IntechOpen

Essential Oils

Bioactive Compounds, New Perspectives and Applications

Edited by Mozaniel Santana de Oliveira, Wanessa Almeida da Costa and Sebastião Gomes Silva

Essential Oils - Bioactive Compounds, New Perspectives and Applications

Edited by Mozaniel Santana de Oliveira, Wanessa Almeida da Costa and Sebastião Gomes Silva

Published in London, United Kingdom







IntechOpen






Supporting open minds since 2005

Essential Oils – Bioactive Compounds, New Perspectives and Applications
http://dx.doi.org/10.5772/intechopen.87266
Edited by Mozaniel Santana de Oliveira, Wanessa Almeida da Costa and Sebastião Gomes Silva

Contributors
Amira El-Anssary, Nada Khazal Kadhim Hindi, Oliviu Vostinaru, Simona Codruta Heghes, Lorena Filip, Ahmed A. Almarie, Hamdy Shaaban, Jorddy Neves Cruz, Adriane Gomes Da Silva, Wanessa Almeida Da Costa, Sebastião Gomes Silva, Willison Eduardo Oliveira Campos, Ely Simone Cajueiro Gurgel, Eloisa Helena De Aguiar Andrade, Marcos Ene Chaves Oliveira, Daniel Santiago Pereira, Antonio Pedro Da Silva Souza Filho, Mozaniel Santana de Oliveira, Mohamed El Hattab, Yannick Stephane Fongang Fotsing, Jean Jules Bankeu Kezetas, Fernando Almeida De Souza, Isadora De Fátima Braga Magalhães, Carla Junqueira Moraga Tellis, Kátia Da Silva Calabrese, Ana Lucia Abreu-Silva, José Weverton Almeida Bezerra, Felicidade Caroline Rodrigues, Ma Aparecida Barbosa Ferreira Gonçalo, Marcos Aurélio Figuereido Dos Santos, Gledson Ferreira Macedo, Janete De Souza Bezerra, Priscilla Augusta De Sousa Fernandes, Emanoel Messias Pereira Fernando, Carlos Henrique Silva De Oliveira, Viviane Bezerra Da Silva, Isabella Hevily Silva Torquato, Niwiarakelly Da Silva Monte, Luciano Temoteo Dos Santos, Henrique Douglas Melo Coutinho, Lubna Abdul Muttalib Al-Shalah, Israa Harjan Mohsen

First published in London, United Kingdom, 2020 by IntechOpen
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, 5 Princes Gate Court, London, SW7 2QJ, United Kingdom
Printed in Croatia

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

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

Essential Oils – Bioactive Compounds, New Perspectives and Applications
Edited by Mozaniel Santana de Oliveira, Wanessa Almeida da Costa and Sebastião Gomes Silva
p. cm.
Print ISBN 978-1-83962-697-5
Online ISBN 978-1-83962-698-2
eBook (PDF) ISBN 978-1-83962-699-9

Meet the editors

Dr. Mozaniel Santana de Oliveira has a Master's degree in Food Science and Technology and PhD also in Food Science and Technology from the Federal University of Pará, Brazil. He has more than 100 scientific contributions among articles in international journals, books, book chapters, and conference abstracts. He has experience in the following scientific areas: engineering, food science and technology, pharmacology and drug discovery, medicinal chemistry, ethnopharmacology and ethnobotany, phytochemistry, extraction methods of bioactive compounds, analytical chemistry, biotechnology of natural products, and allelopathy. Dr Oliveira is currently a researcher in the Institutional Training Program at the Museu Paraense Emílio Goeld, Pará, Brazil.

Dr. Wanessa Almeida da Costa has a PhD in Natural Resources Engineering from the Federal University of Pará (UFPA), Brazil. She also holds a MEng in Chemical Engineering also from the Federal University of Pará, Brazil. Currently, Dr. Wanessa Costa is part of the technical and administrative staff at UFPA, working as a laboratory technician (Chemical Area) at the Faculty of Chemical Engineering. She has experience in Process Simulation, Chemistry, and Food Science and Technology, working mainly in the areas of supercritical extraction; extraction of bioactive compounds of plant origin; applications in allelopathy, and transesterification processes in supercritical medium.

Dr. Sebastião Gomes Silva holds a Master's degree in Organic Chemistry from the Federal University of Pará and PhD in Organic Chemistry, also from the Federal University of Pará, Brazil. He is currently a Class II professor of the state education network of the Pará state, Brazil, and External Collaborating Professor of the Rural Education Course at the Abaetetuba Campus-UFPA. He works in the Chemistry area, with emphasis on Chemistry of Natural Products, mainly in the following themes: extraction of essential oils with supercritical fluids, hydrodistillation, simultaneous distillation and extraction, analysis by gas chromatography/mass spectrometer, and search for of essential oil applications in industries.

Contents

Preface

Plants rich in essential oils (EOs) are viable for use in various human activities and business sectors, particularly the food industry, due to their antioxidant, antimicrobial, phytotoxic, neuroprotective, and anti-inflammatory properties. EOs also have low cytotoxicity, which reduces the risks of intoxication. They are natural volatile fractions extracted from aromatic plants, and are formed in the secondary metabolism of plants. Several classes of volatile substances can be found in their chemical composition, such as fatty acid esters, monoterpenes, sesquiterpenes, phenylpropanoids, alcohols, aldehydes, and in some cases, aliphatic hydrocarbons. This variation in composition depends on physiology, environmental conditions, geographic variations, seasonality, collection period, genetic factors, and plant evolution. As a result, physicochemical properties of EOs can be altered, and oil concentrations in certain plant parts such as stems, leaves, flowers, and fruits can increase or decrease. In nature, EOs play a decisive role in the resistance of plants against phytopathogens and herbivores. EOs are also important in communication, for the plant can use a chemical agent that travels through the atmosphere and may activate defensive genes of other plants. In the oil industry, they are widely studied mainly for their potential applications as agents that promote biological activities. Similarly, volatile compounds have presented over the years several pharmacological properties, such as antioxidant, anticancer, antiprotozoal, antimicrobial, anti-inflammatory, phytotoxic, and neuroprotective activities.
In this sense, this book provides relevant information on applications of essential oils as well as their volatile compounds, and new perspectives on their most diverse uses, aiming to contribute in a systematic way to the dissemination of knowledge in the area of natural products. The book also discusses innovative applications of EOs such as in aromatherapy, control of invasive species, and treatment of breast cancer.

The Academic Editor Dr. Mozaniel Santana de Oliveira thanks PCI-MCTIC/MPEG as well as CNPq for the scholarship process number:302203/2020-6.

Dr. Mozaniel Santana de Oliveira
Museu Emílio Goeldi,
Research Campus Botanical Coordination,
Pará, Brazil

Dr. Wanessa Almeida da Costa and Dr. Sebastião Gomes Silva
Faculty of Chemical Engineering,
Federal University of Pará,
Pará, Brazil

Section 1

Chemical Composition and Biological Activity

Chapter 1

Algae Essential Oils: Chemistry, Ecology, and Biological Activities

Mohamed El Hattab

Abstract

This chapter focuses on the essential oils and volatile fractions of seaweed. It includes an introduction to the essentials and volatile fractions and the main chemical classes found. This part is completed by a presentation of the fundamental aspects of biodiversity and the chemodiversity of the marine environment followed by the taxonomy and systematics of marine macroalgae. The heart of this chapter concerns the chemistry of volatile products extracted from marine algae. It reports the specificities of the marine natural products chemistry in comparison to that of terrestrial organisms. The description of volatile compounds in seaweed is divided into two parts, the first reports the common compounds identified in main volatile fractions and the second cover the specific volatile components. These include C11 hydrocarbons, sulfur compounds, and halogenated hydrocarbons. These latter are playing a very important role in communication and chemical defense. The last part includes aspects of chemical ecology and biological activities of volatile products.

Keywords: essential oils, marine algae, C11 hydrocarbons, sulfur compounds, halogenated sesquiterpenes, chemical ecology, biological activities

1. Introduction

The origin of the distillation methods is an invention attributed to the Arab alchemists and to the Persian scientist Avicenna (980–1037) with the establishment of the steam distillation process. Avicenna invented a setup to prepare essential oils and aromatic waters. Essential oils, sometimes called quintessence, are a very complex mixture of volatile compounds produced by the secondary metabolism in various plant organs (flowers, fruits, seeds, leaves, etc.) and algae. According to ISO and AFNOR standards, essential oils are defined as volatile composition obtained from raw materials by steam distillation and/or by cold expression from citrus peels (known as essences) [1]. The definition of an essential oil excludes other volatile fractions obtained by steam distillation and/or hydrodistillation from the crude extract resulted from solvent extraction, supercritical fluid extraction, solvent- and water-free microwave extraction, ultrasound-accelerated solvent extraction, solid-phase microextraction, and headspace extraction. The chemical composition of essential oils and volatile fraction could be quite similar. Moreover, it should be pointed out the clear difference between the physical and chemical properties of essential oils and fixed or fatty oils. The fixed oils contain mainly triglycerides, esters composed of three saturated fatty acids linked to glycerol, characterized by

high boiling and low volatility. The chemical composition of essential oils is principally composed of terpenes derived from the mevalonate and methylerythritol pathways [2]. Monoterpenes and sesquiterpenes are commonly the main contributor group of compounds identified in several essential oils [3]. Moreover, some essential oils contain other chemical classes, such as phenols (derived from shikimic acid pathway); the saturated and unsaturated fatty acids, acting as biosynthetic precursors; alkanes; and, more rarely, nitrogen and sulfur derivatives [4]. The essential oils play an important role in the allelopathic interaction of plants. They are involved in defense and signaling processes [5] and attraction of pollinating insects [6]. They constitute an important raw material source for the pharmaceutical, food, cosmetics, and perfume industries [7]. The essential oils of different plants exhibit a broad spectrum of biological activities. They show antibacterial activities attributed, in some cases, to the presence of phenolic compounds [8]. The literature reports also the excellent antioxidant [9], anti-inflammatory [10], and cancer chemoprotective activities [11].

2. Marine biodiversity and chemodiversity

More than 70% of the Earth's surface are oceans and seas. It is not surprising to affirm that the marine environment is characterized by an important biodiversity in comparison to terrestrial organisms. In 2010, 230,000 marine species were listed [12]. Consequently, with the increase of biological space (biodiversity), more novel metabolites (high chemodiversity), involved in ecological interactions, are produced in order to ensure easy adaptation of the species [13, 14]. Furthermore, the chemodiversity of the marine ecosystem has no equivalent in terrestrial environment. The large groups of the sea organisms, such as red algae and soft corals, are known to produce a great variety of quite unique secondary metabolites, such as highly halogenated terpenes, definitely due to the high halogen concentration of the sea water, and acetogenins from *Laurencia* (Rhodophyta) [15, 16], toxic polyketide from sponges [17], and prostaglandins from the gorgonian corals [18, 19].

3. Systematics and taxonomy of macroalgae

It was the French botanist Joseph Pitton de Tournefort (1656–1708) who grouped the species into genera and then the Swedish naturalist Carl von Linné (1707–1778), founder of systematics (or taxonomy), who classified the organisms into increasingly large groups: species, genera, families, orders, classes, phylum (or phyla), and kingdoms. Algae, according to Feldmann and Chadefaud [20, 21], are classified into six branches differentiated by the nature of the pigments, the nature and situation of carbohydrate reserves, and the presence or absence, number, and arrangement of flagella:

- Pyrrophycophyta: unicellular marine or freshwater algae
- Euglenophycophyta: unicellular freshwater algae rich in organic matter
- Chrysophycophyta: most are single-celled; freshwater and sea water
- Chlorophycophyta: green algae; single or multi-cell; marine, freshwater, and terrestrial environments

- Phaeophycophyta: brown algae; always multicellular and almost exclusively marine

- Rhodophycophyta: red algae; mainly multicellular and mostly marine

3.1 *Phaeophyceae* (or Fucophyceae)

There are about 2000 species (in 265 genera) of brown algae [22], and less than 1% are known from freshwaters (3–7 genera) [23]. The brown color is due to Fucoxanthin (carotenoid pigment) and in some species to the presence of tannins (phenolic compounds).

3.2 *Chlorophyta*

There are estimated to be at least 600 genera with 10,000 species within the green algae [24] recognized inhabiting mostly in the water's surface of the calmer seas. They are characterized by the presence of chloroplasts with two envelope membranes, stacked thylakoids, and chlorophyll a and b. In their fundamental biochemistry (photosynthetic pigments, storage polysaccharides, etc.), the Chlorophyta resemble the higher plants [24].

3.3 *Rhodophyta*

They are primarily marine in distribution sometimes inhabiting the deep water, with less than 3% (150 species from 20 genera) of the over 6500–10,000 species occurring in truly freshwater habitats [25]. The red algae are characterized by eukaryotic cells, with the complete absence of flagellar structures, food reserves of starch, presence of phycobilins, chloroplasts without stacked thylakoids, and no external endoplasmic reticulum.

4. Chemistry of marine algae volatile compounds

The fragrances of terrestrial plants have aroused human interest since antiquity; they were related to spiritual and civilizational aspects. It is not surprising that the first research work on odorous volatile products was carried out on aromatic plants. Phytochemists have quickly associated the odors emanating from trees and shrubs to terpenes (notably monoterpenes), spices to phenols and derivatives, and fruits and flowers to aldehydes, esters, and ketones. The smell connected with marine flora are much less familiar. Unlike the wide number of terrestrial odoriferous plants, relatively few marine seaweeds possess an attractive odor. Although the natural products chemistry of terrestrial organisms was known before the nineteenth century, the one of the marine derived is more recent, and it has only emerged over the past 75 years. This is due to the complexity to access the marine environment. The marine natural products had become an important subdiscipline of natural products chemistry, which has experienced a particular craze which has led to the isolation and characterization of thousands of secondary metabolites belonging to original chemical skeletons without equivalent in the terrestrial environment.

Historically, volatile oils of terrestrial plants were used in Chinese [26] and Egyptian civilizations [27–29] few centuries ago, whereas the first works on the isolation of volatile products of marine algae were carried out, on the brown alga *Fucus* [30] and the red algae *P. fastigiata* and *P. nigrescens* [31] when the seaweeds are exposed to air, at the beginning of the 1930s, followed later by the Katayama

researches in 1951–1961 [32] and Moore prior to 1966 [33]. The volatile organic compounds in marine algae, as in plants and fungi, released into the seawater, are involved in the chemical communications process; these compounds play an important role as either pheromones or allelochemicals for communication and interaction with the surrounding environment [34, 35]. The species produce the volatile organic compounds in closed relation to their physiology; the algae must adapt to abiotic stresses of their ecosystem [36]. The volatile components of marine algae contain a mixture of chemical classes such as terpenes, hydrocarbons, fatty acids, esters, alcohols, aldehydes, ketones [37–41], C11-hydrocarbons [33, 42], polyphenols and derivatives [43, 44], and halogenated [45] and sulfur compounds [46, 47]. The distinctive ocean smell is due to the presence of terpenes, but particularly, to a fraction of acyclic and cyclic non-isoprenoid C11-hydrocarbons acting as pheromones and playing an important role in the chemical communication [48], it seems to be most abundant in brown algae of the genus *Dictyopteris* [33]. As for terrestrial plants, the monoterpenes identified in algae such as linalool, citral, geraniol, and terpinolene, 1,8-cineole, α-pinene and β-pinene, and eugenol and isoeugenol could be valued in perfumery. While the disagreeable odor is related to amines and halogenated, sulfurous, and other specific compounds [49], the dimethyl sulfide, mainly distributed in Chlorophyta and in some Rhodophyta [50], has a very unpleasant odor molecule. It results from the enzymatic cleavage of dimethyl-2-carboxyethylsulfonium hydroxide, from the green algae species (*E. intestinalis* and *A. centralis*) [51].

4.1 Common volatile organic compounds of macroalgae

4.1.1 Hydrocarbons and oxygenated hydrocarbons

The alkanes and alkenes are common compounds in the majority of volatile fraction and essential oils of marine macroalgae. The chemical composition reveals the presence of the linear and branched saturated hydrocarbons from C_7 to C_{36} [37, 52–54], the unsaturated hydrocarbons from C_8 to C_{19} with the presence of 1 [37, 52–54] to 4 degrees of unsaturation [55] in the volatile fraction obtained by several extraction techniques. We also noted the presence of mono- and di-alcohol of C_4–C_{18} [37, 52–54, 56, 57]. Some short-chain (C_6, C_9) and middle-chain (C_{10}) aliphatic aldehydes are formed in marine algae from fatty acids (C_{20}), whereas they are formed from C_{18} in higher plants [58–60]. Also, it has been reported that long-chain aldehydes (C_{14}, C_{17}) of the green alga *U. pertusa* [61, 62] are formed by decomposition of fatty acids through the corresponding 2-hydroperoxy acid; this later are encountered in a variety of marine algae [63, 64].

In addition to aldehydes, the ketone compounds were commonly reported in the aroma composition of algae [65]; the presence of β-ionone and 6-methyl-5-hepten-2-one which are formed via the oxidative cleavage of carotenoids such as lycopene and phyotene was mentioned [66]. β-ionone, present in several essential earth oils, is a powerful odorant for the perfume industry. 6-methyl-5-hepten-2-one, in addition to its pleasant fragrant note, is often used as an intermediary in the synthesis of several monoterpenes highly valorized in perfumery. In addition, other simple ketone (C_6—C_{19}) compounds such as maltol [53], octan-3-one [57], nonacosan-2-one [67], and undeca-1,4-dien-3-one [42] are identified in the volatile fractions of algae. Saturated fatty acids from C_3 to C_{18} and their ester derivatives have also been identified in the chemical composition of volatile algae fractions [37]. Unsaturated fatty acids and their corresponding esters, in particular Eicosa-5,8,11,14-methyltetraenoate and Eicosa-5,8,11,14,17-methyl-pentaenoate [42], are usually found; this is probably related to their implications in biosynthetic processes of other metabolites.

4.1.2 Amine compounds

The amine compounds have been described several times in marine algae [68–70]; the small amine molecules such as methyl amine, dimethylamine, ethylamine, and propylamine were found in algae [71]. The volatile amines in algae result from decarboxylation of amino acids [71]. Although present in brown and green algae, the amine compounds were especially found in red algae.

4.1.3 Halogen compounds

The volatile halogen compounds are rare in terrestrial plants, but quite habitual in marine algae because of the presence of chlorine and bromine ions at a high concentration in seawater. The red algae possess the highest abundance of halogenated organic compounds, which are found as terpenoid, phenols, carbonyl compounds, and fatty acid-derived metabolites [45]. They were produced in marine algae and emitted into the atmosphere; the highest amounts of brominated compounds released were done by *L. saccharina* [72]. Chemical investigations of marine algae have shown the presence of 2-bromophenol, 2,4-dibromophenol, and 2,4,6-tribromophenol in numerous red, green, and brown algae. It has been reported the biosynthesis of bromophenols in *U. lactuca* via the bromoperoxidases in the presence of precursors such as phenol, 4-hydroxybenzoic acid, and 4-hydroxybenzyl alcohol [73]. The bromoperoxidases are involved in the biosynthesis of brominated alkanes, such as $CHBr_3$, CH_2Br_2, $CHClBr_2$, and others in several marine organisms, among them, the red alga *Asparagopsis* sp. [74]. The biosynthesis of organohalogens has known enormous interest as reported in several literature review [75–77]. As indicated for bromocompounds, the iodoperoxidases are responsible of the production of iodinated compounds in marine algae [78–80]. The chemical investigation of 29 macroalgae species reveals their release of volatile iodocompounds iodoethane, 1-iodopropane, 2-iodopropane, 1-iodo-2-methylpropane, 1-iodobutane, 2-iodobutane, diiodomethane, and chloroiodomethane [81]; it has reported that diiodomethane was the main iodinated compound released by brown macroalgae [82].

4.1.4 Terpenoid compounds

Terpenes, or terpenoids, are a large and diverse class of plant secondary metabolites, produced by numerous varieties of plants and algae from isoprene building blocks; they play a major ecological role, most notably in defense against plant-feeding insects and herbivores [82]. However, some terpenoids are involved in primary metabolism, such as stability of cell membranes and photosynthesis. The terpenes display enormous structural diversity, are the main constituents of essential oils of terrestrial plants and seaweeds [83], and are characterized by their pleasant strong odor. The terpenoids are biosynthesized mainly via two pathways, the mevalonate pathway and the MEP pathway. The chemical screening of volatile fraction and/or essential oils of algae reveals the presence of high content of monoterpenes and sesquiterpenes and rarely diterpenes [42]. The most significant acyclic monoterpenes found in algae are myrcene (1), ocimene (2), geranial (3), neral (4), citronellol (5), and geraniol (6) (**Figure 1**). Moreover, the most odoriferous compounds identified in algae are included in the acyclic group of monoterpenes [84].

Likewise, the most common monocyclic algae volatile oil is 1,8-cineole (8) [84], while α-pinene (9) and β-pinene (10) are the most commonly reported of bicyclic monoterpenes (**Figure 2**) [84, 85]. Sesquiterpenes from marine macroalgae constitute a large group, compared to monoterpenes, of secondary metabolites [86]; some of them are halogenated [87]. Some of the algae sesquiterpenes act as

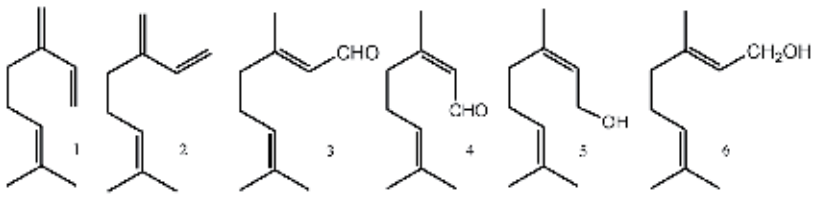

Figure 1.
Common acyclic monoterpenes of algae.

