitory concentration (MIC), 7, 202, 326–339, 341, 404, 406, 417, 418, 420, 421, 423, 427, 428
 - Mitogen-activated protein kinase (MAPK), 123
 - Molecules, 190, 191, 193
 - Monobactams, 188
 - Monoterpenes, 191, 202, 206, 207, 210–218, 224, 226, 227, 231
 - Mucor* spp., 285
 - Multidrug, 193
 - Multidrug resistance, 128
 - Multidrug-resistant microorganisms, 185
 - Mycelium, 330, 332–341, 343–346
 - Mycobacterium tuberculosis*, 186, 243, 245, 246, 251, 258, 264, 266
 - Mycosis, 189
- N**
- N-acetyl-D-glucosamine (NACDG), 123
 - Nanoencapsulation, 45
 - Nanofibers (NFs) mats, 408
 - cellulose acetate-based NF mats, 430
 - electrospinning technique, 430
 - EO enriched dual-function, 428
 - NHDF cells, 429
 - PCL-NF loaded, 429
 - polyurethane polymer, 430
 - wound-dressing materials, 430
 - Nanotechnology, 31–48
 - Natural antimicrobials
 - in vitro* methods, 6
 - in vitro* studies, 10
 - microbial communities, 7
 - Natural products, 285
 - antimicrobial resistance, 185, 186
 - classification and mechanism of action, 187, 188
 - herbal medicines, with antimicrobial action, 188
 - mechanism of action, 187
 - powerful therapy, 185
 - N dimethylformamide (DMF), 72
 - Neisseria gonorrhoeae*, 188, 192
 - Neisseria meningitidis*, 188
 - Nitrogen, 191
 - Nitrogen-containing compounds, 286
 - Nonflowering plants, 16
 - Novel drug, 27
- O**
- Ocimum basilicum*, 221
 - Oleuropein, 75–86
 - Olive leaf extract, 76, 78–83
 - One Health, 54
 - Onychomycosis, 201
 - Oregano, 191
 - Organisms, 187, 192
 - Origanum dictamnus*
 - antimicrobial activity, 96–97
 - antiviral activity, 97
 - chemical constituents, 95–96
 - ethnobotany, 94
 - food preservative action, 97–98
 - history, 94
 - Osteomyelitis, 188
 - Oudemansiella mucida*, 339

- Oxapenins, 188
Oxazolidinones, 188
Oxides, 191
- P**
- Pandemic, 136, 144
Paracoccidioides brasiliensis, 284
Pathogenic bacteria, 185, 192
Pathogenic microorganisms, 324
Pathogen/microbe-associated molecular pattern (P/MAMP) molecules, 376
Pathologies, 186, 189, 193
Pattern recognition receptors (PRRs), 376
Penicillin, 184, 186, 188
Peppermint, 192
Peppers, 191
Persicaria acuminata, 293, 301, 302, 310
Persicaria maculosa, 302
Phagocytosis, 187
Pharmaceutical, 186, 193
Pharmaceutical industries, 186
Phenolic acids, 376, 377, 388
Phenolic compounds, 76, 78, 79, 83, 189–191, 285
Phenolic extract from olive vegetation water (PEOVW), 83
Phenols, 191
Photodiodes, 190, 191
Phytochemicals, 21, 22, 24, 191, 193
Phytolacca tetramera, 297–300
Phytolaccoside B, 299
Phytolaccoside E, 299
Phytolaccoside F, 299
Phytopathogenic fungi, 307, 308
Pink rock-rose, 100–107
Pistacia lentiscus (Anacardiaceae), 93, 107
 ethnopharmacology, 108
 history, 108
 mastic antimicrobial properties
 clinical data, 112
 in vitro, 111–113
 natural polymer, 109
 resin, 93, 108, 109, 113
 triterpenoid types, 109
Plant antimicrobial activity
 antimicrobial action
 essential oils with, 191, 192
 extracts, 189, 190
 phytoconstituents with, 190, 191
Plant antimicrobial peptides, 375, 378–380
Plant secondary metabolites, 375, 377–380
Pleurotus spp., 329
Pneumonia, 188
Polygonum, 301, 302
Polyketides, 185
Polymeric microcapsules, 421–422
Polyols, 186
Polyphenols, 190
Preservatives, 191
Proanthocyanidins, 189
Production, 324, 326, 331, 342, 344–347
Propionates, 187
Protozoa, 186
Pseudomonas aeruginosa, 186, 190–192, 243, 245, 249, 250, 254–258, 260, 261, 263–266
Psycho-social effects, 201
Pteridophytes, 16
 activity of ferns, 22
 bacteria, 22, 24
 fungi, 24, 25
 viruses, 25
 antimicrobial medicines, 21
 bioactive compounds, 21
 challenges, 26
 diversity and distribution, 17–20
 human beings, 16
 nepalese ferns, 26
 nonflowering plants, 16
 opportunities, 27
Public policies, 186
Purity, 191
Pyocyanin, 359, 363–367
- Q**
- Quantitative structure–activity relationship (QSAR), 210
Queen bee acid, 387
Quinolones, 188
Quorum quenching (QQ), 356, 359–362
Quorum-sensing (QS), 60, 356
 AHL-deactivating enzymes, 360
 autoinducers (AIs), 357
 compounds inhibiting, 359
 measurement of enzymatic activity, 361
 QQ, 359
 qualitative and quantitative measurements, 360
 Vibrio harveyi BB170 method, 361
 inhibitor, 5
 low cell density, 357
 mechanisms, 358
 mushrooms, 362
 AHL inhibition, 368
 antibiofilm activity, 366
 biofilm formation, 365