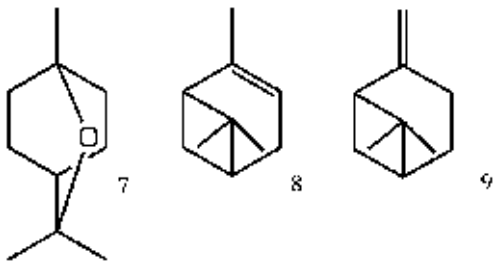

Figure 2.
Most representative monocyclic and bicyclic monoterpenes of algae.

semiochemicals, chemical defense agents, and/or pheromones. They may be acyclic, cyclic, or bicyclic, including several original structures. Among all marine macroalgae, the genus *Laurencia* (red algae) is the most potent source of sesquiterpenes.

The most common sesquiterpenes reported in marine algae (10–53) are grouped in **Table 1** and illustrated in **Figure 3**. The only diterpene and triterpene described as volatile compounds are, respectively, phytol and squalene. Phytol is a degradation product of chlorophyll and the precursor of vitamin E. The squalene is via the epoxy squalene, the biosynthetic precursors of triterpenes and steroids.

4.2 Specific volatile compounds of macroalgae

4.2.1 Odoriferous C11 hydrocarbons from brown algae (Phaeophyta)

The brown algae produce a variety of volatile derivatives whose chemical nature and biological function are different from those of red algae. They are hydrocarbons with 11 carbon atoms without halogens which can be classified according to their chemical structure into four groups [94]: (a) derivatives of cyclopropane, (b) derivatives of cyclopentene, (c) derivatives of cycloheptadiene, and (d) acyclic olefins. The only volatile hydrocarbon with eight carbon atoms identified in brown algae is fucoserratene. These metabolites, which are known in all the species of *Phaeophyceae*, are not specific to an order or a family. They have been isolated from diverse groups of brown algae (e.g., the *Zonaria*, *Desmarestia*, *Dictyota*, *Ectocarpus*, *Laminaria*, and *Fucus*); it appears to be most abundant in brown algae of the genus *Dictyopteris* [95].

They are involved in the reproduction process of the alga; they are sex pheromones. To date, it has been revealed that these algal pheromones are involved at least in three well-defined ecological interactions [96]: (i) synchronization of the mating of male and female cells by the controlled release of male spermatozoids, (ii) enhancement of the mating efficiency by attraction, and (iii) chemical defense of the plant due to the presence of high amounts of pheromones within and release from the thalli into the environment. Furthermore, the relationship between

N°	Compounds	Species and References	N°	Compounds	Species and References
10	(E)-Farnesene	*D. m.*[88] *D. d.* [89]	32	Germacrene D	*D. m.*[92] *D. div.*[96]
11	Hexahydro Farnesyl-acetone	(*B. f., C. m., C. g., C. e., P. d., L. p., L. c.*).[90] *C. v.*[91]	33	Sativene	*D. m.*[92]
12	α-Cubebene	*D. m.*[92] *D. d.*[89]	34	Zonarene	*D. m.*[92]
13	β Cubebene	*D. m.*[92] *D. d.*[89]	35	Vulgarol	*D. m.*[92]
14	α-Copaene	*D. m.*[92] *D. d.*[89]	36	4,4a,5,6,7,8-hexahydro-5-methyl 8 (1 methylethyl) 2(3H)naphtalenone	*D. m.*[92]
15	(-)-Copaene	*D. Div.*[93]	37	1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methyl ethyl) Naphtalène	*D. m.*[92]
16	δ-Cadinene	*D. m.*[92] *Z. m.*[93] *D. d.*[89]	38	Epi bicyclo sesqui phellandrene	*D. m.*[92]
17	γ-Cadinene	*D. div.*[94] *D. d.*[89]	39	Cyclosativene	*D. d.*[89]
18	α Cadinol	*P. t.*[74] *U. p.*[94]	40	α-Ylangene	*D. d.*[89]
19	δ-Cadinol	*Z. m.*[93] *D. div.*[94]	41	γ-Muurolene	*D. d.*[89]
20	1,10-di-epi-cubebol	*D. m.*[92]	42	β-Cadinene	*D. d.*[89]
21	Cubenol	*D. div.*[94], *D. p.*[96], *P. t.*[74], *U. p.*[94]	43	Ar curcumene	*D. d.*[89]
22	Epi-Cubenol	*Z. m.*[93]	44	Bicyclogermacrene	*D. d.*[89]
23	Cadalene	*D. div.*[93]	45	Epizonarene	*D. d.*[89]
24	α-Calacorene	*D. m.*[92]	46	α-Muurolene	*D. d.*[89]
25	β-Bourbonene	*D. m.*[92], *D. d.*[89]	47	δ-Selinene	*D. d.*[89]
26	Azulene	*D. m.*[92]	48	trans-γ-Bisabolene	*H. f.*[95]
27	Axenol	*D. m.*[92]	49	trans-Cadina-1,4-diene	*D. d.*[89]
28	Aromadendrene	*D. m.*[92] *D. d.*[89]	50	α-Cadinene	*D. d.*[89]
29	α-Amorphene	*D. m.*[92] *D. d.*[89]	51	Germacrene B	*D. d.*[89]
30	Alloicanol	*D. m.*[92]	52	Dendrolasin	*H. f.*[95]
31	β Elemene	*D. d.*[89]	53	τ-Muurolol	*D. d.*[89]

Abbreviation: D. m., Dictyopteris membranacea; D. d., Dictyota dichotoma; B. f., Bangia fuscopurpurea; C. m., Cystoseira mediterranea; C. g., Callithamnion granulatum; C. e., Cystoseira elegans; P. d., Polysiphonia denudata; L. p., Laurencia papillosa; L. c., Laurencia coronopus; C. v., Cladophora vagabunda; D. div., Dictyota divaricata; Z. m., Zostera marina; P. t., Pyropia tenera; U. p., Ulva pertusa; D. p., Dictyota prolifera; H. p., Halopteris filicina.

Table 1.
Most common sesquiterpenes of macroalgae [88–93].

structures of pheromones and the taxonomic classifications of algae are still not established. Until now, a series of 12 (54–65) hydrocarbons and epoxides (**Figure 4**) have been characterized, and more than 50 stereoisomers are known within the pheromone bouquets of more than 100 different species of brown algae [48, 96–99].

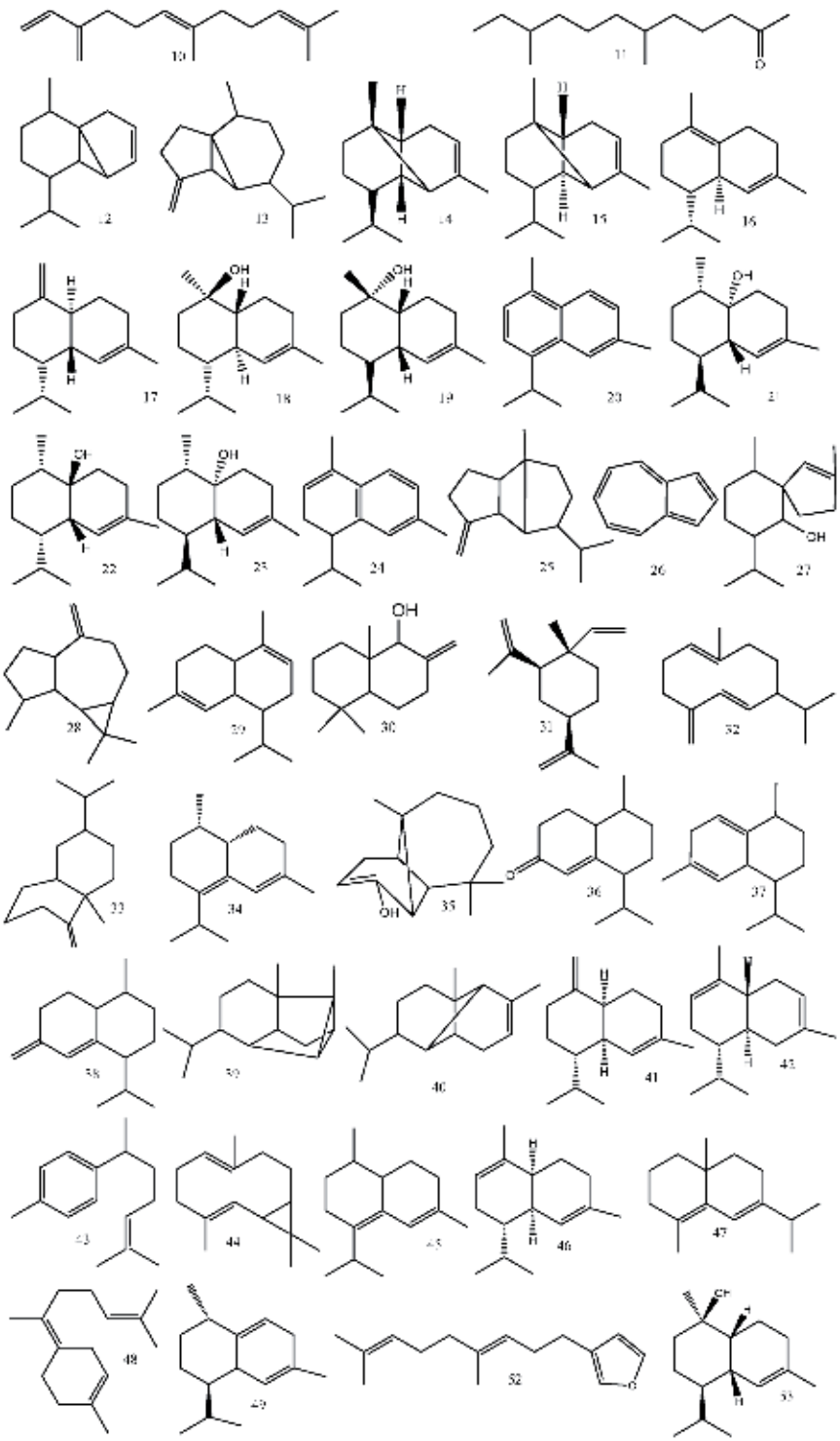

Figure 3.
Common sesquiterpenes described in volatile oil of marine algae.

Moreover, the presence of C11 hydrocarbons is not only limited to marine brown algae. The same compounds have been reported in cultures of diatoms [100], the volatile fraction released during blooms of microalgae in freshwater lakes [101] and,

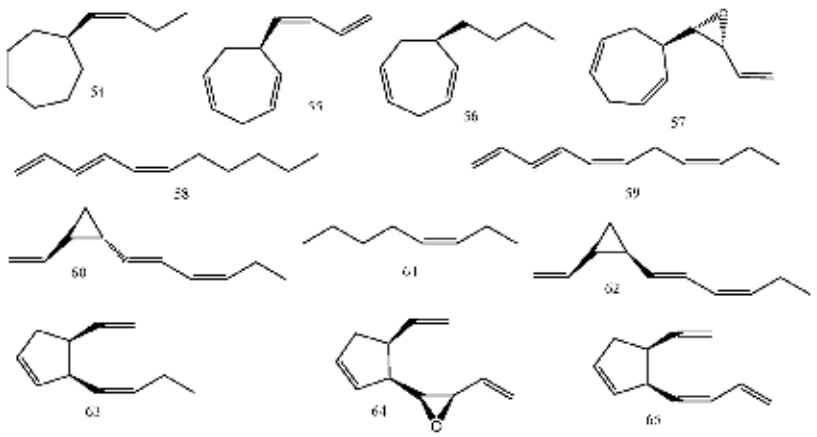

Figure 4.
Pheromones of brown algae.

inquisitively, in higher plants [102, 103]. **Table 2** reports the pheromones described in **Figure 4**, the algae from which they are derived, as well as their attraction or release activities. In comparison to the number of brown algae species, the chemodiversity of pheromones is relatively limited, so, the semiochemical activity of the same molecule is noted in more than one species. Female gametes secrete a mixture of products, not just one pheromone and depending on species; released pheromones are either optically pure or enantiomeric mixtures.

N°	Pheromones	Release/ Attraction (R/A)	Algal species
54	Teloecarpene	A	*Scytosiphon sp. E. fasciculatus, A. tuberculoris, S. rigidula*
55	Desmarestene	A/R A	*D. aculeata, D. viridis* *C. spongiosus, D. firma*
56	Dictyotene	A	*D. dichotoma , D. diemensis, D. prolifera*
57	Lamoxirene	A/R	*L. angustata, L. nuclari* *L. japonica, L. digitata, L. hyperborea* *L. saccharina, Pleurophycus, A. clavigera, A. esculenta, A. marginata, E. radiata, E. arborea, P. california, U. pinnatifida, D. reticulata, L. variegata, L. litoralis, M. integrifolia, M. pyrifera, N. luetkeana, P. porra, A. arbuscula, C. triplicata, H. sessile, K. gyrata*
58	Cyclophorene	A	*C. subquasa.*
59	Finavarrene	R	*P. calliarthra*
60	Hormosirene	A	*H. banksii, X. chondrophylla, X. gladiata, D. antarctica, D. pottsiana, D. willana, C. peregrina, C. bullosa, A. mirabilis, M. simplex, S. lomentaria.*
61	Fucoserratene	A	*F. serratus, F. spiralis, F. vesiculosus.*
62	Prefucocarpene	A	*F. vilkidosus*
63	Multifidene	A/R	*C. multifida, Z. angustata, C. tomentosa*
64 65	Caudoxirene Viridene	R A R/A	*P. caudata, D. fenicalaceus.* *S. phillmeyi* *D. viridis*

Table 2.
C11 and C8 pheromone activities from marine brown algae.

However, it has been verified that the biological activity is associated with a single constituent which may not be the major product. These by-products sometimes play a role of modulator of response of the gametes, and in general, they do not have a determined biological function [94].

4.2.2 Sulfur compounds in the genus Dictyopteris

The organic sulfur compounds are widespread in terrestrial and marine plants [104]. Due to the relatively high sulfate concentration in seawater, and the particularly high sulfide concentration in anoxic environments, it was expected that many sulfides would occur in the marine environment [104]. They are reported in few taxa and act as chemical defenses against herbivores [105]. As part of this single group, some *Dictyopteris* species (*Phaeophyceae, Dictyotales*) are acknowledged to produce considerable amounts of sulfur-containing compounds (**Figure 5**); many of them were found in *D. polypodioides* [106]. Among the first seaweeds discovered to produce organic sulfur compounds were the Hawaiian brown algae

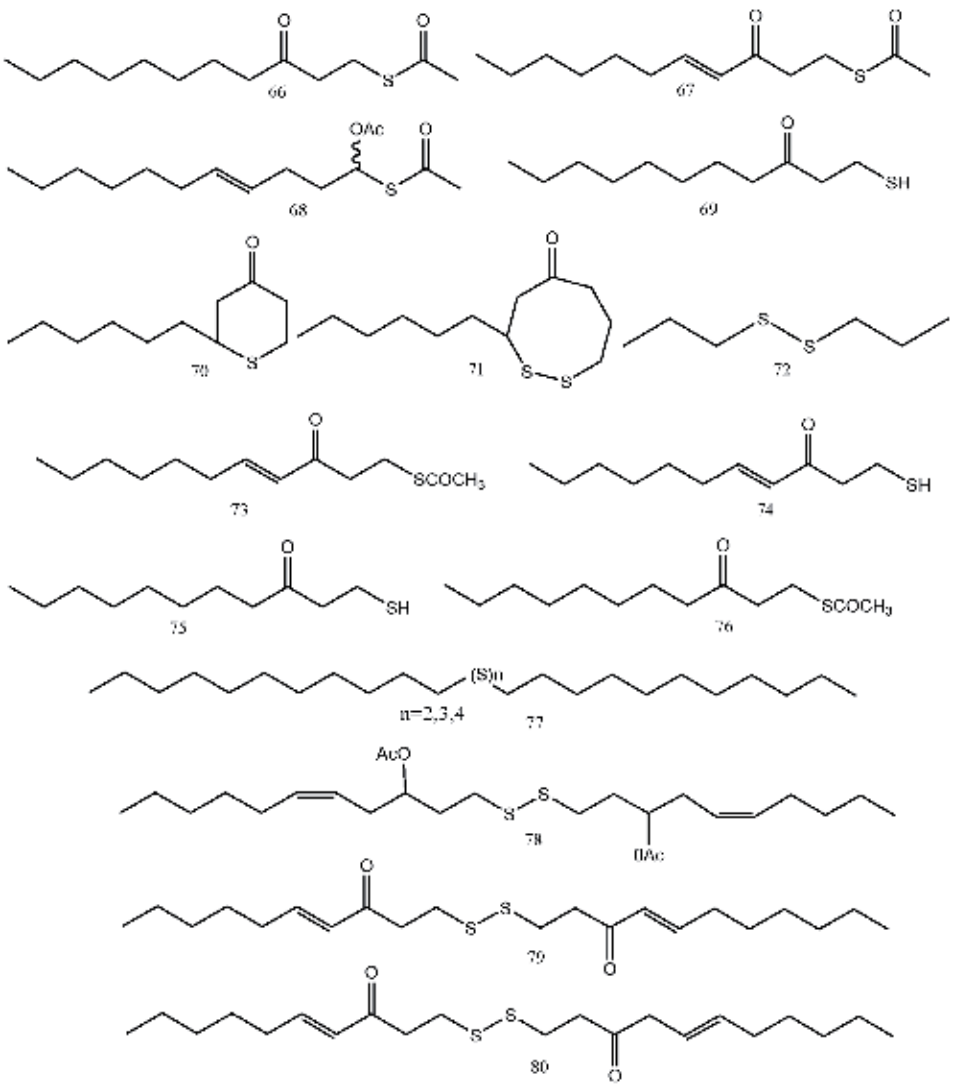

Figure 5.
Sulfur compounds of the genus Dictyopteris.

D. plagiogramma and *D. australis* [107]. Eight compounds containing a C11 unit attached to a sulfur atom with oxygen substituent at C-3 have been isolated and characterized [47]; most of these compounds appear to be biosynthetically related to C11 hydrocarbon pheromones and may originate from oxidative degradation of highly unsaturated eicosanoids (arachidonic acid) via oxygenated intermediates. The 1-undecen-3-ol, present in essential oils from *Dictyopteris* spp., may represent the common precursor to both classes of C11 compounds [95, 107]. The C11 sulfur metabolites seem to be restricted to the *Dictyopteris* genus.

4.2.3 Halogenated terpenes from red algae (Rhodophyta)

As noted previously, the halogenated compounds are common in the marine environment. They are formed among diverse species such as bacteria, sponges, molluscs, algae, and several marine worms. Among all marine algae, the Rhodophyta class possesses a privileged biosynthetic pathway for organohalogen compounds. A huge number of organohalogens have been isolated from most genera of Rhodophyta [108, 109]. The genus *Laurencia* is the most prolific source of sesquiterpenes among all marine macroalgae, most notably, the halogenated sesquiterpenes belonging to a variety of chemical skeletons including chamigrane, bisabolane, laurane, snyderane, and brasilane along with some rearranged derivatives [110, 111]. Inquisitively, bromine is the most occurring halogen in marine natural products, despite that its concentration in seawater is lower than that of chlorine. To the best of our knowledge, the isolation of halogenated monoterpenes is limited to three families of marine red algae, the *Plocamiaceae* and *Rhizophyllidaceae* [112, 113], and *Ceramiaceae* [114]. The chemical structure of Rhodophyta monoterpenes is characterized by multiple halogen substitutions (chlorine and bromine) and by uncommon carbon cycle structures in the case of cyclic compounds. All halogenated acyclic seaweed monoterpenes appear to be derived from the halogenation of myrcene or ocimene [114]. As indicated in the rich bibliography dedicated to this purpose [45, 113, 115–117], the almost majority of halogenated terpenoids (monoterpenes, sesquiterpenes, and diterpenes) described in red algae are isolated from crude solvent extracts. Monoterpenes, even halogenated, are characterized by high volatility; they are the main constituents of essential oils and volatile fractions. The selective supercritical fluid extraction, by adjusting time and pressure, of Santa Cruz *P. cartilagineum* [118] has allowed the isolation of eight halogenated monoterpenes (81–87) (**Figure 6**).

The same species collected along the central coast of Chile [119] conduct to the isolation of eight monoterpenes (88–95), four of which are based on the 1-(2-chlororovinyl)-2,4,5-trichloro-1,5-dimethylcyclohexane skeleton (**Figure 7**). As in the genus *Plocamium*, the chemical study of the genera *Portieria* [120], *Ochtodes* [121], and *Microcladia* [114, 122] has led to the isolation of over 100 of acyclic, cyclic, and tetrahydrofuran halogenated monoterpenes. A large number of halogenated sesquiterpenes, more than monoterpenes, were described in red algae especially in the genus *Laurencia* (Ceramiales). Although the sesquiterpenes are also volatile compounds, we describe in this paragraph only the ones reported in the chemical composition of essential oils and volatile fraction of red algae.

The first brominated sesquiterpene (**Figure 8**) ketone spirolaurenone (96), chamigrane skeleton, was described in the essential oil of *L. glandulifera* (Japan) in 1970 [123], followed by the 10-Bromo-7-chamigren-2-one (97) in the same species [124]. The preintricatol (98), found in *L. gracilis* [125], seem to be the precursor of halogenated sesquiterpenes of chamigrene type. The Puertitols A (99) and B (100) were isolated from *L. obtusa* [126] as well as the metabolites (101) and (102) from *L. caespitosa* [127]. An important halosesquiterpene characteristic of the family

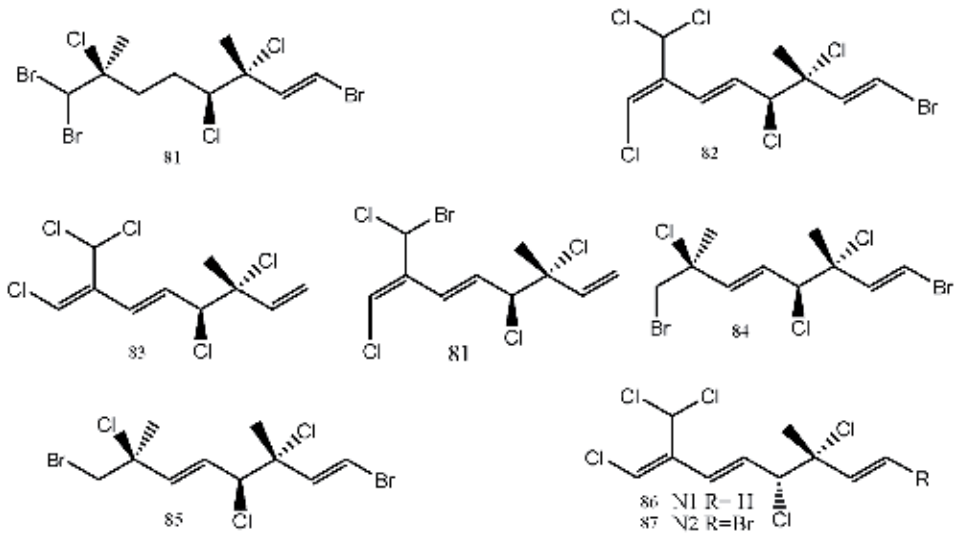

Figure 6.
Monoterpenes isolated Santa Cruz Plocamium cartilagineum.

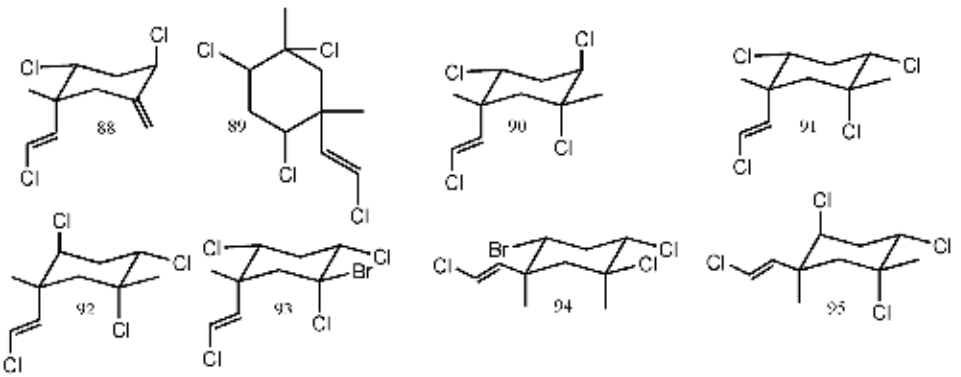

Figure 7.
Monoterpenes isolated from Plocamium cartilagineum *(Chile).*

Rhodomelaceae is elatol (103); it is isolated from *L. elata* [128] and from several other species of *Laurencia* [129]. An exhaustive literature review has described the chemical structure data and biological activities of the halogenated sesquiterpenes of red algae [130, 131].

4.2.4 Ecology

The volatile compounds play an important role in the inter- and intraspecies chemical communication in marine algae. They act as pheromones [97] or allelochemicals, chemical defenses against herbivores [132, 133], and inhibition of bacterial and fungal biofilms [134]. The genus *Dictyopteris* produce a high amount of C11 hydrocarbons, some of which act as pheromones that stimulate gamete release or attract sperm during sexual reproduction [96]. The first male-attracting metabolite was elucidated as ectocarpene (54) [135] which shows a moderate activity at 10 mM. A subsequent study revealed that the real pheromone used by the female gamete was pre-ectocarpene (62) which is active at 5 pM. In fact, the alga produces pre-ectocarpene which undergoes a thermal rearrangement (Cope rearrangement)

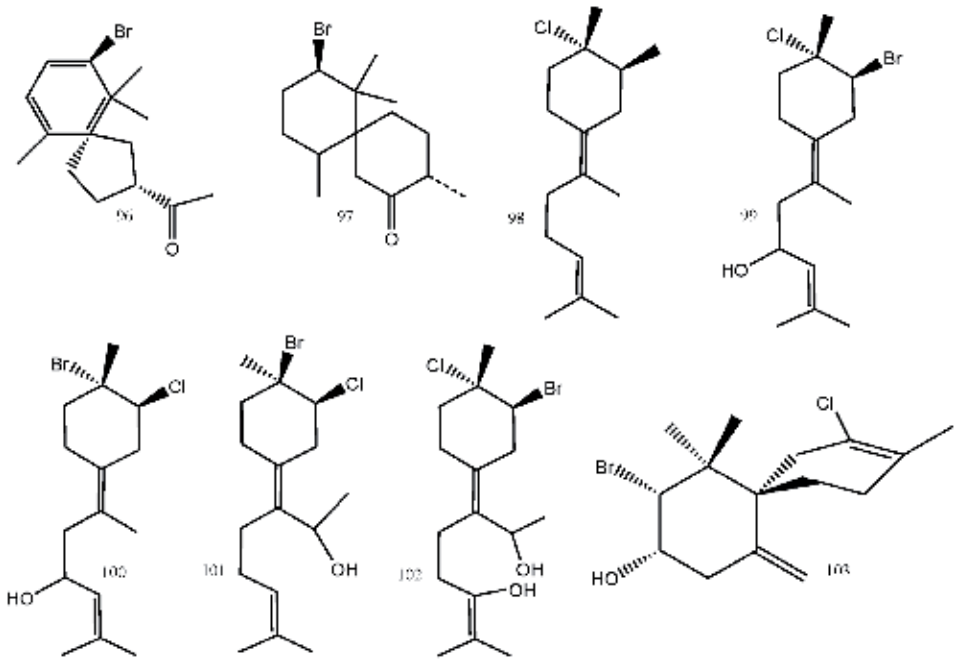

Figure 8.
Halogenated sesquiterpenes from red the genus Laurencia.

to lead to ectocarpene [136]. The Cope rearrangement occurs between the time of the releasing and attraction of the pheromone; the sigmatropic transformation serves as a natural control mechanism for deactivation of the pheromone [136]. The genus *Dictyopteris* produces significant amounts of C11-sulfur compounds which are involved in chemical defense [137].

In green algae, the volatile compounds, such as (Z)-8- heptadecane, act also as allochemicals [61]. In the genus *Caulerpa*, the caulerpenyne is the most abundant cytotoxic sesquiterpene produced by *C. taxifolia* and *C. racemosa* [138, 139]. It is involved either in the chemical defense of the plant against herbivore or within the framework of interspecific competition as antifeedant and/or antifouling activities [140]. In red algae, the halogenated organic compounds are produced, probably, to be involved in the defense system against microorganism infection [141], herbivore attack [141], space competitors [142], and harmful fouling by different types of epiphytes [142].

4.2.5 Biological activities

There are several reports of secondary metabolites, among them are numerous volatile compounds, derived from macroalgae which exhibit a broad range of biological activities such as antibiotics [40, 143].

The essential oil of *D. membranacea* has shown a strong antibacterial activity against *Staphylococcus aureus* and *Agrobacterium tumefaciens*, which is translated by an MIC of 1519 μg/mL [106]. The volatile oil of *P. pavonica* possesses a moderate antimicrobial activity against *Staphylococcus aureus* and *Candida albicans* [144]; antifungal against *Macrophomina phaseolina*, *Rhizoctonia solani*, and *Fusarium solani* [145]; cytotoxicity against KB cells [146]; and antitumor activity against lung and human carcinoma cell lines [147]. On the other hand, the volatile of *H. clathratus* showed a pronounced antimicrobial activity against *S. cerevisiae* compared with Canesten as reference material [148].

The cytotoxicity is the most common activity observed for halogenated organic compounds isolated from the family Rhodomelaceae. A large number of these compounds were shown to be cytotoxic to a wide range of cancer cell lines [115].

Among many of the halogenated sesquiterpenes evaluated for their in vitro cytotoxic effects against HeLa and HEP-2 cancer cell lines, and against nontumoral VERO cells, during both lag- and log-phase cell growth [149], elatol (103) turned out the most active compound with IC50 values of 4.1 and 1.3 μM to HeLa, 2.4 and 2.0 μM to HEP-2, and 2.3 and 25.0 μM to VERO cells, in lag- and log-phase, respectively [150]. Further studies were carried on the evaluation of the cytotoxicity against several tumor cell lines of chamigrane [150] and Laurane- and Cuparane-type sesquiterpenes and were found to display a wide range of potency levels [151, 152]. Other activities of halosesquiterpenes such as antibacterial activity [153], antifungal activity [154], and antiviral activity [155] were investigated and conducted to promising results.

5. Conclusion

Essential oils from terrestrial plants have been known for a very long time. They have been applied in different domain, particularly in aromatherapy. Essential oils from seaweed are much more recent. The fragrant note of marine origin is becoming more and more interesting among perfumers, the species of the genus *Dictyopteris* and *Dictyota* could be considered as the best example. This importance is related to the great biodiversity and chemodiversity of the marine environment compared to the terrestrial environment. The chemical composition of essential oils and volatile fractions of macroalgae contains compounds usually found in terrestrial essential oils. These include hydrocarbons, oxygenated hydrocarbons, terpenes, and fatty acids. However, they contain specific products such as halogenated products, C11 hydrocarbons, sulfur compounds, and halogenated terpenes. The specific compounds play a very important role on the chemical ecology; they are involved in defense mechanisms and chemical communication. The volatile fractions of algae show a broad spectrum of biological activity, such as antibacterial, antifungal, anticancer, and antibiotic activities.

Acknowledgement

This work is supported by the General Directorate of Scientific Research and Technological Development (DGRSDT)—Algeria.

Author details

Mohamed El Hattab
Laboratory of Natural Products Chemistry and Biomolecules, Chemistry Department, Faculty of Sciences, University of Blida 1, Algeria

*Address all correspondence to: elhattab@univ-blida.dz

References

[1] Abdul Aziz ZA, Ahmad A, Mohd Setapar SH, Karakucuk A, Azim MM, Lokhat D, et al. Essential oils: Extraction techniques, pharmaceutical and therapeutic potential – A review. Current Drug Metabolism. 2018;**19**:1-12. DOI: 10.2174/1389200219666180723144850

[2] Hirsch AH, Diederich F. The non-Mevalonate pathway to isoprenoid biosynthesis: A potential source of new drug targets. Chimia. 2008;**62**(4): 226-230. DOI: 10.2533/chimia.2008.226

[3] Hüsnü Can Baser K, Buchbauer G. Handbook of Essential Oils: Science Technology and Applications. 2nd ed. Boca Raton London: Taylor and Francis; 2016

[4] Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils—A review. Food and Chemical Toxicology. 2008;**46**:446-475. DOI: 10.1016/j.fct.2007.09.106

[5] Vallet M, Strittmatter M, Murúa P, Lacoste S, Dupont J, Hubas C, et al. Chemically-mediated interactions between macroalgae, their fungal endophytes, and protistan pathogens. Frontiers in Microbiology. 2018;**9**(3161):13. DOI: 10.3389/fmicb.2018.03161

[6] Cseke LJ, Kaufman PB, Kirakosyan A. The biology of essential oils in the pollination of flowers. Natural Product Communications. 2007;**2**:1317-1336. DOI: 10.1177/1934578X0700201225

[7] Sarkic A, Stappen I. Essential oils and their single compounds in cosmetics—A critical review. Cosmetics. 2018;**5**(11): 1-21. DOI: 10.3390/cosmetics5010011

[8] Golkar P, Moattar F. Essential oil composition, bioactive compounds, and antioxidant activities in Iberis Amara L. Natural Product Communications. 2019;**40**(5):1-8. DOI: 10.1177/1934578X19846355

[9] Koh K, Pearce A, Marshman G, Finlay-Jones J, Hart P. Tea tree oil reduces histamine-inducedsk in inflammation. The British Journal of Dermatology. 2002;**147**:1212-1217. DOI: 10.1046/j.1365-2133.2002.05034.x

[10] Maruyama N, Sekimoto Y, Ishibashi H, Inouye S, Oshima H, Yamaguchi H, et al. Suppression of neutrophil accumulation in mice by cutaneous application of geranium essentialoil. Journal of Inflammation. 2005;**2**:1-21. DOI: 10.1186/1476-9255-2-1

[11] Edris AE. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: A review. Phytotherapy Research. 2007;**21**:308-323. DOI: 10.1002/ptr.2072

[12] Bouchet P. The magnitude of marine biodiversity. In: Duarte CM, editor. The Exploration of Marine Biodiversity. Scientific and Technological Challenges. Bilbao: Fundacion BBVA; 2006. pp. 31-62. ISBN: 84-96515-27-3

[13] Becerra JX, Noge K, Venable DL. Macroevolutionary chemical escalation in an ancient plant-herbivore arms race. Proceedings of the National Academy of Sciences. 2009;**106**(43):18062-18066. DOI: 10.1073/pnas.0904456106

[14] Becerra JX. Synchronous coadaptation in an ancient case of herbivory. Proceedings of the National Academy of Sciences. 2003;**100**(22):12804-12807. DOI: 10.1073/pnas.2133013100

[15] Aydogmus Z, Imre S, Ersoy L, Wray V. Halogenated secondary metabolites from *Laurencia obtusa*. Natural Product Research. 2004;**18**(1):43-49. DOI: 10.1080/1057563031000122086

[16] Wanke T, Philippus AC, Zatelli GA, Vieira LFO, Lhullier C, Falkenberg M. C15 acetogenins from the Laurencia complex: 50 years of research – An overview. Revista Brasileira de Farmacognosia. 2015;**25**:569-587. DOI: 10.1016/j.bjp.2015.07.027

[17] Epifanio RDA, Pinheiro LS, Alves NC. Polyketides from the marine sponge plakortis angulo spiculatus. Journal of the Brazilian Chemical Society. 2005;**16**(6B):1367-1371. DOI: 10.1590/S0103-50532005000800010

[18] Di Costanzo F, Di Dato V, Ianora A, Romano G. Prostaglandins in marine organisms: A review. Marine Drugs. 2019;**17**:428. DOI: 10.3390/md17070428

[19] Imbs AB. Prostaglandins and oxylipins of corals. Russian Journal of Marine Biology. 2011;**37**:325-334. DOI: 10.1134/S1063074011050075

[20] Chandler CJ, Wilts BD, Brodie J, Vignolini S. Structural color in marine algae. Advanced Optical Materials. 2017;**5**:11. DOI: 10.1002/ adom. 201600646

[21] Pereira L, Neto JM. Marine Algae Biodiversity, Taxonomy, Environmental Assessment, and Biotechnology. Boca Raton: CRC Press Taylor & Francis Group; 2015. p. 397

[22] Van den Hoek C, Mann DG, Jahns HM. Algae. An Introduction to Phycology. Cambridge U.K.: Cambridge Univ. Press; 1995. ISBN: 0521304199 – 0521316871

[23] Silberfeld T, Rousseau F, de Reviers B. An updated classification of Brown algae (*Ochrophyta*, *Phaeophyceae*). Cryptogamie Algologie. 2014;**35**(2):117-156. DOI: 10.7872/crya. v35.iss2.2014.117

[24] Fang L, Leliaert F, Zhang Z-H, Penny D, Zhong B-J. Evolution of the Chlorophyta: Insights from chloroplast phylogenomic analyses. Journal of Systematics and Evolution. 2017;**55**(4):322-332. DOI: 10.1111/ jse.12248

[25] Guiry MD, Guiry GM. AlgaeBase. World-wide Electronic Publication. National University of Ireland, Galway; 2017. Available from: http://www. algaebase.org. [Accessed: 20 May 2017]

[26] Gnatta JR, Kurebayashi LFS, Turrini RNT, Silva MJP. Aromatherapy and nursing: Historical and theoretical conception. Revista da Escola de Enfermagem da U.S.P. 2016;**50**(1):127-133. DOI: 10.1590/ S0080-623420160000100017

[27] Aboelsoud NH. Herbal medicine in ancient Egypt. Journal of Medicinal Plant Research. 2010;**4**(2):82-86. DOI: 10.5897/JMPR09.013

[28] Farag RS, Daw ZY, Hewedi FM, El-Baroty GSA. Antimicrobial activity of some Egyptian spice essential oils. Journal of Food Protection. 1989;**52**(9):665-667. DOI: 10.4315/0362-028X-52.9.665

[29] Viuda-Martos M, Mohamady M, Fernández-López J, El Razik KA, Omer E, Pérez-Alvarez J, et al. In vitro antioxidant and antibacterial activities of essentials oils obtained from Egyptian aromatic plants. Food Control. 2011;**22**:1715-1722. DOI: 10.1016/j.food cont.2011.04.003

[30] Heavisides E, Rouger C, Reichel AF, Ulrich C, Wenzel-Storjohann A, Sebens S, et al. Seasonal variations in the Metabolome and bioactivity profile of *Fucus vesiculosus* extracted by an optimised, pressurised liquid extraction protocol. Marine Drugs. 2018;**16**:503. DOI: 10.3390/md16120503

[31] Battley EH. Advances in microbial ecology. Volume 11. K. C. Marshall. The Quarterly Review of Biology. 1992;**67**(2):209. DOI: 10.1086/417584

[32] Katayama T. Volatile Constituents. In: Lewin RA, editor. Physiology and Biochemistry of Algae. New York: American Press; 1962. pp. 467-472

[33] Moore RE. Volatile compounds from marine algae. Accounts of Chemical Research. 1977;**10**:40-47. DOI: 10.1021/ar50110a002

[34] Dudavera N, Negre F, Nagegowda DA, Orlova I. Plant volatiles: Recent advances and future perspectives. Critical Reviews in Plant Sciences. 2006;**25**:417-440. DOI: 10.1080/07352680600899973

[35] Wiesemeier T, Hay M, Pohnert G. The potential role of wound activated volatile release in the chemical defence of the brown alga *Dictyota dichotoma*: Blend recognition by marine herbivores. Aquatic Sciences. 2007;**69**:403-412. DOI: 10.1007/s00027-007-0889-y

[36] Kajiwara T, Akakabe Y, Matsui K, Kodama K, Koga H, NagakuraT. (+)-(3S, 4S)-3-butyl-4-vinylcyclopentene in brown algae of the genus Dictyopteris. Phytochemistry. 1997;**45**(3):529-532. DOI: 10.1016/S0031-9422(96)00884-9

[37] Sugisawa H, Nakamura K, Tamura H. The aroma profile of the volatiles in marine green algae (*Ulva pertusa*). Food Reviews International. 1990;**6**(4):573-589. DOI: 10.1080/87559129009540893

[38] Kajiwara T, Hatanaka A, Tanaka Y, Kawai T, Ishihara M, Tsuneya T, et al. Specificity of the enzyme system producing long chain aldehydes in the green alga, *Ulva pertusa*. Phytochemistry. 1989;**28**(2):636-639. DOI: 10.1016/0031-9422(89)80070-6

[39] Kawasaki W, Matsui K, Akakabe Y, Itai N, Kajiwara T. Volatiles from *Zostera marina*. Phytochemistry. 1998;**47**(1):27-29. DOI: 10.1016/S0031-9422(97)88555-X

[40] Ozdemir G, Horzum Z, Sukatar A, Karabay-Yavasoglu N. Antimicrobial activities of volatile components and various extracts of Dictyopteris membranaceae and *Cystoseira barbata* from the coast of Izmir, Turkey. Pharmaceutical Biology. 2006;**44**(3):183-188. DOI: 10.1080/13880200600685949

[41] Giogios I, Grigorakis K, Nengas I, Papasolomontos S, Papaioannoub N, Alexis MN. Fatty acid composition and volatile compounds of selected marine oils and meals. Journal of the Science of Food and Agriculture. 2009;**89**:88-100. DOI: 10.1002/jsfa.3414

[42] El Hattab M, Culioli G, Piovetti L, Chitour SE, Valls R. Comparison of various extraction methods for identification and determination of volatile metabolites from the brown alga *Dictyopteris membranacea*. Journal of Chromatography. A. 2007;**1143**(1-2):1-7. DOI: 10.1016/j.chroma. 2006.12.057

[43] De Carvalho LR, Roque NF. Fenóishalogenados e/ou sulfatados de macroalgasmarinhas. Quimica Nova. 2000;**23**(6):757-764. DOI: 10.1590/s0100-40422000000600009

[44] Fleury BG, Kelecom A, Pereira RC, Teixeira VL. Polyphenols, terpenes and sterols in Brazilian dictyotales and fucales (*Phaeophyta*). Botanica Marina. 1994;**37**(5):457-462. DOI: 10.1515/botm.1994.37.5.457

[45] Kladi M, Vagias C, Roussis V. Volatile halogenated metabolites from marine red algae. Phytochemistry Reviews. 2004;**3**(3):337-366. DOI: 10.1515/botm.1994.37.5.457