- Quorum-sensing (QS) (*cont.*)
 Chaga mushroom, 367
 chloroform extracts, 367
 edible mushrooms, 367
 ethanolic extracts, 366
 hot water extracts, 367
 inhibition by extracts, 363–365
 mechanism, 362
 methanolic extracts, 365–367
 signalling molecules, 367–368
 violacein inhibition, 367
 signalling molecules, 358
- R**
Ramaria species, 330
 Resistance genes, 186
 RNA interference (RNAi), 380, 381
 Rosemary, 191
 Rosmarinic acid, 190
 Royalisin, 387
- S**
Saccharomyces cerevisiae, 283
Salix babylonica, 248, 259–261
Salix humboldtiana, 248, 255, 259–261
Salmonella spp., 242
Salmonella typhi, 191
 Salvianolic acid B, 190
Satureja montana, 221
 Scanning electron microscope (SEM), 127
 Scopus database, 244
 Secondary metabolites (SMs), 21, 189,
 191, 377
 antimicrobial peptides (AMPs), 378
 anionic (AAMPs), 379
 cationic (CAMPs) peptides, 379
 defensins, 380
 discriminated and illicit use, 192
 polyphenols, 377
 flavonoids, 377
 hydroxyl groups, 378
 proteins and peptides, 378
 practical and negligent use, 192
 therapeutic purposes, 193
 volatile SMs (Terpenoids), 378
 Sedatives, 191
 Self-nanoemulsifying delivery systems
 (SNEDDS), 411
Senna macranthera (Collad), 189
Senna spectabilis DC., 189
 Septic arthritis, 188
- Sesquiterpenes, 191, 202, 210, 219, 224, 226,
 230, 231
Sideritis (Lamiaceae)
 antimicrobial activity, 99–100
 chemical constituents, 99
 essential oil, 99
 ethnobotany, 98
 history, 98
 iron wort, 98
 mountain tea, 98
S. clandestina, 98–100
S. montana, 100
S. raeseri, 99
S. scardica, 100
S. syriaca, 100
 Signal molecules, 356–359, 361
 Significant effect, 25
 Spasmolytic, 191
 Specific pathogen effectors, 376
Staphylococcus aureus, 85, 127, 243, 245,
 246, 249–251, 253–258, 260–267
Staphylococcus epidermidis, 128
Staphylococcus haemolyticus, 128
Streptococcus agalactiae, 126
Streptococcus pneumoniae, 188
Streptococcus pyogenes, 126
 Streptogramins, 188
 Structure–activity relationships (SAR)
 approach, 4
 Sugars, 187
 Sulfamethazine, 188
 Sulfonamides, 188
 Sulfur, 191
 Superoxide dismutase (SOD), 121
 Synergism, 210–218, 222, 230, 231, 305
 Synergistic effects, 190, 305, 306
 Syphilis, 188
Syzygium cumini, 192
- T**
 Tanshinone IIA, 146
 Temperature, 193
 Terpenes, 385
 Terpenic, 192
 Terpenoids, 185, 285
 Tetracyclines, 188
 Therapeutic, 185, 186, 193
 Therapy, 32–34
 Thionins, 379, 380
 Thyme, 191, 192
Thymus capitatus, 221
Thymus vulgaris, 192

- TITLE-ABS-KEY, 244
Traditional Chinese medicine (TCM),
120, 146–157
Traditional herbal medicine (THM), 98
Toxicity, 186
Toxins, 187
Translation, 186
Treponema pallidum, 188
Trianthema decandra, 191
Trichophyton mentagrophytes, 122, 210
Trichophyton spp., 284
- V**
Vancomycin, 186
Vancomycin-resistant enterococci
(VRE), 384
Varicella-zoster virus (VZV), 384
Vibrio cholerae, 125, 191
Vibrio harveyi BB170 method, 361
- Vicks VapoRub™, 218
Violacein pigment inhibition method, 367
Virucidal, 191
Virulence factors, 81, 86
Viruses, 186, 192
Viruses and diseases in humans, 137–143
- Y**
Yeast extract–peptone–dextrose (YPD), 123
Yeasts, 122–124
Yi Gan Kang, 158
YouDuJing cream, 146
Yunnan Baiyao TCM formulation, 159
- Z**
Zedoary turmeric oil (ZTO), 411
Zuccagnia punctata, 286, 294–296
Zuccagnia punctata, 295