[46] Roller P, Kalfred A, Moore RE. Isolation of S-(3-oxoundecyl) thioacetate, bis-(3-oxoundecyl) disulphide, (−)-3-hexyl-4,5-dithiacycloheptanone, and S-(trans-3-oxoundec-4-enyl) thioacetate from Dictyopteris. Chemical Communications. 1971;**273**:503-504. DOI: 10.1039/C29710000503

[47] Schnitzler I, Boland W, Hay ME. Organic sulfur compounds from Dictyopteris spp. deter feeding by an herbivorous amphipod (*Ampithoe longimana*) but not by an herbivorous sea urchin (*Arbacia punctulata*). Journal of Chemical Ecology. 1998;**24**(10):1715-1732. DOI: 10.1023/ A:1020876830580

[48] Boland W, Müller DG. On the odor of the Mediterranean seaweed *Dictyopteris membranacea*: New C11 hydrocarbons from marine brown algae. Tetrahedron Letters. 1987;**28**(3):307-310. DOI: 10.1016/ S0040-4039(00)95714-9

[49] Mouritsen OG. Seaweeds Edible, Available and Sustainable. London: The University of Chicago Press, Ltd.; 2013

[50] Haas P. The liberation of methyl sulphide by seaweed. The Biochemical Journal. 1935;**29**:1297-1299. DOI: 10.1042/ bj0291297

[51] Challenger F, Simpson MI. Studies on biological methylation. Part XII. A precursor of the dimethyl sulphide evolved by *Polysiphonia fastigiata*. Dimethyl-2-carboxyethylsulphonium hydroxide and its salts. Journal of the Chemical Society. 1948;**1948**:1591-1597. DOI: 10.1039/JR9480001591

[52] Karabay-Yavasoglu NU, Sukatar A, Ozdemir G, Horzum Z. Antimicrobial activity of volatile components and various extracts of the red alga. Janiarubens. Phytotherapy Research. 2007;**21**:153-156. DOI: 10.1002/ptr.2045

[53] Yamamoto M, Baldermann S, Yoshikawa K, Fujita A, Mase N, Watanabe N. Determination of volatile compounds in four commercial samples of Japanese green algae using solid phase microextraction gas chromatography mass spectrometry. Scientific World Journal. 2014;**2014**:1-8. DOI: 10.1155/2014/289780

[54] Terezinha M, Neta SL, Narain N. Volatile components in seaweeds. Marine Biology and Oceanography. 2018;**2**(2):195-201. DOI: 10.31031/ EIMBO.2018.02.000535

[55] Kajiwara T, Yoshikawa H, Matsui K, Hatanaka A, Kawai T, Ishihara T, et al. Specificity of the enzyme system producing long chain aldehydes in the green alga, *Ulva pertusa*. Phytochemistry. 1989;**28**(2):636-639. DOI: 10.1016/0031-9422(89)80070-6

[56] Beauchêne D, Grua-Priol J, Lamer T, Demaimay M, Quémeneur F. Concentration by pervaporation of aroma compounds from *Fucus serratus*. Journal of Chemical Technology and Biotechnology. 2000;**75**(6): 451-458. DOI: 10.1002/1097-4660(200006)75:6<451: AID-JCTB231>3.0.CO;2-U

[57] Le Pape MA, Grua-Priol J, Prost C, Demaimay M. Optimization of dynamic headspace extraction of the edible red algae *Palmaria palmata* and identification of the volatile components. Journal of Agricultural and Food Chemistry. 2004;**52**(3):550-556. DOI: 10.1021/jf030478x

[58] Boonprab K, Matsui K, Akakabe Y, Yotsukura N, Kajiwara T. Hydroperoxy-arachidonic acid mediated n-hexanal and (Z)-3- and (E)-2-nonenal formation in *Laminaria angustata*. Phytochemistry. 2003;**63**(6):669-678. DOI: 10.1016/S0031-9422(03)00026-8

[59] Boonprab K, Matsui K, Akakabe Y, Yoshida M, Yotsukura N, Chirapart A, et al. Formation of aldehyde flavor (n-hexanal, 3Z-nonenal and 2E-nonenal) in the brown alga, *Laminaria angustata*. Journal of Applied Phycology. 2006;**18**(3-5):409-412. DOI: 10.1007/s10811-006-9038-6

[60] Akakabe Y, Matsui K, Kajiwara T. 2,4-Decadienals are produced via (R)-11-HPITE from arachidonic acid in marine green alga *Ulva conglobata*. Bioorganic & Medicinal Chemistry.

2003;**11**:3607-3609. DOI: 10.1016/S0968-0896(03)00364-X

[61] Akakabe Y, Kajiwara T. Bioactive volatile compounds from marine algae: Feeding attractants. Journal of Applied Phycology. 2008;**20**:661-664. DOI: 10.1007/978-1-4020-9619-8_26

[62] Akabe Y, Matsui K, Kajiwara T. Enantioselective α-hydroperoxylation of long chain fatty acids which crude enzyme of marine green alga *Ulva pertusa*. Tetrahedron Letters. 1999;**40**:1137-1140. DOI: 10.1016/S0040-4039(98)02547-7

[63] Akakabe Y, Matsui K, Kajiwara T. α-Oxidation of long-chain unsaturated fatty acids in the marine green alga *Ulva pertusa*. Bioscience, Biotechnology, and Biochemistry. 2000;**64**:2680-2681. DOI: 10.1271/bbb.64.2680

[64] Akakabe Y, Matsui K, Kajiwara T. Enantioselective 2- hydroperoxylation of long-chain fatty acids in marine green algae. Fisheries Science. 2001;**67**:328-332. DOI: 10.1046/j.1444-2906.2001.00235.x

[65] Vilara EG, O'Sullivan MG, Kerry JP, Kilcawleya KN. Volatile compounds of six species of edible seaweed: A review. Algal Research. 2020;**45**:101740. DOI: 10.1016/j.algal.2019.101740

[66] Firouzi J, Gohari A, Rustaiyan A, Larijani K, Saeidnia S. Composition of the essential oil of *Nizamuddin zanardinii*, a Brown alga collected from Oman gulf. Journal of Essential Oil Bearing Plants. 2013;**16**(5):689-692. DOI: 10.1080/0972060X.2013.862072

[67] Püttmann W. Thermodesorption-gas chromatography-mass spectrometric analysis of biological materials for potential molecular precursors of the constituents of the crude oils. Journal of Chromatography. 1991;**552**:325-336. DOI: 10.1016/S0021-9673(01)95949-7

[68] Steiner M, Hartmann T. The occurrence and distribution of volatile amines in marine algae. Planta. 1968;**79**(2):113-121. DOI: 10.1007/BF00390154

[69] Percot A, Yalçin A, Aysel V, Erdugan H, Dural B, Guven KC. β-Phenylethylamine content in marine algae around Turkish coasts. Botanica Marina. 2009;**52**:87-90. DOI: 10.1515/BOT.2009.031

[70] Barwell CJ. Pharmacologically-active amines in some marine algae and algal food products. Journal of Home & Consumer Horticulture. 2008;**1**(1):77-82. DOI: 10.1300/J280v01n01_04

[71] Takaoka M, Ando Y. Essential oil of seaweeds. I. Composition of the oil of *Dictyopteris divaricata*. Nippon Kagaku Kaishi (1921-1957). 1951;**72**:999-1003. DOI: 10.1246/nikkashi1948.72.999

[72] Flodin C, Whitfield F. 4-Hydroxybenzoic acid: A likely precursor of 2,4,6-tribromophenol in *Ulva lactuca*. Phytochemistry. 1999;**51**(2):249-255. DOI: 10.1016/S0031-9422(98)00754-7

[73] Flodin C, Whitfield F. Biosynthesis of bromophenols in marine algae. Water Science and Technology. 1999;**40**(6):53-58. DOI: 10.1016/S0273-1223(99)00537-5

[74] Marshall RA, Hamilton JTG, Dring MJ, Harper DB. Do vesicle cells of the red alga Asparagopsis (Falkenbergiastage) play a role in bromocarbon production? Chemosphere. 2003;**52**:471. DOI: 10.1016/S0045-6535(03)00197-8

[75] Garson MJ. The biosynthesis of marine natural products. Chemical Reviews. 1993;**93**:1699-1733. DOI: 10.1021/cr00021a003

[76] Moore BS. Biosynthesis of marine natural products: Microorganisms (part a). Natural Product Reports. 2005;**22**:580-593. DOI: 10.1039/b404737k

[77] Moore BS. Biosynthesis of marine natural products: Macroorganisms (part B). Natural Product Reports. 2006;**23**:615-629. DOI: 10.1039/ b508781n

[78] Le Blanc C, Colin C, Cosse A, Delage L, La Barre S, Morin P, et al. Biochimie. 2006;**88**:1773-1785. DOI: 10.1016/j.biochi.2006.09.001

[79] Moore RM. Methyl halide production and loss rates in sea water from field incubation experiments. Marine Chemistry. 2006;**101**:213-219. DOI: 10.1016/j.marchem.2006.03.003

[80] Giese B, Laturnus F, Adams FC, Wiencke C. Release of volatile iodinated C1–C4 hydrocarbons by marine macroalgae from various climate zones. Environmental Science & Technology. 1999;**33**(14):2432-2439. DOI: 10.1021/ es980731n

[81] Carpenter LJ, Malin G, Liss PS, Kupper FC. Novel biogenic iodine-containing trihalomethanes and other short-lived halocarbons in the coastal East Atlantic. Global Biogeochemical Cycles. 2000;**14**:1191-1204. DOI: 10.1029/2000GB001257

[82] Michelozzi M. Defensive roles of terpenoid mixtures in conifers. Acta Botanica Gallica. 1999;**146**(1):73-84. DOI: 10.1080/ 12538078.1999.10515803

[83] Kumari S, Pundhir S, Priya P, Jeena G, Punetha A, Chawla K, et al. EssOilDB: A database of essential oils reflecting terpene composition and variability in the plant kingdom. Database. 2014;**2014**:1-14. DOI: 10.1093/ database/bau120

[84] Naylor S, Hanke FJ, Manes LV, Crews P. Chemical and biological aspects of marine monoterpenes. Fortschritte der Chemie Organischer Naturstoffe. 1983;**44**:189-241. DOI: 10.2307/2107215

[85] Marmulla R, Harder J. Microbial monoterpene transformations–a review. Frontiers in Microbiology. 2014;**5**:346-359. DOI: 10.3389 /fmicb.2014.00346

[86] Soares AR. Extraction, isolation, and identification of Sesquiterpenes from Laurencia species. In: Natural Products from Marine Algae Methods and Protocols. Vol. 1308. New York: Humana Press; 2015. pp. 225-240

[87] Ji NY, Li XM, Li K, Ding LP, Gloer JB, Wang BG. Diterpenes, sesquiterpenes, and a C15-acetogenin from the marine red alga *Laurencia mariannensis*. Journal of Natural Products. 2007;**70**(12):1901-1905. DOI: 10.1021/np070378b

[88] Jerković I, Marijanović Z, Roje M, Kuś PM, Jokić S, ČozÏ-Rakovac R. Phytochemical study of the headspace volatile organic compounds of fresh algae and seagrass from the Adriatic Sea (single point collection). PLoS One. 2018;**13**(5):1-13. DOI: 10.1371/journal. pone.0196462

[89] Kamenarska Z, Ivanova A, Stancheva R, Stoyneva M, Stefanov K, Dimitrova-Konaklieva S, et al. Volatile compounds from some Black Sea red algae and their chemotaxonomic application. Botanica Marina. 2006;**49**(1):47-56. DOI: 10.1515/ BOT.2006.006

[90] Elenkov I, Georgieva T, Hadjieva P, Dimitrova-Konaklieva S, Popov S. Terpenoids and sterols in *Cladophora vagabunda*. Phytochemistry. 1995;**38**(2):457-459. DOI: 10.1016/0031-9422(94)00704-W

[91] Yamada K, Tan H, Tatematsu H, Ojiva M. Dictyoprolene and neodictyoprolene, two new odoriferous compounds from the brown alga *Dictyopteris prolifera*: Structures and synthesis. Tetrahedron. 1986;**42**(14):3775-3780. DOI: 10.1016/ S0040-4020(01)87531-1

[92] Kajiwara T, Kashibe M, Matsui K, Hatanaka A. Volatile compounds and

long-chain aldehydes formation in conchocelis filaments of a red alga, *Porphyra tenera*. Phytochemistry. 1990;**29**(7):2193-2195. DOI: 10.1016/0031-9422(90)83036-Z

[93] Fujimura T, Kawai T, Shiga M, Kajiwara T, Hatanaka A. Long-chain aldehyde production in thalli culture of the marine green alga *Ulva pertusa*. Phytochemistry. 1990;**29**(3):745-747. DOI: 10.1016/0031-9422(90)80011-5

[94] Maier I, Muller DG. Sexual pheromones in algae. The Biological Bulletin. 1986;**170**:145-175. DOI: 10.2307/1541801

[95] Moore RE. Volatiles compounds from marine algae. Accounts of Chemical Research. 1977;**10**:40-47. DOI: 10.1021/ar50110a002

[96] Boland W. The chemistry of gamete attraction: Chemical structures, biosynthesis, and abiotic degradation. Proceedings of the National Academy of Sciences of the United States of America. 1995;**92**:37-43. DOI: 10.1073/pnas.92.1.37

[97] Pohnert G, Boland W. The oxylipin chemistry of attraction and defense in brown algae and diatoms. Natural Product Reports. 2002;**19**:108-122. DOI: 10.1039/a806888g

[98] Fink P. Ecological functions of volatile organic compounds in aquatic systems. Marine and Freshwater Behaviour and Physiology. 2007;**40**:155-168. DOI: 10.1080/10236240701602218

[99] Amsler CD, Fairhead VA. Defensive and sensory chemical ecology of brown algae. Advances in Botanical Research. 2006;**43**:1-91. DOI: 10.1016/S0065-2296(05)43001-3

[100] Derenbach JB, Pesandeo D. Investigations into a small fraction of volatile hydrocarbons: III. Two diatom cultures produce ectocarpene, a pheromone of brown algae. Marine Chemistry. 1986;**19**:337-432. DOI: 10.1016/0304-4203(86)90054-X

[101] Juttner F, Wurster K. Evidence of ectocarpene and dictyopterenes a and C in the water of a freshwater lake. Limnology and Oceanography. 1984;**29**:1322-1324. DOI: 10.4319/lo.1984.29.6.1322

[102] Boland W, Jaenicke L, Brauner A. Vinyl olefines and sesquiterpenes in the root oil of *Senecio isatideus*. Zeitschrift für Naturforschung. 1982;**37C**:5-9. DOI: 10.1515/znc-1982-1-202

[103] Wang Y, Li X, Jiang Q, Sun H, Jiang J, Chen S, et al. GC-MS analysis of the volatile constituents in the leaves of 14 composite plants. Molecules. 2018;**23**:166. DOI: 10.3390/molecules23010166

[104] Faulkner J. Interesting aspects of marine natural products chemistry. Tetrahedron. 1977;**33**:1421-1443. DOI: 10.1016/0040-4020(77)88001-0

[105] Hay ME, Duffy JE, Fenical W, Gustaíson K. Chemical defense in the seaweed *Dictyopteris delicatula*: Differential effects against reef fishes and amphipods. Marine Ecology Progress Series. 1988;**48**:185-192. DOI: 10.3354/meps048185

[106] Riad N, Zahi MR, Trovato E, Bouzidi N, Daghbouche Y, Utczas M, et al. Chemical screening and antibacterial activity of essential oil and volatile fraction of *Dictyopteris polypodioides*. Microchemical Journal. 2020;**152**:104415. DOI: 10.1016/j.microc.2019.104415

[107] Moore RE. Chemotaxis and the odor of seaweed. Lloydia. 1976;**39**:181-190. DOI: 10.1016/B978-0-12-505050-0.50014-8

[108] Blunt JW, Copp BR, Munro MHG, Northcote PT, Prinsep MR. Marine natural products. Natural Product

Reports. 2003;**20**:1-48. DOI: 10.1039/b207130b

[109] Faulkner DJ. Marine natural products. Natural Product Reports. 2001;**18**:1-49. DOI: 10.1039/B009029H

[110] Li XD, Ding W, Miao FP, Ji NY. Halogenated chamigrane sesquiterpenes from *Laurencia okamurae*. Magnetic Resonance in Chemistry. 2012;**50**(2): 174-177. DOI: 10.1002/mrc.2870

[111] Kim SK. Handbook of Marine Macroalgae Biotechnology and Applied Phycology, 1st ed. Chichester, West Sussex: Wiley-Blackwell John Wiley& Sons; 2012. p. 592. DOI: 10.1002/9781119977087

[112] Coll JC, Wright AD. Tropical marine algae. I. New halogenated monoterpenes from Chondrococus hornemannii (Rhodophyta, Gigartinales, Rhizophyllidaceae). Australian Journal of Chemistry. 1987;**40**:1893-1900. DOI: 10.1071/CH9871893

[113] Cikoš MA, Jurin M, Rakovac RC, Jokic S, Jerkovic I. Update on monoterpenes from red macroalgae: Isolation, analysis, and bioactivity. Marine Drugs. 2019;**17**:537. DOI: 10.3390/md17090537

[114] Crews P, Ng P, Kho-Wiseman E, Pace C. Halogenated monoterpenes of the red alga Microcladia. Phytochemistry. 1976;**15**:1707-1711. DOI: 10.1016/S0031-9422(00)97461-2

[115] Wang BW, Gloer JB, Ji N-Y, Zhao J-C. Halogenated organic molecules of Rhodomelaceae origin: Chemistry and biology. Chemical Reviews. 2013;**113**:3632-3685. DOI: 10.1021/cr9002215

[116] Cabrita MT, Vale C, Pilar RA. Halogenated compounds from marine algae. Marine Drugs. 2010;**8**:2301-2317. DOI: 10.3390/md8082301

[117] Kamada T, Phan CS, Vivian Shi-Ting Sien VST, Vairappan CS. Halogenated chamigrane sesquiterpenes from *Bornean Laurencia* majuscula. Journal of Applied Phycology. 2018;**30**:3373-3378. DOI: 10.1007/s10811-018-1452-z

[118] Gao D, Okuda R. Supercritical fluid extraction of halogenated monoterpenes from the red alga *Plocamium cartilagineum*. Journal of AOAC International. 2001;**84**:1313-1331. PMID: 11601448

[119] San-Martin A, Negrete R, Rovirosa J. Insecticide and acaricide activities of polyhalogenated monoterpenes from Chilean *Plocamium cartilagineum*. Phytochemistry. 1991;**30**:2165-2169. DOI: 10.1016/0031-9422(91)83607-M

[120] Wright AD, König GM, Sticher O. Five new monoterpenes from the marine red alga *Portieria hornemannii*. Tetrahedron. 1991;**47**:5717-5724. DOI: 10.1016/S0040-4020(01)86524-8

[121] Paul VJ, McConnell OJ, Fenical W. Cyclic monoterpenoid feeding deterrents from the red marine alga *Ochtodes crockeri*. The Journal of Organic Chemistry. 1980;**45**:3401-3407. DOI: 10.1021/jo01305a006

[122] Wise ML, Rorrer GL, Polzin JJ, Croteau R. Biosynthesis of marine natural products: Isolation and characterization of a myrcene synthase from cultured tissues of the marine red alga *Ochtodes secundiramea*. Archives of Biochemistry and Biophysics. 2002;**400**(1):125-132. DOI: 10.1006/abbi.2002.2780

[123] Suzuki M, Kurosawa E, Irie T. Spirolaurenone, a new sesquiterpenoid containing bromine from *Laurencia glandulifera* Kützing. Tetrahedron Letters. 1970;**11**:4995-4998. DOI: 10.1016/S0040-4039 (00)89329-6

[124] Suzuki M, Kurosawa E, Irie T. Three new sesquiterpenoids containing

bromine, minor constituents of *Laurencia glandulifera* Kützing. Tetrahedron Letters. 1974;**15**:821-824. DOI: 10.1016/S0040-4039(01)82342-X

[125] Konig M, Wright D. New C15 Acetogenins and Sesquiterpenes from the red alga Laurencia sp. cf. *L. gracilis*. Journal of Natural Products. 1994;**57**:477. DOI: 10.1021/np50106a006

[126] Vazquez JT, Chang M, Nakanishi K, Martin JD, Martin VS, Perez R. Puertitols: Novel sesquiterpenes from *Laurencia obtusa*. Structure elucidation and absolute configuration and conformation based on circular dichroism. Journal of Natural Products. 1988;**51**(6):1257-1260. DOI: 10.1021/ np50060a036

[127] North M, Fernandez JJ, Padilla A. Bisabolane halogenated sesquiterpenes from Laurencia. Phytochemistry. 1992;**31**:326-327. DOI: 10.1016/ 0031-9422(91)83065-S

[128] Sims JJ, Lin GHY, Wing RM. Marine natural products X elatol, a halogenated sesquiterpene alcohol from the red alga *Laurencia elata*. Tetrahedron Letters. 1974;**15**:3487-3490. DOI: 10.1016/S0040-4039(01)91944-6

[129] Lhullier C, Donnangelo A, Caro M, Palermo JA, Horta PA. Isolation of elatol from *Laurencia microcladia* and its palatability to the sea urchin Echinometralucunter. Biochemical Systematics and Ecology. 2009;**37**:254-259. DOI: 10.1016/j.bse.2009.04.004

[130] Al-Massarani SM. Phytochemical and biological properties of Sesquiterpene constituents from the marine red seaweed Laurencia: A review. Natural Products Chemistry and Research. 2014;**2**(5):1-13. DOI: 10.4172/2329-6836.1000147

[131] Dembitsky VM, Tolstikov GA. Natural halogenated sesquiterpenes from marine organisms. Chemistry for Sustainable Development. 2004;**12**:1-12

[132] Hay ME, Piel J, Boland W, Schnitzler I. Seaweed sex pheromones and their degradation products frequently suppress amphipod feeding but rarely suppress sea urchin feeding. Chemoecology. 1998;**8**(2):91-98. DOI: 10.1007/PL00001809

[133] Pelletreau K, Muller-Parker G. Sulfuric acid in the phaeophyte alga *Desmarestia mundadeter* feeding by the sea urchin *Strongylocentrotus droebachiensis*. Marine Biology. 2002;**141**:1-9. DOI: 10.1007/s00227-002-0809-6

[134] Bakus GJ, Targett NM, Schulte B. Chemical ecology of marine organisms: An overview. Journal of Chemical Ecology. 1986;**12**:951-987. DOI: 10.1007/ BF01638991

[135] Muller DG, Jaenicke L, Donike M, Akintobi T. Sex attractant in a brown alga - chemical structure. Science. 1971;**171**:1132. DOI: 10.1126/science. 171.3976.1132

[136] Ponhert G, Boland W. Pericyclic reactions in nature: Synthesis and cope rearrangement of thermolabile bis-alkenylcyclopropanes from female gametes of marine brown algae (Phaeophyceae). Tetrahedron Letters. 1997;**53**(40):13681-13694. DOI: 10.1016/ S0040-4020(97)00886-7

[137] Schnitzler I, Pohnert G, Hay ME, Boland W. Chemical defense of brown algae (Dictyopteris spp.) against the herbivorous amphipod Ampithoelongimana. Oecologia. 2001;**126**(4):515-521. DOI: 10.1007/ s004420000546

[138] Valls R, Artaud J, Amade P, Vincente N, Piovetti L. Determination of caulerpenyne, a toxin from the green alga *Caulerpa taxifolia* (Caulerpaceae). Journal of Chromatography. A. 1994;**663**(1):114-118. DOI: 10.1016/0021-9673(94)80502-4

[139] Dumay O, Pergent G, Pergent-Martini C, Amade P. Variations in caulerpenyne contents in *Caulerpa taxifolia* and *Caulerpa racemosa*. Journal of Chemical Ecology. 2002;**28**(2):343-352. DOI: 10.1023/a:1017938225559

[140] Paul VJ, Fenical W. Chemical defense in tropical green algae, order Caulerpales. Marine Ecology Progress Series. 1986;**34**:157-169. DOI: 10.3354/meps034157

[141] Goodwin KD, North WJ, Lidstrom ME. Production of bromoform and dibromomethane by Giant kelp: Factors affecting release and comparison to anthropogenic bromine sources. Limnology and Oceanography. 1997;**42**(8):1725-1734. DOI: 10.4319/lo.1997.42.8.1725

[142] Dworjanyn SA, De Nys R, Steinberg PD. Localisation and surface quantification of secondary metabolites in the red alga *Delisea pulchra*. Marine Biology. 1999;**133**:727-736. DOI: 10.1007/s002270050514

[143] Scheuer PJ. Some marine ecological phenomena: Chemical basis and biomedical potential. Science. 1990;**248**:173-177. DOI: 10.1126/science.2183350

[144] Kamenarska Z, Gasic MJ, Zlatovic M, Rasovic A, Sladic D, Kljajic Z, et al. Chemical composition of the brown alga *Padina pavonia* (L.) Gaill. From the Adriatic Sea. Botanica Marina. 2002;**45**:339-345. DOI: 10.1515/BOT.2002.034

[145] Sultana V, Ehteshamul-H S, Ara J, Athar M. Comparative efficacy of brown, green and red seaweeds in the control of root infecting fungi and okra. International Journal of Environmental Science and Technology. 2005;**2**(2):129-132. DOI: 10.1007/BF03325866

[146] Ktari L, Guyot M. A cytotoxic oxysterol from the marine Red Sea alga *Padina pavonica* (L.) Thivy. Journal of Applied Phycology. 1990;**11**:511-513. DOI: 10.1023/A:1008162624027

[147] Awad NE, Selim MA, Metawe HM, Matloub AA. Cytotoxic xenicane diterpenes from the brown alga *Padina pavonia* (L.) Gaill. Phytotherapy Research. 2008;**22**:1610-1613. DOI: 10.1002/ptr.2532

[148] Awad NE, Motawe HM, Selim MA, Matloub AA. Volatile constituents of the brown algae *Padina pavonia* (L.) Gaill. And *Hydroclathrus clathratus* (C. Agardh) Howe and their antimicrobial activity. Medicinal and Aromatic Plant Science and Biotechnology. 2009;**3**(1):12-15

[149] Dias T, Brito I, Moujir L, Paiz N, Darias J, Cueto M. Cytotoxic sesquiterpenes from *Aplysia dactylomela*. Journal of Natural Products. 2005;**68**:1677-1679. DOI: 10.1021/np050240y

[150] Juagdan EG, Kalidindi R, Scheuer P. Two new chamigranes from an hawaiian red alga, *Laurencia cartilaginea*. Tetrahedron. 1997;**53**(2):521-528. DOI: 10.1016/S0040-4020(96)01002-2

[151] Kladi M, Vagias C, Furnari G, MoreauD,RoussakisC,RoussisV.Cytotoxic cuparene sesquiterpenes from *Laurencia microcladia*. Tetrahedron Letters. 2005;**46**:5723-5726. DOI: 10.1016/j.tetlet.2005.06.076

[152] Kladi M, Vagias C, Papazafir P, Furnari G, Serio D, Roussis V. New sesquiterpenes from the red alga *Laurencia microcladia*. Tetrahedron. 2007;**63**:7606-7611. DOI: 10.1016/j.tet.2007.05.051

[153] Vairappan CS. Potent antibacterial activity of halogenated metabolites from Malaysian red algae, *Laurencia majuscula* (Rhodomelaceae, Ceramiales). Biomolecular Engineering.

2003;**20**:255-259. DOI: 10.1016/S1389-0344(03)00067-4

[154] Kurata K, Amiya T. Bis(2,3,6-tribromo-4,5-dihydroxybenzyl)ether from the red alga, *Symphyocladia latiuscula*. Phytochemistry. 1980;**19**:141-142. DOI: 10.1016/0031-9422(80)85032-1

[155] Kimura J, Kamada N, Tsujimoto Y. Fourteen Chamigrane derivatives from a red alga, *Laurencia nidifica*. Bulletin of the Chemical Society of Japan. 1999;**72**:289-292. DOI: 10.1246/bcsj.72.289

Chapter 2

Essential Oils

Lubna Abdul Muttalib Al-Shalah,
Nada Khazal Kadhim Hindi and Israa Harjan Mohsen

Abstract

Modern science has tended to use several natural substances that have little or no side effects in daily use or to treat many diseases. Among these materials are essential oils that represent one of the secondary metabolic products of many plants such as Terpenes and Terpenoids, Alkaloids, and the Phenolic compounds, which are extracted by special methods from different parts of the plants. Several applications were using the essential oils such as in the nutrition, cosmetic manufacture, and alternatives to synthetic medication that uses to treatment several infections and diseases as disinfection, as an anti-inflammatory, mouthwashes, as well as in cleaning and calm mood and pesticides. This review describes essential oils, methods of their extraction, and ways of utilization and their application.

Keywords: essential oils, extraction, health benefit, antibacterial activity

1. Introduction

Plants produce thousands types of chemicals materials and included two types of metabolites (primary and secondary). Primary metabolites are macromolecules like carbohydrates, fats, proteins, nucleic acids, chlorophylls, hemes this molecular are required for their basic metabolic processes [1] plants, fungi and bacteria of definite genera and families create a number of organic compounds which are not included in primary metabolism that important in essential activity of organism (photosynthesis, respiration, and protein and lipid metabolism) and seem to have no function in growth and development of them [2]. Such compounds are called secondary metabolites (secondary plant products or natural products), there are other name which known as Phytogenic feed additives (PFA) or phytobiotics and botanicals, are commonly defined as various plant secondary compounds (PSC) and metabolites with beneficial effects on animal health and production, including feed and animal products. These compounds are accessary rather than central to the functioning of the plants in which they are found. These compounds are produced in small quantities and their extraction from the plant is difficult and expensive.

There important product of plant secondary metabolites as natural products: Terpenes and Terpenoids (25,000 types), Alkaloids (12,000 types) and the Phenolic compounds (8000 types) [3].

Essential oils (EOs) represent a major group of phytogenic feed additives (PFA). Plant oils and extracts have been used for a wide variety of purposes for many thousands of years [4], Due to their strong aromatic features and bioactivity, EOs have been widely used since ancient times in aromatherapy, as flavor and fragrances in cosmetics and foods, and more recently as pharmaceuticals, natural preservatives, additives, and biopesticides [5–7]. There are many defines of EO, like EO is a

mixture of low molecular weight constituents that are responsible for its characteristic aroma, or Essential oils are concentrated liquids of complex mixtures of volatile compounds and can be extracted from several plant organs [8]. The chemistry of EO constituents includes terpenoid and non-terpenoid hydrocarbons and their oxygenated derivatives [5, 9, 10]. Essential oils are concentrated plant extracts that retain the natural smell and flavor, or "essence," of their source Essential oils, also called volatile odoriferous oil, are aromatic oily liquids extracted from different parts of plants, for example, leaves, peels, barks, flowers, buds, seeds, and so on [11].

They may be found in different parts of the plant. Some EOs could be found in leaves (oregano), seed (almond), flower (jasmine), peel (bergamot), berries (juniper), rhizome (galangal ginger), root (angelicaarchangelica), bark (sassafras), wood (agar wood), resin (frankincense), andpetals (rose) [12].

2. How do essential oils work

Essential oils include biological compounds like growth factors, hormones, and neurotransmitters that are concentrated from the plant. Several researches have been performed on PEOs in order to confirm their biological efficacy against bacteria and fungi [13]; PEOs have an antibacterial activity where they cause damage to cells via an interface with plasma membrane components which lead to leak the most important compounds and damage the transportation channels, especially of potassium ion [12, 13]. Volatiles from PEOs not only work on single target site in the cell but also they bind to protein structures of the cell. Some of the PEOs and their volatiles are found to be responsible in inhibiting the enzymatic proteins in some bacterial pathogens [14–16].

Essential oils used all over the world for disinfection, as anti-inflammatory, relaxing, and stimulating substances, and with potential and modern exploitation in clinical medicine are most commonly used in the practice of aromatherapy, in which they are inhaled, or be swallowed, or can interact with your body in several ways when applied to your skin, some plant chemicals are absorbed also commonly used in food and cosmetic industries [17] (**Figure 1**).

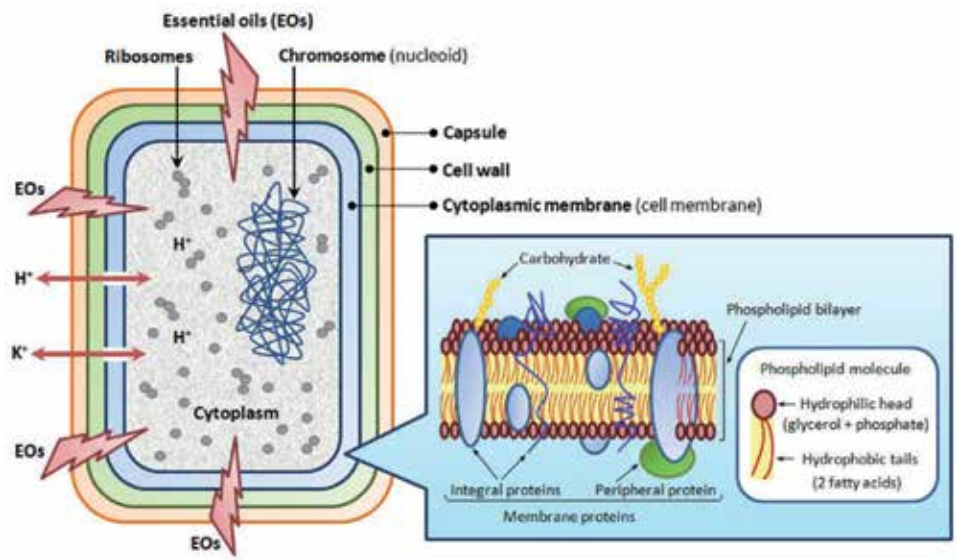

Figure 1.
Schematic illustration for the effect of essential oils on bacteria cell. Source: Ref. [10].

3. Extraction of essential oils

Essential oils are produce from valuable plant products, generally of complex composition including the volatile principles contained in the plant and the more or less modified during the preparation process. The earliest recorded mention of the techniques and methods used to produce essential oils is believed to be that of Ibnal-Baitar (1188–1248). The oil droplets being stored in the oil glands or sacs can be removed by either accelerate diffusion through the cell wall or crush the cell wall [17–20]. The assumed techniques depend on the part of the plants where the oil is to be extracted, the stability of the oil to heat and susceptibility of the oil constituents to chemical reactions. An extract is derived when the plant material is soaked in a substance such as water, alcohol or other liquid for long periods of time so that its flavor, aroma or medicinal properties, infuses into the liquid [11].

Common techniques used for the extraction of essential oils are:

- Hydrodistillation
- Hydrodiffusion
- Effleurage
- Cold pressing
- Steam distillation
- Solvent extraction
- Microwave Assisted Process (MAP)
- Carbondioxide extraction

3.1 Hydrodistillation

Hydrodistillation represents one of the most used traditional and a commonly methods of extraction used method of extracting essential oils from plant samples (wood and flower). This method may be further classified into the subcategories of steam distillation, water distillation, or a combination of water and steam distillation. Hydrodistillation include saturation of the powdered wood in the water in the first step, while the next step is to use the steam that result from the heating of the water reservoir then finally gather the oil that result after the reservoir cold. The advantage of this technique is that the required material can be distilled at a temperature below 100°C. There are many studies provided that the different extraction processes on yield and properties of essential oil from rosemary (*Rosmarinus officinalis* L.) by HD and solvent-free microwave extraction (SFME) [21]. While Golmakani and Rezaei (2008) [22] researched the microwave-assisted HD (MAHD), which is an advanced HD technique utilize a microwave oven in the extraction procedure.

MAHD was preferable in extraction time (75 min, compared to 4 h in HD). Ohmic-assisted HD (OAHD) is another advanced HD technique [23]. OAHD method had the extraction time of 24.75 min, while HD took 1 h for extraction of essential oil. No changes in the compounds of the essential oils obtained by OAHD were found in comparison with HD.

3.2 Hydro diffusion

Hydro Diffusion method for extraction of oils is a type of steam distillation and is only different in the way in which steam enters the container of the still. This method is preferred when the plant material has been dried and is not deteriorate at boiling temperature [24]. The steam in this method is drenched from the topmost onto the phytophagous matter and thus leads to impregnate the plants even more and less time. Hydrodiffusion method is superior to steam distillation because of shorter processing time and a higher oil yield with less steam used [11].

3.3 Steam distillation

One of the most widely used methods of extracting essential oils from their sources is steam distillation, and it is one of the preferred methods due to its low cost. In this method, the essential oil and aromatic components of the plant are gathered by utilize heating water or vapor and thus cause damage to plant cells and lead to release these materials [21, 25]. This method is applied in isolation of essential oils at temperatures approach to 100°C, and next with condensation to produce an immiscible fluid that can clarify it to isolate the oil, so it is used in the conservation of compounds that might rupture at elevated temperatures [23, 26, 27].

3.4 Solvent extraction

In this method, the extraction of the essential oil occur after utilization of a hydrocarbon solvent which is acts as dissolving material when added to the plant and leads to the formation of mixture contain the essential oil with other material. The next step includes purification by concentration and filtration of the mixture and then added of absolute alcohol that when evaporate left the oil behind it. This method represents the best in the formation of big amounts from the product oil because of the least display to high temperatures or air but on the other hand the residue that results by the way after the extraction had bad effects on the health and immunity [28].

3.5 Cold pressing

Uses large machinery to either grind the seeds/fruits to squeeze out the oil or pierce the rind and peel of the fruit while it is rotating to extract the oil. Cold pressing is used to extract the essential oils from citrus rinds such as orange, lemon, grapefruit and bergamot. In this method the heat is generated internally where it result from the revolving of the mixture and the friction lead to disconnect of the oil from the mixture and the next step include filtration of the oil to discard any residue and ensure the purification process [29].

3.6 Enfleurage

An intense and classical method of elicit essential oil from petals of flowers. The procedure includes coating fats above petals of the flower which act on the soak up the oils, and then utilize alcohol to isolate the essential oils from the fat, then let alcohol be evaporated and thus the essential oils are gathered [29].

3.7 Carbondioxide extraction

This method is traditional where it uses the liquid CO_2 via pressurized it and the essential oils of the plants dissolved in this liquid. The next step includes return

back of CO_2 to the state of gases thus gathered left the oil, so the positive feature of this method was kept the essential oil from damage by high temperatures [29].

4. List of essential oils

This list is organized alphabetically by the common essential oil name (**Table 1**).

NO.	Essential oils	NO.	Essential oils
1	Allspice (Pimento Berry, Jamaica Pepper)	23	Cassia
2	Amyris (Torchwood, West Indian Sandalwood)	24	Catnip
3	Angelica Root	25	Cedarwood Atlas
4	Anise (Aniseed)	26	Cedarwood Himalayan
5	Arborvitae (Western Red Cedar)	27	Cedarwood Virginian
6	Balsam Peru (Peru Balsam)	28	Celery Seed
7	Basil (Sweet Basil, Basil Linalool	29	Cilantro (Coriander Leaf)
8	Bay Leaf	30	Cinnamon Bark
9	Bergamot	31	Cinnamon Leaf
10	Bergamot Mint	32	Cistus (Labdanum, Rock Rose)
11	Birch (Sweet Birch)	33	Citronella
12	Black Pepper	34	Clary Sage
13	Black Spruce	35	Clementine
14	Blue Cypress Blue Tansy	36	Clove Bud
15	Buddha Wood (Desert Rosewood)	37	Coffee
16	Blue Tansy (Moroccan Blue Chamomile)	38	Copaiba (Copaiba Balsam)
17	Cajeput (Cajuput, White Tea Tree)	39	Coriander Seed
18	Camphor (White Camphor, Camphor Laurel)	40	Cumin
19	Cannabis	41	Cypress
20	Caraway Seed	42	Davana
21	Carrot Seed	43	Dill Weed/Dill Seed
22	Cardamom	44	Douglas Fir
NO.	Essential oils	NO.	Essential oils
45	Elemi	73	Juniper Berry (Juniper)
46	*Eucalyptus dives*	74	Key Lime
47	*Eucalyptus globulus*	75	Kunzea
48	*Eucalyptus radiata*	76	Labdanum
49	*Eucalyptus smithii*	77	Laurel Leaf (Bay Leaf, Bay Laurel, Sweet Bay)
50	Everlasting (see Helichrysum)	78	Lavandin
51	Fennel (Sweet Fennel)	79	Lavender
52	Fir Balsam(Canadian Fir Needle)	80	Lavender (Spike Lavender)

NO.	Essential oils	NO.	Essential oils
53	Fir Needle (Siberian Fir)	81	Lemon Balm
54	Frankincense (Olibanum)	82	Lemon Eucalyptus
55	Galangal Root (Greater Galangal, Siamese Ginger)	83	Lemongrass
56	Galbanum	84	Lemon Myrtle
57	Geranium	85	Lemon Tea Tree
58	Geranium Bourbon	86	Lime Expressed
59	German Chamomile (Blue Chamomile)	87	Lime Distilled
60	Ginger	88	Mandarin
61	Gingergrass	89	Manuka (New Zealand Tea Tree)
62	Goldenrod	90	Marjoram (Sweet Marjoram)
63	Grapefruit	91	May Chang (Litsea)
64	Helichrysum (Everlasting, Immortelle)	92	Melaleuca
65	Hemlock (Spruce Hemlock, Tsuga)	93	Melissa (Lemon Balm)
66	Hemp (Cannabis)	94	Mountain Savory (Winter Savory)
67	Ho Leaf	95	Myrrh
68	Ho Wood	96	Myrtle
69	Hyssop	97	Neroli (Orange Blossom)
70	Immortelle	98	Niaouli
71	Jack Pine	99	Nutmeg
72	Jasmine Absolute	100	Opoponax (Sweet Myrrh, Opopanax)
NO.	**Essential oils**	**NO.**	**Essential oils**
101	Orange (Blood Orange)	**127**	Spearmint
102	Orange Blossom	**128**	Spikenard (Nard, Jatamansi)
103	Orange (Sweet Orange, Wild Orange)	**129**	Spruce Hemlock
104	Oregano	**130**	Star Anise
105	Palmarosa	**131**	Sweet Myrrh
106	Palo Santo	**132**	Tangerine
107	Patchouli	**133**	Tarragon
108	Peppermint	**134**	Tea Tree
109	Petitgrain	**135**	Thyme (Thyme Linalool,Thyme Thymol)
110	Pine (Scotch Pine, Scots Pine)	**136**	Tsuga
111	Pink Pepper	**137**	Turmeric
112	Plai	**138**	Valerian
113	Ravensara	**139**	Vanilla Absolute (Vanilla Oleoresin)
114	Ravintsara (Ho Leaf)	**140**	Verbena (Honey Verbena, Wild Verbena)
115	Rock Rose	**141**	Vetiver
116	Rosalina (Lavender Tea Tree)	**142**	White Fir
117	Rose Absolute (Rose Otte, Bulgarian Rose, Damask Rose)	**143**	Wintergreen

NO.	Essential oils	NO.	Essential oils
118	Rose Absolute (Provence Rose, Cabbage Rose)	**144**	Winter Savory
119	Rose Geranium	**145**	Yarrow
120	Rosemary	**146**	Ylang Ylang
121	Rosewood (Bios de Rose)	**147**	Yuzu
122	Sage (Dalmatian Sage, Common Sage)		
123	Sandalwood		
124	Saro (Mandravasarotra)		
125	Siberian Fir		
126	Silver Fir (Silver Fir Needle, Silver Spruce, White Fir)		

Table 1.
The most common name of the essential oil.

4.1 The application of oils essential

There were several applications using the essential oils such as in the nutrition, cosmetic manufacture and alternatives to synthetic medication that uses to treatment several infections and diseases [28]. Essential oils were used all over the world for disinfection, as anti-inflammatory, relaxing, and stimulating substances, and with potential and modern exploitation in clinical medicine and oil can act as antibacterial agent against a wide spectrum of pathogenic bacteria strains including: *Listeria monocytogenes*, *Listeria Linnocua*, *Salmonella typhimurium*, *Shigella dysenteriae*, *Bacillus cerus*, and *Staphylococcus aureus*. Mouth washes containing essential oils could also be used as part of plaque-control routine since they can penetrate the plaque biofilm and kill pathogenic-wall. It is also had antibacterial activity especially versus the pathogenic bacteria in dental and mouth and this feature help in adding the essential oil in washes of the mouth to keep the oral health and improve the odor of mouth, especially when mixed with chlorhexine gluconate which is an act in preventing the transmission of pathogenic bacteria.

Essential oil with high concentration of thymol and carvacrol e.g., oregano, savory and thyme, usually inhibit gram positive more than gram-negative pathogenic bacteria.However the antibacterial activity against gram- negative Haemophilus influenza and *Pseudomonas aeruginosa* respiratory pathogens, while gram-positive streptococcus pyrogens was the most resistant to the oil.

Recently, the sciences show several danger effects of the synthetic flavoring and preservatives materials in the food onto the human health so they directed to utilize alternatives such as essential oils as natural materials for its features in preservatives the food for long time, antibacterial effects, and decrease food deterioration [29]. Pathogenic microbes in storage food or food products are responsible to degrade or deteriorate the quality of food products, resulting in the emerging foodborne diseases in various regions of the world [30]. It is well known that some essential oils exert antimicrobial and antioxidant properties. Significant variations in the chemical composition of rosemary essential oils have been reported in relation to the geographic origin [27, 28]. Moreover, variations in the antioxidant and antimicrobial properties of rosemary oils from natural populations were also detected. Its applications are represented in the medicinal and therapeutics, such as aromatherapy, phytotherapy, antibacterial and antifungal uses, hypolipidemic, antitumor, etc.

5. Ways of utilization

The chemical structure of the essential oils is different from one the other and this feature influences their utilization ways and leads to variation in their absorption and the ways of their uses by the body. So, essential oils are generally used in four ways as follows.

5.1 Aromatically

In this way, the oils are inhaled via diluted and then using the diffuser which is act on lightening the oil and make it easy to broad into the air so, this help in treated the external respiratory passage, improve the emotional and mental state and decrease of anxiety, where some researches indicated that the inhalation of some essential oils such as lavender act as quite sedatives where it incorporated with cells of the brain via the receptors of smell or have effects on some hormones and enzymes and do its works as a relaxer, Uplifting Mood, Calm Mood and Meditation [31, 32].

5.2 Topically

This method includes applying the essential oil on the skin where it is absorbed easily. Some oils must be diluted before using while others need a carrier oils. The topical ways used in the support of stress when it used in massage and also it is used in the beauty products such as lotions and wax, but it may cause allergies to some peoples who suffering from skin sensitive so must be careful when using it and applied in small area of the skin after taking advice of the physician [32].

5.3 Internally

Some essential oils have medication features when ingested orally, that is, it act internally where it transported via the stream of blood to various parts of the body, they may acts as an anti-inflammatory, treats digestive disorders, improves digestion, and as a gas expeller. Efficient ways of internal implementation are by mixed the essential oils with some water or milk or by ingesting it as a capsule or in cooking [32].

5.4 Externally

The external way that uses essential oils (i.e., around the home) such as cleaning the home or dishes by add drops from the oil with the cleaning product for its activity as detergents, more effective in cleaning clothes, add nice smell and some act as expeller of insects [32].

6. Conclusions

Essential oils are natural substances extracted from several parts of plants by various methods. They are safe as food preservatives because they do not have side effects on human health. In addition, its bactericidal characteristics encourage utilizing them as medications and in beauty care products.

Author details

Lubna Abdul Muttalib Al-Shalah[1], Nada Khazal Kadhim Hindi[2*] and Israa Harjan Mohsen[2]

1 University of Babylon, Babylon Province, Iraq

2 Department of Basic and Medical Science, College of Nursing, University of Babylon, Babylon Province, Iraq

*Address all correspondence to: nadakhzal@yahoo.com

References

[1] Abdollahi M, Salehnia A, Mortazavi S. Antioxidant, antidiabetic, antihyperlipidermic, reproduction stimulatory properties and safety of essential oil of Satureja khuzesstanica in rat in vivo. Medical Science Monitor. 2003;**9**:331-335

[2] Pagare S, Bhatia M, Tripathi N, Pagare S, Bansal YK. Secondary metabolites of plants and their role: Overview. Current Trends in Biotechnology and Pharmacy. 2015;**9**(3):293-304

[3] Hamid AA, Aiyelaagbe OO, Usman LA. Essential oils: Its medicinal and pharmacological uses. International Journal of Current Research. 2011;**33**(2):086-098

[4] Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. Journal of Applied Microbiology. 1999;**86**:985-990

[5] Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils -a review. Food and Chemical Toxicology. 2008;**46**:446-475

[6] Suryawanshi MA, Mane VB, Kumbhar GB. Methodology to extract essential oils from lemongrass. In: Leaves: Solvent Extraction Approach. IRJET. 2012;**3**:1775-1780

[7] Aspen Leave a Comment. What is the difference between essential oil and extract (Active Ingredients from Aromatic and Medicinal Plants); 2016

[8] Tripathi AK, Upadhyay S, Bhuiyan M, Bhattacharya PR. A review on prospects of essential oils as biopesticide in insect-pest management. Journal of Pharmacognosy and Phytotherapy. 2009;**1**(5):52-63. Available from: http://www.academicjournals.org/jpp

[9] Bajpai VK, Baek KH, SCH K. Review control of Salmonella in foods by using essential oils: A review. Food Research International. 2012;**45**(2012):722-734

[10] Tongnuanchan P, Benjakul S. Essential oils: Extraction, bioactivities, and their uses for food preservation. Journal of Food Science. 2014;**79**(7):1231-1249

[11] Burt SA. Essential oils: Their antibacterial properties and potential applicationsin foods—A review. International Journal of Food Microbiology. 2004;**94**:223-253

[12] Cox SD, Mann CM, Markham JL, Bell HC, Gustafson JE, Warmington JR, et al. The mode of antimicrobial action of essential oil of *Melaleuca alternifola* (tea tree oil). Journal of Applied Microbiology. 2000;**88**:170-175

[13] Lambert RJW, Skandamis PN, Coote P, Nychas GJE. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. Journal of Applied Microbiology. 2001;**91**:453-462

[14] Juven BJ, Kanner J, Schved F, Weisslowicz H. Factors that interact with the antibacterial action of thyme essential oil and its active constituents. The Journal of Applied Bacteriology. 1994;**76**:626-631

[15] Sikkema J, De Bont JAM, Poolman B. Mechanisms of membrane toxicity of hydrocarbons. Microbiological Reviews. 1995;**59**(2):201-222

[16] Wendakoon CN, Sakaguchi M. Inhibition of amino acid decarboxylase activity of *Enterobacter aerogenes* by active components in spices. Journal of Food Protection. 1995;**58**(3):280-283

[17] Firenzuoli F, Jaitak V, Bassolé IHN, Horvath G, Setzer WN, Gori L. Essential oils: New perspectives in human health and wellness evidence-based complementary and alternative medicine. 2014;**2014**:467363. DOI: 10.1155/2014/467363

[18] Rao VPS, Pandey D. Extraction of essential oil and its applications. In partial fulfillment of the requirements of Bachelor of Technology. Chemical Engineering. 2006

[19] Okoh OO, Sadimenko AP, Afolayan AJ. Comparative evaluation of the antibacterial activities of the essential oils of Rosmarinus officinalis L. obtained by hdrodistillation and solvent free microwave extraction methods. Food Chemistry. 2010;**120**:308-312

[20] Golmakani M-T, Rezaei K. Comparison of microwave-assisted hydrodistillationwiththe traditional hydrodistillation method in the extractionof essential oils from Thymus vulgaris L. Food Chemistry. 2008;**109**:925-930

[21] Perineau F, Ganou L, Vilarem G. Studying production of lovage essential oils in a hydrodistillation pilot unit equipped with a cohobation system. Journal of Chemical Technology and Biotechnology. 1992;**53**:165-171

[22] Gavahian M, Farahnaky A, Javidnia K, Majzoobi M. Comparison of Ohmic-assisted hydrodistillation with traditional hydrodistillation for the extraction of essential oils from Thymus vulgaris L. Innovative Food Science and Emerging Technologies. 2012;**14**:85-91

[23] Donelian A, Carlson LHC, Lopes TJ, Machado RAF. Comparison of extraction of patchouli (Pogostemoncablin) essential oil with supercritical CO22and by steam distillation. Journal of Supercritical Fluids. 2009;**48**:15-20

[24] Vian MA, Fernandez X, Visinoni F, Chemat F. Microwave hydrodiffusion and gravity, anew technique for extraction of essential oils. Journal of Chromatography. A. 2008;**1190**:14-17

[25] Babu KGD, Kaul VK. Variation in essential oil composition of rose-scented geranium (Pelargonium sp.) distilled by different distillation techniques. Flavour and Fragance Journal. 2005;**20**:222-231

[26] Guan W, Li S, Yan R, Tang S, Quan C. Comparison of essential oils of clove buds extracted with supercritical carbon dioxide and other three traditional extraction methods. Food Chemistry. 2007;**101**:1558-1564

[27] Farhat A, Fabiano-Tixier A-S, Maataoui ME, Maingonnat J-F, Romdhane M, Chemat F. Microwave steam diffusion for extraction of essential oil from orange peel: Kinetic data, extract's global yield and mechanism. Food Chemistry. 2011;**125**:255-261

[28] Morsy NFS. Chemical structure, quality indices and bioactivity of essential oil constituents. 2017. DOI: 10.5772/66231

[29] Fern J, Viuda-Martos LM. Application of essential oils in food systems. Foods. 2017;**6**:59

[30] Jeffrey, Slater V. Guide to aromatherapy & essential oils. International Research Journal of Engineering and Technology (IRJET). 2016;**3**(08):2395-2472

[31] Available from: http://www.umm.edu/health/medical/altmed/treatment/aromatherapy

[32] Available from: www.sustainablebabysteps.com

Chapter 3

Safety Profile of Essential Oils

Oliviu Vostinaru, Simona Codruta Heghes and Lorena Filip

Abstract

Essential oils are complex mixtures of terpenes and phenylpropanoid compounds, present in multiple species of aromatic plants. They are extensively used in food and cosmetic industries in order to give flavor to food and drinks or as natural fragrances. Moreover, several compounds present in essential oils are important for the pharmaceutical industry due to their antioxidant, antimicrobial, anxiolytic or spasmolytic effects. Although many essential oils are generally recognized as safe, a series of adverse reactions have been reported after their use either by internal or external routes. The aim of this chapter is to increase the awareness of healthcare professionals concerning possible safety issues of essential oils. Common adverse effects of essential oils like sensitization and dermatitis but also more severe phenomena like neurotoxicity will be presented in detail, concerning their epidemiology, mechanism and clinical significance. A thorough understanding of the safety profile of essential oils is necessary for healthcare and food industry professionals in order to maximize their beneficial effects while minimizing the risk for the users.

Keywords: essential oils, terpenes, sensitization, neurological toxicity, endocrine disrupting potential

1. Introduction

Essential oils (EOs) are complex mixtures of aromatic terpenes (monoterpenes and sesquiterpenes) and other aromatic or aliphatic compounds, formed as secondary metabolites in specialized secretory tissues of aromatic plants [1]. Various parts of the aromatic plants (leaves, flowers, fruits, roots, bark) could be used for essential oil extraction by multiple techniques including steam distillation, solvent extraction or supercritical fluid extraction [2]. A verified botanical origin (chemical composition) and protection against contamination and oxidative degradation provided by adequate recipients are key factors influencing essential oils quality. Essential oils from peppermint, lavender, jasmine or ylang-ylang have been used from Antiquity in European and Asian traditional medicine for the prevention and treatment of several diseases but also for food flavoring. Nowadays, over 3000 compounds have been identified in EOs and more than 300 essential oils are commercially available [3] (**Table 1**).

Essential oils are extensively used as food flavors, as fragrances in cosmetic industry but also, a para-medicinal use like aromatherapy has become increasingly popular in the last decades (**Figure 1**). Moreover, due to a complex chemical composition, they are capable to interact with multiple pharmacological targets (receptors, ion channels or enzymes), being studied with promising results for the development of new drug candidates. Some essential oils like peppermint oil are already used in clinical settings for the treatment of functional dyspepsia or irritable bowel

No.	Plant species	Common name of EO
1.	*Boswellia carterii*	Frankincense
2.	*Cananga odorata*	Ylang-Ylang
3.	*Carum carvi*	Caraway
4.	*Cinnamomum zeylanicum*	Cinnamon
5.	*Citrus aurantium* var. *Amara*	Neroli
6.	*Cupressus sempervirens*	Cypress
7.	*Cymbopogon citratus*	Lemongrass
8.	*Eletaria cardamomum*	Cardamom
9.	*Foeniculum vulgare*	Fennel
10.	*Gaultheria fragrantissima*	Wintergreen
11.	*Juniperus communis*	Juniper
12.	*Melaleuca alternifolia*	Tea tree
13.	*Melaleuca viridiflora*	Niaouli
14.	*Mentha x piperita*	Peppermint
15.	*Rosmarinus officinalis*	Rosemary
16.	*Thymus vulgaris*	Thyme
17.	*Zingiber officinale*	Ginger

Table 1.
A selection of commercially available essential oils and their botanical origin (in alphabetical order) [1, 2].

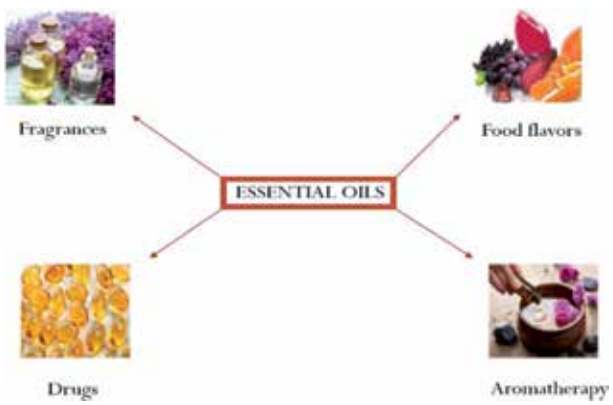

Figure 1.
Main uses of essential oils.

syndrome. Numerous *in vitro* and *in vivo* experiments have proved significant antioxidant, antimicrobial, anxiolytic, spasmolytic or anti-inflammatory effects for several essential oils, which could be also translated in human medicine [4, 5].

Despite their extensive use, key information concerning the safety profile of essential oils are not known to the general public or practitioners of aromatherapy. A significant proportion of the general public mistakenly believes that all essential oils are completely safe for human use, being hailed as "natural and risk-free medicines." Therefore, the aim of this chapter is to increase the awareness of healthcare and food industry professionals, but also general public, concerning possible safety issues of essential oils. Common adverse effects of essential oils like sensitization

and dermatitis but also more severe phenomena like neurotoxicity will be presented in detail, concerning their epidemiology, mechanism and clinical significance. A thorough understanding of the safety profile of essential oils is necessary in order to maximize their beneficial effects while minimizing the risk for the users.

2. Primary routes of systemic absorption of essential oils

Essential oils have a significant lipophilicity due to a high content of monoterpenes, being capable of easily passing through several biological barriers. Thus, a systemic absorption of specific chemical constituents is possible after oral, cutaneous or pulmonary administration of essential oils with beneficial therapeutic effects but also with toxicological implications [6].

After an oral administration of EOs, the systemic absorption of several molecules present in their chemical composition could be significant. A study in rats showed that after oral administration of radio-labeled trans-anethole, over 90% of the substance was absorbed from the digestive tract into the bloodstream, being subsequently metabolized and excreted in feces and urine [7]. Recently, a study from 2018 showed that an immediate release formulation with geraniol orally administered in Sprague-Dawley rats showed an absolute bioavailability of 92%, thus showing an increased systemic absorption [8]. In humans, the absorption of 1,8-cineole from the digestive tract was clearly demonstrated in a study which used enteric coated capsules with a mixture of three terpenoids: limonene, 1,8-cineole and α-pinene [9].

The contact of essential oils with the skin, frequently encountered in aromatherapy massage, could also lead to a systemic absorption of the chemical constituents, depending on the contact time, size of exposed skin surface and concentration of the compound. Essential oils and their volatile constituents can penetrate the skin barrier and facilitate the absorption of other topically applied drugs by inducing a conformational modification of intercellular proteins in the corneal layer and by increasing the drug partitioning [10]. Transdermal absorption was demonstrated for several monoterpenes like α-pinene, camphor or limonene, other structurally related compounds being also capable of passing the skin barrier and generating systemic effects [6].

The volatility of essential oils makes them ideal for pulmonary administration, suitable in the treatment of respiratory diseases. Nevertheless, a fraction of the inhaled compounds could be rapidly absorbed at alveolar level and through airway mucosa, with the apparition of plasmatic concentrations and possible systemic effects. The pulmonary absorption was confirmed for α-pinene, camphor and menthol, the rate of absorption depending on the nature and concentration of inhaled volatile substances and local physiological factors like breathing mechanics [6].

3. General aspects of essential oil toxicity

Essential oils are easily available in pharmacies, supermarkets or online, being used by large segments of the general public. A recent study found that 11% of Australians have used essential oils in 2016, for medicinal purposes, usually self-prescribed [11]. Despite their popularity and extensive use, the safety profile of essential oils has not been fully determined to date. Chemical complexity of essential oils is challenging when investigating which individual components are responsible for certain unwanted effects. Nevertheless, some necessary steps have already been undertaken.

Potential toxic effects of some essential oils and their components were tested on laboratory animals, usually rodents. Acute toxicity was evaluated by LD_{50} test (median lethal dose) in rats, which revealed that most essential oils have a LD_{50} of 1–20 g/kg, indicating a low toxicity. In humans, some essential oils like lemon oil have an LD_{50} of above 5 g/kg. Thus, the lethal dose would be 350 g for an adult of 70 kg, difficult to reach in normal circumstances [12, 13].

A few notable exceptions are EOs from *Boldo* leaf, *Chenopodium*, *Mentha pulegium* (pennyroyal), *Satureja hortensis* (savory) and *Thuja* who presented an LD_{50} between 0.1 and 1 g/kg in rats, signaling a significant toxicity which recommends necessary precautions for their use [12].

Essential oils are susceptible to oxidative degradation, some of the resulting molecules like oxidation products of limonene being potential skin sensitizers [14]. Therefore, a proper storage of essential oils is necessary to conserve their effectiveness and reduce the risk of adverse reactions. Essential oils should be stored in a refrigerator or in a cool, dark place in tightly sealed recipients (brown bottles).

Although most essential oils received the GRAS (generally recognized as safe) status, granted by Flavor and Extract Manufacturers Association (FEMA), it should be pointed out that they were evaluated as flavors with a very low concentration in the tested products. For a concentrated essential oil, certain toxic effects, local or systemic, could develop in specific circumstances [12].

4. Acute intoxication with essential oils

Acute intoxication (poisoning) with essential oils almost invariably results from an oral ingestion of large quantities of undiluted oil, usually accidental. The intoxicated person may present polypnea, convulsions, nausea and vomiting or even death in rare cases. Tea tree oil and the oils of wintergreen, clove, cinnamon and eucalyptus are responsible for most cases, although acute intoxication with other essential oils is possible [13].

In the US, 966 intoxication cases due to tea tree oil ingestion were recorded in 2006, most subjects being represented by children up to 6 years old [13]. In Australia, a recent study identified 1387 cases of essential oil poisoning between 2014 and 2018 [15]. The exposures were accidental or due to a confusion between liquid cough medicines and essential oils. In young children, oral ingestion of 0.6–5 mL of pure eucalyptus oil is sufficient to cause severe symptoms, a fatal case being reported after the ingestion of 30 mL of the oil by an 8-month-old infant [16]. In acute intoxication, infants and young children are particularly at risk due to their reduced body weight combined with the immaturity of enzymatic systems capable of metabolizing essential oils.

Essential oil poisoning was reported also in dogs and cats treated topically with tea tree oil used in large doses for dermatological conditions. The animals presented depression, weakness, motor incoordination and tremors but they recovered after supportive treatment was given [17].

In order to reduce acute intoxication risk, it is recommended that essential oils are kept in child proof recipients, with droppers, separated from oral medication, to avoid confusion.

5. Dermatological toxicity of essential oils

In aromatherapy, essential oils, usually diluted in a carrier oil, are applied directly to the skin. The most important dermatological adverse reactions that may occur include irritation, sensitization and photosensitization [18, 19].

The severity of a dermatological reaction is variable, according to factors like applied substance (aldehydes, phenols), used vehicle, quality/adulteration of the essential oil, method of application, dilution, anatomical site of exposure, integrity of the skin and age of the subject. Environmental conditions could play also an important role. The presence of ultraviolet (UV) light is the decisive factor in photosensitization. Also, ambient temperature and humidity can influence general sensitivity, warm and humid conditions being more favorable for increased severity of adverse reactions [18, 19].

5.1 Skin irritation

Cutaneous irritation is the biological response of the skin to a variety of external stimuli that can induce skin inflammation. The main pathological mechanisms of irritancy include skin barrier disruption, induction of a cytokine cascade and involvement of the oxidative stress network [20]. Primary irritation (contact dermatitis) occurs rapidly the first time an essential oil is used, manifesting as a red wheal or burn and is more likely to occur when essential oils contain large amounts of compounds like phenol, carvacrol and thymol (oregano, savory or thyme), phenolic ethers like eugenol and anethole (clove) or aromatic aldehydes like cinnamaldehyde (cinnamon) (**Figure 2**). Skin reaction is usually limited to the area where the essential oil is applied [21].

The plants whose essential oils are potentially irritant to the skin are listed in **Table 2**. Considering all these aspects, it is recommended that a patch test should be performed before using these oils.

5.2 Skin sensitization

In contrast to irritation, skin sensitization is a response of the adaptive immune system to certain chemical substances called sensitizers or haptens, which can modify skin proteins and induce a delayed T-cell-mediated allergic response [23]. Some of the ingredients which may trigger allergic reactions are listed in the seventh amendment of directive 76/768 CEE (directive 2003/15/CE) and include benzyl alcohol, cinnamyl alcohol, citral, eugenol, hydroxycitronellal, isoeugenol, benzyl salicylate, cinnamaldehyde, coumarin, geraniol, anisyl alcohol, benzyl cinnamate, farnesol, linalool, benzyl benzoate, citronellol, or limonene [24, 25]. Skin sensitization occurs on first exposure to a substance, with only a slight (or absent) effect on the skin. Subsequent exposure to the same compound/compounds will produce a severe inflammatory reaction caused by T-lymphocytes.

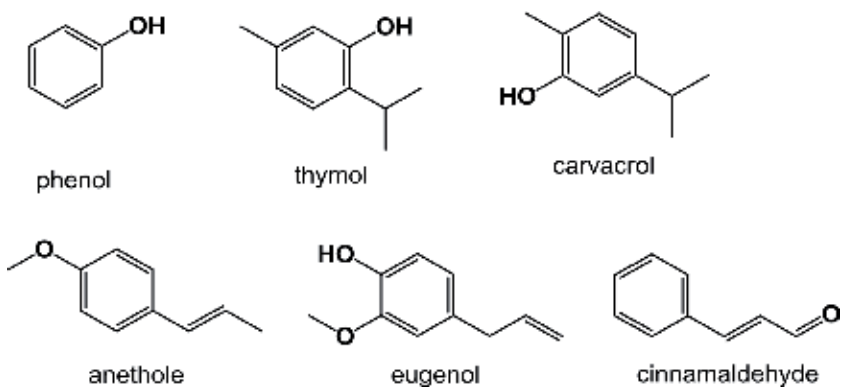

Figure 2.
Chemical structures of the main skin irritant compounds.

Latin name (botanical family)/common name	Part used in EO extraction
Cuminum cyminum (*Apiaceae*)/Cumin	Fruits
Tagetes minuta (*Asteraceae*)/Marigold	Leaves
Origanum vulgare (*Lamiaceae*)/Oregano	Aerial parts
Satureja hortensis (*Lamiaceae*)/Summer savory	Leaves
Satureja montana (*Lamiaceae*)/*W*inter savory	Leaves
Thymus capitatus ct. carvacrol or thymol (*Lamiaceae*)/Spanish oregano	Aerial parts
Thymus serpyllum (*Lamiaceae*)/Wild thyme	Aerial parts
Thymus vulgaris ct. phenol (*Lamiaceae*)/Red thyme	Aerial parts
Cinnamomum cassia (*Lauraceae*)/Chinese cinnamon	Barks
Cinnamomum zeylanicum (*Lauraceae*)/True cinnamon	Leaves, barks
Pimenta racemosa (*Myrtaceae*)/Bay rum tree	Fruits, leaves
Syzygium aromaticum (*Myrtaceae*)/Clove	Buds, leaves, stems
Cymbopogon citratus (*Poaceae*)/Citronella	Aerial parts
Cymbopogon nardus (*Poaceae*)/Lemongrass	Aerial parts
Lippia citriodora (*Verbenaceae*)/Lemon verbena	Leaves

Table 2.
Essential oils potentially irritant to the skin (in alphabetical order of the botanical family) [22].

Symptoms include a bright red rash, which may be painful to some individuals and sometimes a pigmentation of the skin, more frequently in Asians [21, 24]. In order to prevent sensitization, it is recommended to avoid known dermal sensitizers and avoid application of the same essential oil every day for a long period of time.

Table 3 lists some of the oils considered to be dermal sensitizers, but the sensitization process can occur for any essential oil [22, 25].

Essential oils obtained from different species of *Pinus* and *Abies* should only be used when the level of peroxides is kept to the lowest practical level, preferably by adding anti-oxidants at the time of production [19].

Skin sensitization reactions are idiosyncratic, identification of the causative allergen(s) and their subsequent withdrawal generally leading to a resolution of the problem. Some standard mixtures (fragrance mixture) can be used in a patch test to screen for allergic reactions in susceptible individuals, but not all the allergies can be predicted by this method [26, 27].

5.3 Photosensitization

Photosensitization is a reaction between a phototoxin from an essential oil that is applied to the skin in the presence of sunlight or ultraviolet A (UVA) light. The interaction with the light may be either phototoxic or photoallergic.

Photoallergy is an immune-mediated skin reaction, while phototoxicity may lead to photocarcinogenesis. Furanocoumarins (psoralens) appear to be primarily responsible for phytophototoxic reactions in humans. Reactions can vary from pigmentation, blistering, to severe full-thickness burns. Furanocoumarins occur mainly in expressed citrus peel oils (*C. bergamia*, *C. aurantium, C. limon*, *C. aurantifolia*) although they are also found in angelica root (*Angelica archangelica*), rue (*Ruta graveolens*), cumin (*Cuminum cyminum*) parsley leaf (*Petroselinum crispum*), and marigold (*Tagetes minuta*) essential oils [19, 28].

The most common compounds are bergapten and psoralen (**Figure 3**). They are not found in distilled citrus peel oils [21].

Latin name (botanical family)/common name	Part used in EO extraction
Cananga odorata (*Annonaceae*)/Ylang ylang	Flowers
Inula helenium (*Annonaceae*)/Elecampane	Flowers, leaves
Saussurea costus (*Asteraceae*)/Costus	Roots
Commiphora erythraea (*Burseraceae*)/Opoponax	Resin
Myroxylon pereirae (*Fabaceae*)/Peru balsam	Resin
Liquidambar styraciflua (*Hamamelidaceae*)/Styrax	Resin
Cinnamomum cassia (*Lauraceae*)/Chinese cinnamon	Barks
Cinnamomum zeylanicum (*Lauraceae*)/True cinnamon	Leaves, barks
Melissa officinalis (*Lamiaceae*)/Lemon balm	Leaves
Backhousia citriodora (*Myrtaceae*)/Lemon myrtle	Leaves
Melaleuca alternifolia (*Myrtaceae*)/Tea tree	Leaves
Pimenta racemosa (*Myrtaceae*)/Bay rum tree	Fruits, leaves
Syzygium aromaticum (*Myrtaceae*)/Clove	Buds, leaves, stems
Pinus spp. (*Pinaceae*)/Turpentine	Leaves
Cymbopogon nardus (*Poaceae*)/Lemongrass	Aerial parts
Lippia citriodora (*Verbenaceae*)/Lemon verbena	Leaves

Table 3.
Essential oils considered to be dermal sensitizers (in alphabetical order of the botanical family) [21, 22].

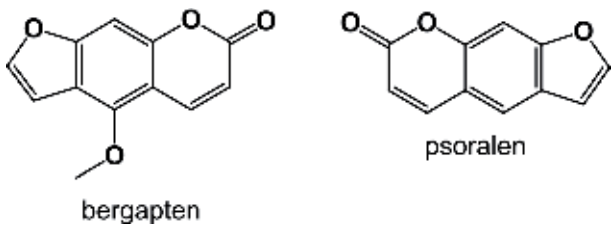

Figure 3.
Chemical structure of the most common furanocoumarins involved in photosensitization.

The factors influencing risk of photosensitization include the amount of essential oil applied topically and the area of exposure. It is considered there is no risk of photosensitization if the skin is covered to prevent exposure to UVA light for at least 2 h [28].

6. Neurological toxicity of essential oils

Essential oils could easily pass the blood-brain barrier, reaching the central nervous system after a systemic absorption. In an experimental setting, essential oils from *Salvia officinalis* and *Hyssopus officinalis* evoked convulsions after intraperitoneal administration in rats at doses of 0.5 g/kg and 0.13 g/kg, respectively [29].

In humans, essential oils from *Salvia officinalis*, *Thuja plicata*, *Cedrus* spp., *Hyssopus officinalis*, *Eucalyptus* spp., *Mentha pulegium*, *Cinnamonum camphora* and *Anethum graveolens* produced tonic-clonic convulsions, particularly in children and especially in those with a history of epileptic syndromes, according to several reports [30, 31].

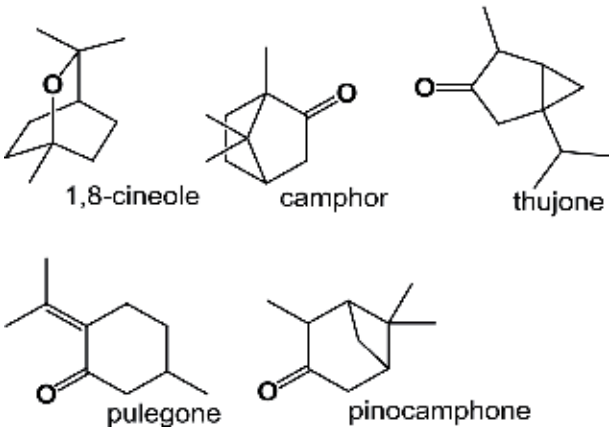

Figure 4.
Chemical structure of terpenes with epileptogenic potential.

The identified chemical constituents responsible for convulsions were usually 1,8-cineole, camphor, thujone, pulegone and pinocamphone (**Figure 4**) [29, 30].

Molecular mechanisms of the convulsant effect of essential oils and their constituents were investigated in laboratory animals. According to a study, some essential oils resemble pentylentetrazole, a powerful convulsive agent, modifying tissue gradients of Na and K and leading to increased cellular excitability in the brain [32]. In another experimental study, thujone one of the frequently incriminated pro-convulsant terpenes, suppressed GABA-induced peak currents in rat dorsal root ganglion neurons, with the subsequent apparition of convulsions, terminated by diazepam or phenobarbital [33]. On the contrary, other research proved that different terpenes could have an anticonvulsant effect. Menthol, another terpene derivative found in the chemical composition of some essential oils enhanced electric currents induced by low concentrations of GABA and directly activated $GABA_A$ receptors in laboratory animals [34].

In the context of potential neurological toxicity of essential oils, European Medicines Agency (EMA) reviewed the safety of suppositories containing terpenes used in seven European countries (France, Belgium, Portugal, Spain, Italy, Luxembourg and Finland) for the treatment of respiratory diseases. The report concluded that terpenes could induce convulsions in children less than 30 months, recommending they should be contraindicated in this particular segment of patients [35].

7. Endocrine disrupting potential of essential oils

In the last decade, there has been an accumulation of evidence suggesting a possible endocrine disrupting effect of some essential oils. Initially, a report signaled the apparition of idiopathic male prepubertal gynecomastia in patients with topically applied lavender oil or tea tree oil [36]. The experimental data showed that both lavender oil and tea tree oil effects are produced by the activation of estrogenic receptors (ER), with a potency of 50% of estradiol, being attenuated in the presence of fulvestrant, a pure antagonist of ER receptors.

Recently, another study published in 2019, confirmed the mentioned data, showing that a continuous exposure to lavender-fragranced products induced a

premature thelarche in four patients [37]. The chemical constituents from the essential oils were individually tested concerning their capacity of stimulating ERα estrogen response element (ERE)-mediated activity. The most active compounds with estrogenic activity were α-terpineol, 4-terpineol and linalool. Further research on a more statistically significant population is needed to confirm the relevance of these findings [37].

8. Essential oils in pregnancy and lactation

The main concerns of the use of essential oils during pregnancy is related to the risk of chemical compounds crossing the placental barrier with direct effects on the product of conception, but also to the direct abortive effect. The use of essential oils during pregnancy is a controversial topic and one that is yet to be fully understood.

Some essential oils are abortifacients, being capable of inducing miscarriage/abortion. Essential oils like persil oil (*Petroselinum sativum*) rich in apiole, pennyroyal oil (*Mentha pulegium*) rich in pulegone, plectranthus oil (*Plectranthus amboinicus*), Spanish sage oil (*Salvia lavandulifolia*) or savin oil (*Juniperus sabina*) rich in sabinyl acetate should be avoided during pregnancy (**Figure 5**). The amounts required to induce an abortion may also pose toxicity risks to the mother, including kidney and liver damage (could be the reason of pregnancy termination in pennyroyal oil case) or even death [12, 38].

Due to their chemical properties (low molecular weight, lipophilicity), it is likely that certain essential oil components could cross the placental barrier, reaching fetal circulation. Following a possible biotransformation into polar molecules, they can accumulate in the fetus due to a reduced glomerular filtration rate and low content of plasma proteins capable of binding xenobiotics [13, 38].

Essential oils should not be used in pregnancy (or breastfeeding) if they contain large amounts of the following components: (E)-anethole (aniseed-*Pimpinella anisum*, star anise-*Illicium verum*, fennel-*Foeniculum vulgare,* dill-*Anethum graveolens*), apiole (persil-*Petroselinum sativum*), β-eudesmol (cypress-*Cupressus sempervirens*), camphor (Spanish lavender-*Lavandula stoechas*), methyl salicylate (sweet birch-*Betula lenta*), pinocamphone (hyssop-*Hyssopus officinalis*), or thujone (mugworth-*Arthemisia vulgaris,* savin-*Juniperus sabina,* thuja-*Thuja occidentalis*) [12, 38].

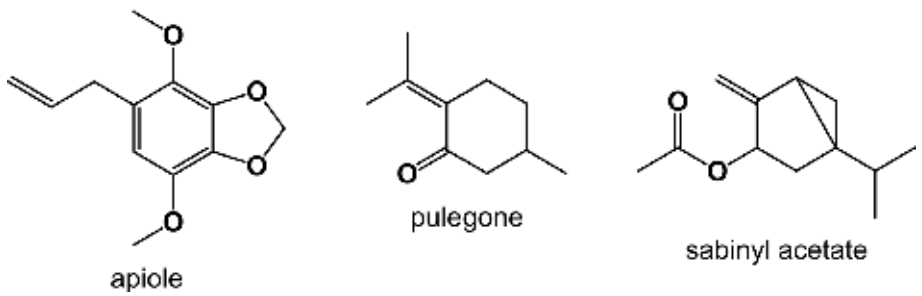

Figure 5.
Chemical compounds responsible for the abortifacient effect.

9. Conclusions

Essential oils have gained an increased attention in the last decades, being used as flavors, fragrances, medicines or in aromatherapy. Although generally considered as "natural and safe," some essential oils could cause significant adverse effects like skin sensitization and contact dermatitis, neurological toxicity or endocrine dysregulations.

An increased awareness of healthcare professionals and general public concerning the safety profile of essential oils is needed in order to correctly exploit their diverse biological effects.

Conflict of interest

The authors declare no conflict of interest.

Author details

Oliviu Vostinaru[1*], Simona Codruta Heghes[2] and Lorena Filip[3]

1 Department of Pharmacology, Physiology and Physiopathology, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2 Department of Drug Analysis, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

3 Department of Bromatology, Hygiene, Nutrition, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

*Address all correspondence to: oliviu_vostinaru@yahoo.com

References

[1] Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils—A review. Food and Chemical Toxicology. 2008;**46**(2): 446-475. DOI: 10.1016/j.fct.2007.09.106

[2] Ribeiro-Santos R, Andrade M, Sanches-Silva M, Ramos de Melo N. Essential oils for food application: Natural substances with established biological activities. Food and Bioprocess Technology. 2018;**11**(1):43-71. DOI: 10.1007/s11947-017-1948-6

[3] Hyldgaard M, Mygind T, Meyer RL. Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. Frontiers in Microbiology. 2012;**3**:1-24. Article 12. DOI: 10.3389/fmicb.2012.00012

[4] Nieto G. Biological activities of three essential oils of the Lamiaceae family. Medicines (Basel). 2017;**4**(3):E63. DOI: 10.3390/medicines4030063

[5] Heghes SC, Vostinaru O, Rus LM, Mogosan C, Iuga CA, Filip L. Antispasmodic effect of essential oils and their constituents: A review. Molecules. 2019;**24**:1675. DOI: 10.3390/molecules24091675

[6] Kohlert C, Van Rensen I, Marz R, Schindler G, Graefe EU, Veit M. Bioavailability and pharmacokinetics of natural volatile terpenes in animals and humans. Planta Medica. 2000;**66**: 495-505. DOI: 10.1055/s-2000-8616

[7] Bounds SV, Caldwell J. Pathways of metabolism of [1'^{14}C]-trans-anethole in the rat and mouse. Drug Metabolism and Disposition. 1996;**24**:717-724

[8] Pavan B, Dalpiaz A, Marani L, Beggiato S, Ferraro L, Canistro D, et al. Geraniol pharmacokinetics, bioavailability and its multiple effects on liver antioxidant and xenobiotic-metabolizing enzymes. Frontiers in Pharmacology. 2018;**9**:18. DOI: 10.3389/fphar.2018.00018

[9] Zimmerman T, Seiberling M, Thomann P, Karabelnik D. Untersuchungen zur relative bioverfugbarkeit und zur pharmacokinetic von myrtol standardisiert. Arzneimittel Forschung. 1995;**45**:1198-1201

[10] Herman A, Herman AP. Essential oils and their constituents as skin penetration enhancer for transdermal drug delivery: A review. The Journal of Pharmacy and Pharmacology. 2015;**67**(4):473-485. DOI: 10.1111/jphp.12334

[11] Harnett JE, McIntyre E, Steel A, et al. Use of complementary medicine products: A nationally representative cross-sectional survey of Australian adults. BMJ Open. 2019;**9**:e024198. DOI: 10.1136/bmjopen-2018-024198

[12] Lis-Balchin M. Aromatherapy Science: A Guide for Healthcare Professionals. 1st ed. London: Pharmaceutical Press; 2005. p. 528. ISBN: 9780857111340

[13] Tisserand R, Young R. Essential Oil Safety, A Guide for Health Care Professionals. 2nd ed. London: Churchill Livingstone; 2014. 780 p. DOI: 10.1016/C2009-0-52351-3

[14] Karlberg AT, Magnusson K, Nilsson U. Air oxidation of d-limonene (the citrus solvent) creates potent allergens. Contact Dermatitis. 1992;**26**:332-340

[15] Lee KA, Harnett JE, Cairns R. Essential oil exposures in Australia: Analysis of cases reported to the NSW poisons information Centre. The Medical Journal of Australia. 2020;**212**(3): 132-133. DOI: 10.5694/mja2.50403

[16] Woolf A. Essential oil poisoning. Clinical Toxicology. 1999;**37**(6):721-727. DOI: 10.1081/CLT-100102450

[17] Villar D, Knight MJ, Hansen SR, Buck WB. Toxicity of melaleuca oil and related essential oils applied topically on dogs and cats. Veterinary and Human Toxicology. 1994;**36**(2):139-142

[18] Michalak M. Aromatherapy and methods of applying essential oils. Archives of Physiotherapy and Global Researches. 2018;**22**:25-31. DOI: 10.15442/apgr.22.2.3

[19] Burfield T. Safety of essential oils. International Journal of Aromatherapy. 2000;**10**:16-29. DOI: 10.1016/S0962-4562(00)80005-3

[20] Fluhr JW, Darlenski R, Angelova-Fischer I, Tsankov N, Basketter D. Skin irritation and sensitization: Mechanisms and new approaches for risk assessment. 1. Skin irritation. Skin Pharmacology and Physiology. 2008;**21**:124-135. DOI: 10.1159/000131077

[21] Buckle J. Clinical Aromatherapy: Essential Oils in Healthcare. 3rd ed. London: Churchill Livingstone; 2014. p. 412. 9780702064890

[22] Price L. Power and hazards. In: Price S, Price L, editors. Aromatherapy for Health Professionals. 4th ed. London: Churchill Livingstone; 2011. pp. 61-76. ISBN: 9780702035647

[23] Basketter D, Darlenski R, Fluhr JW. Skin irritation and sensitization: Mechanisms and new approaches for risk assessment—2. Skin sensitization. Skin Pharmacology and Physiology. 2008;**21**:191-202. DOI: 10.1159/000135635

[24] Vigan M. Essential oils: Renewal of interest and toxicity. European Journal of Dermatology. 2010;**20**:685-692. DOI: 10.1684/ejd.2010.1066

[25] Heisterberg MV, Menné T, Johansen JD. Contact allergy to the 26 specific fragrance ingredients to be declared on cosmetic products in accordance with the EU cosmetics directive. Contact Dermatitis. 2011;**65**:266-275. DOI: 10.1111/j.1600-0536.2011.01962.x

[26] De Groot AC, Schmidt E. Essential oils, part IV: Contact allergy. Dermatitis. 2016;**27**:170-175. DOI: 10.1097/DER.0000000000000197

[27] Uter W, Schmidt E, Geier J, Lessmann H, Schnuch A, Frosch P. Contact allergy to essential oils: Current patch test results (2000-2008) from the information network of departments of dermatology (IVDK)*. Contact Dermatitis. 2010;**63**:277-283. DOI: 10.1111/j.1600-0536.2010.01768.x

[28] Vangipuram R, Mask-Bull L, Kim SJ. Cutaneous implications of essential oils. World Journal of Dermatology. 2017;**6190**:27-31. DOI: 10.5314/wjd.v6.i2.27

[29] Millet Y, Jouglard J, Steinmetz M, Tognetti P, Joanny P, Arditti J. Toxicity of some essential plant oils. Clinical and experimental study. Clinical Toxicology. 1981;**18**:1485-1498

[30] Burkhard PR, Burkhardt K, Haenggeli C-A, Landis T. Plant-induced seizures: Reappearance of an old problem. Journal of Neurology. 1999;**246**:667-670

[31] Halicioglu O, Astarcioglu G, Yaprak I, Aydinlioglu H. Toxicity of *Salvia officinalis* in a newborn and a child: An alarming report. Pediatric Neurology. 2011;**45**:259-260. DOI: 10.1016/j.pediatrneurol.2011.05.012

[32] Steinmetz M, Vial M, Millet Y. Actions de l'huile essentielle de romarin et de certains de ses constituents (eucalyptol et camphre) sur le cortex cerebrale de rat in vitro. Journal de Toxicologie Clinique et Expérimentale. 1987;**7**:259-271

[33] Hold KM, Sirisoma NS, Ikeda T, Narahashi T, Casida JE. A-thujone (the active component of absinthe): γ-Aminobutyric acid type A receptor modulation and metabolic detoxification. Proceedings of the National Academy of Sciences of the United States of America. 2000;**97**:3826-3831

[34] Kolassa N. Menthol differs from other terpenic essential oil constituents. Regulatory Toxicology and Pharmacology. 2013;**65**:115-118. DOI: 10.1016/j.yrtph.2012.11.009

[35] European Medicines Agency. Assessment report for suppositories containing terpenic derivatives. EMA/67070/2012. Available from: https://www.ema.europa.eu/en/documents/referral/terpenic-derivatives-article-31-referral-assessment-report_en.pdf

[36] Henley DV, Mueller S, Korach KS. Prepubertal gynecomastia linked to lavender and tea tree oils. The New England Journal of Medicine. 2007;**356**:479-485. DOI: 10.1056/NEJMoa064725

[37] Ramsey JT, Li Y, Arao Y, Naidu A, Coons LA, Diaz A, et al. Lavender products associated with premature thelarche and prepubertal gynecomastia: Case reports and endocrine-disrupting chemical activities. The Journal of Clinical Endocrinology and Metabolism. 2019;**104**(11):5393-5405. DOI: 10.1210/jc.2018-01880

[38] Black JM. Essential oils and miscarriage. Midwifery Today with International Midwife. 2000;**56**:5-68

Chapter 4

Essential Oils' Potential in Breast Cancer Treatment: An Overview

Isadora de Fátima Braga Magalhães, Carla Junqueira Moraga Tellis, Kátia da Silva Calabrese, Ana Lucia Abreu-Silva and Fernando Almeida-Souza

Abstract

Essential oils are widely used in the pharmaceutical industry for their antimicrobial, antiviral, antifungal, antiparasitic, and insecticidal properties. Their anticancer activity has been increasingly explored as the natural constituents of essential oils play an important role in cancer prevention and treatment. The chemical composition of essential oils includes monoterpenes, sesquiterpenes, oxygenated monoterpenes, phenolic sesquiterpenes, and others. Several mechanisms of action such as antioxidant, antimutagenic, antiproliferative, enhancement of immune functions, modulation of multidrug resistance, and synergistic mechanism of volatile constituents are responsible for their chemotherapeutic properties. This review focuses on the activity of essential oils and their chemical composition in regard to breast cancer.

Keywords: essential oils, antitumor activity, chemical composition, antitumor mechanism, breast cancer

1. Introduction

Cancer is a disease in which normal cells change into a type of cell that can continuously proliferate and, by a process named metastasis, migrate to distant parts of the body [1]. Breast cancer (BC) is the most common cancer in women in the world, presenting high morbidity and mortality [2]. It causes a major public health problem, and the incidence is increasing all over the world [3].

The treatment used for cancer causes many side effects; besides, there are a large number of cases of resistance toward anticancer drugs [4]. These are the main causes that limit the success of treatment in aggressive BC cases. Thus, the need to have novel therapeutic agents is urgent [2].

Natural products, such as plants, may hold the future of BC treatment as the source for new drugs that can interfere with certain processes and ultimately result in clinical usage as an adjuvant therapy [1].

Essential oils (EOs) act as protective mechanism for plants against bacteria, viruses, insects, and even herbivores [5]. They are widely used by the population to treat cancer and can change the metabolism of cancer cells in very low doses, besides provide energy for synthetic processes [6]. This way, EOs are being considered as a promising agent opening venues for novel anticancer therapy as a way to defeat side

effects and the high cost of chemotherapy approaches in BC [7]. This review focuses on apoptosis as an action mechanism by EOs in breast cancer cells, antitumoral activity of EOs and their bioactive compounds, and optimization of EOs' use and their potential as an alternative for side effects reduction during breast cancer treatment.

2. Induction of apoptosis in breast cancer cells by essential oils

Apoptosis is a cellular process involved in physiological and pathological conditions. The mechanism of apoptosis plays an important role in the pathogenesis of many diseases, such as cancer, in which it can be reduced by the cells as a mechanism of survival so they can continue to proliferate, leading to metastasis and resistance to drugs. Caspases can act as initiators and executers of this process and can be activated in an intrinsic or extrinsic way. The intrinsic, or mitochondrial, pathway is controlled by proteins from the Bcl-2 family, which can be proapoptotic proteins such as Bax, Bak, Bad, Bcl-Xs, Bid, Bik, Bim, and Hrk, or antiapoptotic such as Bcl-2, Bcl-XL, Bcl-W, Bfl-1, and Mcl-1 [8].

Collected from Kerman, Golestan, and East Azerbaijan provinces from Iran, EO from the seed of *Foeniculum vulgar* Mill, known as fennel (FN), increased the expression of a proapoptotic factor Bax and decreased antiapoptotic factor Bcl2 gene expression, which leads to cytotoxic effects on MCF7 [7]. FN also had action against MDA-MB [9]. But the FN from Tajikistan presented low cytotoxicity when compared to doxorubicin [10].

Another plant in Iran, *Oliveria decumbens,* is used as a vegetable and medicinal plant by the population to treat cancer-related symptoms. Its EO (OEO) inhibited viability of murine mammary carcinoma 4T1, promoting apoptosis in vitro and led to a TH1 anticancer response in 4T1 tumor-challenging mice [11]. OEO and its main component, thymol, also showed anticancer properties in MDA-MB-231 BC monolayers by activation of intrinsic and maybe extrinsic apoptosis [12]. Also, in Iran, all EOs obtained from the aerial parts of *Zhumeria majdae* collected from five different localities were active and did not show cytotoxicity variability for MCF7 [13].

EO of *Decatropis bicolor* (Zucc.) Radlk, empirically used in Mexico for BC treatment, showed a selective cytotoxic effect toward MDA-MB-231 by activation of Bax and caspases 9 and 3 through intrinsic apoptosis pathway [14]. The apoptosis of MCF7 was stimulated by *Ocimum sanctum* EO with the regulation of apoptotic genes p53 and Bid and elevation of Bax/Bcl-2 [15]. Similar results were found with MCF7 apoptosis induction by *Tetraclinis articulata* [16] and *Myrtus communis* L., commonly used in Morocco for culinary purposes [17]. Carvacrol is the major ingredient of *Zataria Multiflora* EO and induced apoptosis in 2D and 3D cell cultures of MDA-MB-231, MCF7, and T47D with selectivity and increased reactive oxygen species (ROS) generation, loss of mitochondrial membrane potential ($\Delta\Psi m$), caspase 3 activation, and DNA damage [18]. *Thymus vulgaris* L. EO was also proapoptotic to MCF7 and MDA-MB-231 cells [6].

Nuclear factor-κB (NF-κB) is involved in tumor development by regulation of cell proliferation, apoptosis, and cell migration, and its activation is associated with both inflammation and development of cancer, processes that seem to be linked [19]; therefore, its influence by components can be used as a target. Justified by the use already made by the local population, research has shown that *Cyphostemma juttae* (Dinter & Gilg) Desc. EO decreased NF-κB activation with suppressive action on triple negative breast cancer cell lines (TNBCs) [20].

The inhibition effect of EO from *Erythrina corallodendron* L. (ECEO) seems to be mediated by the suppression of the epithelial-mesenchymal transition (EMT) process, implicated with metastasis in cancer progression. Another aspect that

points to it being a possible good target for a new chemotherapeutic agent is that ECEO had greater cytotoxicity to breast cancer cells MDA-MB-231 and MCF7 than to normal human mammary epithelial cells (HMLEs). Although it was not as good as the positive drugs, it may qualify as an adjuvant drug [21].

MCF7 cells treated with frankincense EO (FCO), pine needle, and geranium activated the 5′-adenosine monophosphate-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) signaling pathway, which controls cell growth, proliferation, and autophagy and is deregulated in cancer [22]. As a consequence, there was suppression of cell viability, proliferation, migration, and invasion activity. FCO was also effective in inhibiting tumor growth and inducing apoptosis in human BC mouse model [2].

Aquilaria spp. can provide agarwood, and its EO has proven to reduce the cell number of MCF7, suggesting an effect on cell death and attachment inhibition. Although there have not been any reports of its traditional use for cancer treatment, many reports show its use for inflammatory-associated diseases [23]. On the other hand, *Boswellia* sp. gum has proved to have anti-neoplastic effects and is very commonly used for aroma therapy. EO of *B. sacra* induced cell death in T47D, MCF7, and MDA-MB-231, and the EO hydrodistilled at 100°C was more potent than the one prepared at 78°C, which demonstrates the importance of the form of preparation in the effect of the EO [24].

3. Antitumoral activity of EOs and bioactive compounds

Terpenes (TPs) are usually part of EOs' constituents. Terpenoids (TPNs) are a modified class of terpenes that can be classified according to the number of units of isoprene. Monoterpenes (MTs) are the TPNs with only 2 isoprene units and 10 carbon atoms, sesquiterpenes (STs) have 3 isoprene units and 15 carbon atoms, diterpenes have 4 isoprene units and 20 carbon atoms, triterpenes (TTs) have 6 isoprene units and 30 carbon atoms, and tetraterpenes have 8 isoprene units and 40 carbon atoms [25]. The biosynthesis of terpenoids is shown in **Figure 1**.

Sesquiterpenes (STs) are produced in plants in a way to interact with other plants and as a response to herbivores. These compounds are widely distributed, have been exploited in research for their phytomedicinal potential [26], and are associated with decreasing progression of cancer.

Many plants have demonstrated antiproliferative activity on MCF7 cells and have MT as a major constituent of their EO composition, such as the following : *Schefflera heptaphylla* (β-pinene) [27]; *Heteropyxis dehniae* (linalool) [28]; *Schinus molle* and *Schinus terebinthifolius* (α-phellandrene) [29]; *Melaleuca alternifolia* (terpinen-4-ol) [30]; *Citrus limon*, *Citrus medica*, and *Citrus sinensis* (limonene) [31]; and *Cunila angustifolia* (pulegone and isomenthol) [32], *Satureja khuzistanica* Jamzad (carvacrol) [33], *Satureja intermedia* C.A. Mey (γ-terpinene, thymol, and p-cymene) [34], *Melaleuca armillaris* (Sol Ex Gateau) (1,8-cineole) [35], *Monodora myristica*, *Xylopia aethiopica*, *X. parviflora* [36], *Laurus nobilis*, *Origanum syriacum, O. vulgare*, *Salvia triloba* [37], and berries of *Schinus molle* L. and *S. terebinthifolius* Raddi (more active) (α-phellandrene, β-phellandrene, α-terpineol, α-pinene, β-pinene, and ρ-cymene) [29].

Bisabolene isomers are the main constituents of opoponax (*Commiphora guidotti*); therefore, a ST named β-bisabolene and an alcoholic analogue, α-bisabolol, were tested for their in vitro and in vivo influence on BC. Only β-bisabolene exhibited selective cytotoxic activity for mouse cells MG1361 and human BC cells MCF7, MDA-MB-231, SKBR3, and BT474 with a 37.5% reduction of the growth of transplanted 4T1 mammary tumors [38].

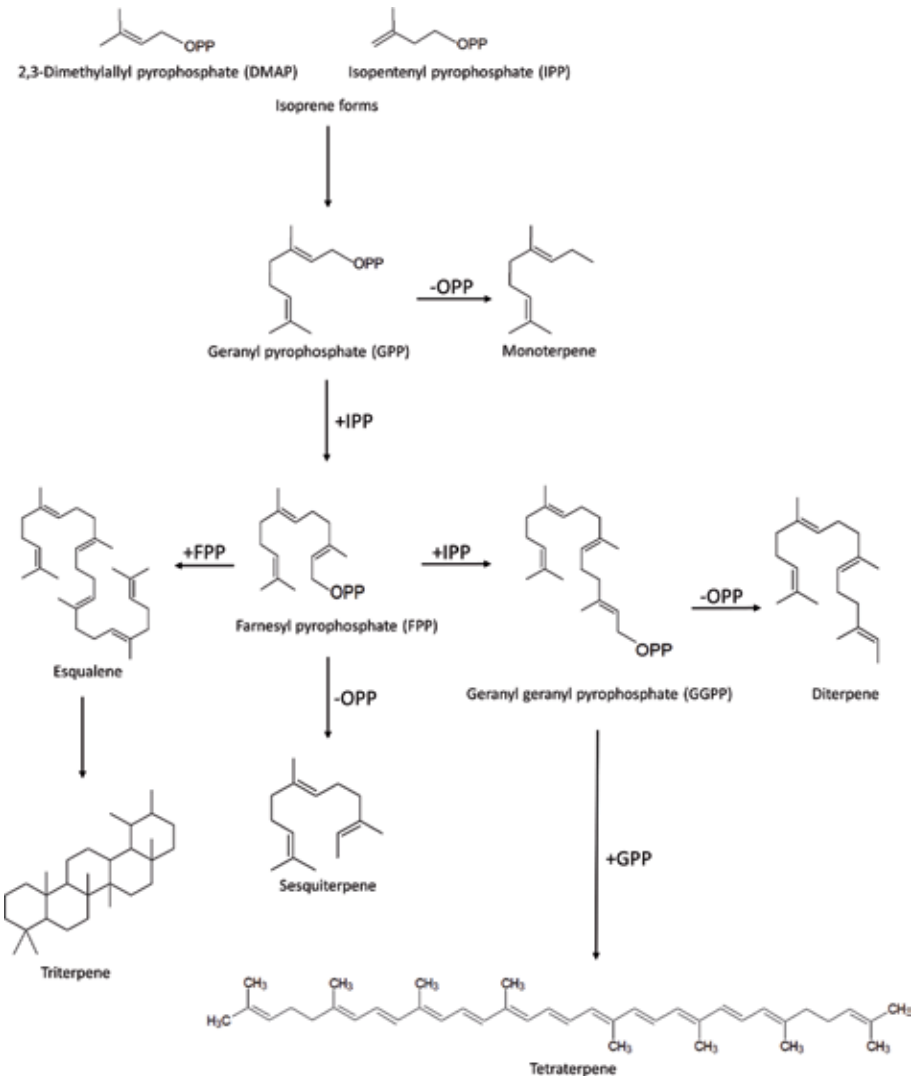

Figure 1.
Terpeneoids' biosynthesis.

EO of *Myrcia splendens* (Sw.) DC. (Myrtaceae) from Amazonian Ecuador has its anticancer activity in MCF7 attributed to α-bisabolol in its composition [39], and EO from leaves of *Anaxagorea* mainly composed of β-eudesmol, α-eudesmol, and β-bisabolene showed similar effect [40].

EO from leaves of *Schinus terebinthifolius* Raddi (Anacardiaceae) collected in Brazil, with germacrene D as one of the major compounds, and fractions were tested in vitro against MCF7. All of them had anticancer activity and that may be due to α- and β-pinene structures [41]. EO of *S. molle* made in Costa Rica was active in breast carcinoma EMT-6 cell line and also had beta-pinene and alpha-pinene as major components [42]. Similar results were not found in EO from leaves of *Porcelia macrocarpa* R. E. Fries (Annonaceae), with main compounds germacrene D and bicyclogermacrene, which did not have significant effect on human breast adenocarcinoma SKBr [43].

β-Elemene is the major active component of the EO from a traditional plant from China, *Curcuma wenyujin* Y.H. Chen et C. Ling, and showed significant cytotoxicity in multidrug-resistant cell line MCF7/adriamycin through inhibition of mTOR

activity, related with cell proliferation and cancer, with the presence of autophagy. However, it only showed effects at high concentrations and the EO had problems regarding stability [44].

Different parts of the same plant can have different chemical constitution and biological activity. For example, the EO of *Garcinia atroviridis* Griff. ex T. Anders showed different results when different parts of plants were used. The essential oil from stem bark (EO-SB) had 79.8% of fatty acid including palmitoleic acid and palmitic acid, and the leaf oil (EO-L) had 86.3% of STs. While EO-SB did not induce cytotoxic effect, the EO-L stimulated the growth of BEAS-2B normal cells, but not in MCF7 cancerous cells, proving the medicinal effects of STs. But the best result was noticed when EO-L was associated with tamoxifen, which demonstrated better activity than the treatment with the drug alone [45]. The EO of *Pallines spinose* flower (F-PSEO) showed different composition than the leaf EO (L-PSEO). F-PSEO contained 96.39% of STs with 78.63% of the oil as oxygenated derivatives such as acorenone B, α-muurolol, and α-cadinol. The L-PSEO was composed of 51.60% of oxygenated STs and 34.06% of SQ hydrocarbons. F-PSEO had stronger anticancer results for MCF7 and MDA-MB-231 and both EOs induced a caspase-dependent and caspase-independent apoptosis and altered the levels of Bcl-2 and Bax proteins [46].

A component that can optimize the anticancer effects when combined with chemotherapy or reduce side effects of the current treatment is a target for many researches. *Rhizoma curcumae* is a plant known to possess activity against different types of cancer cells [47] and is common in Chinese medicine for the treatment of cancer [48]. Curcumol, a guaiane type ST lactone, is the major component of *R. curcumaeis* and, in combination with doxorubicin, made MDA-MB-231 cells more sensible to the action of doxorubicin through the activation of transcription factor NFAT1 and through the bind of the promoter region of miR-181b-2-3p, which is implicated in motility of BC [49] and less survival in breast cancer patients [50]. Curcumol also demonstrated in vivo suppress of tumor growth [49].

EO of *Blepharocalyx salicifolius* was cytotoxic against the MDA-MB-231 cell not by mechanisms related to apoptosis but by preventing cell metabolism reactions. Its main constituents identified were STs bicyclogermacrene, globulol, viridiflorol, γ-eudesmol, and α-eudesmol [51].

Leaves of *Garcinia celebica* L, popularly used in Malaysia and known as "manggishutan," provide an ST-rich EO composed of α-copaene (61.25%), germacrene D (6.72%), and β-caryophyllene (5.85%) with antiproliferative action to MCF7 cells [52]. A similar result was found with the leaf of *Phoebe bournei* (Hemsl.) Yang, which is also composed mainly of STs such as α-copaene, α-muurolene, α-cadinene, and 1s-calamenene [53].

A ST isolated from the EO of *Rhizoma curcumae* named Furanodiene (FD) is associated with anticancer activities in various types of cancers in humans. FD also showed action on chemo-resistant breast cancer cells [54]. EO of *R. curcumae* and the main bioactive component FD were assessed on doxorubicin-resistant MCF7 cell line; although it showed inhibitory effects on cell viability it did not work on ABC transporters [47], which promote the efflux of chemotherapeutic compounds from cells leading to reduction of drug levels inside the cancer cells and insensitivity to the treatment associated with resistance [55]. Furthermore, FD induced apoptosis via intrinsic/extrinsic-dependent and NF-κB-independent pathways [54].

Multidrug-resistant human BC cells MCF7/ADR were treated with EO of *Inula japonica* (IJO) or its ST component isoalantolactone (ISO) or *Angelicae dahuricae* EO (ADO). IJO, ISO, and ADO may reverse the cancer cell by down-regulating ABCB1 expression [56]. This gene encodes a transporter that changes the phenotype of the cells into a multidrug resistance type associated with worse prognosis in BC patients [57].

EO of *Lycopus lucidus* Turcz. var. hirtus Regel was mainly composed of STs α-humulene, β-caryophyllene, and humulene epoxide II, which resulted in a significant dose-dependent inhibition of cell growth human BC cell lines MDA-MB-435S and ZR-75-30, possibly due to the presence of STs in its composition [58].

A primary alcohol named 2-phenylethanol was the main constituent in the EO of *Magnolia virginiana,* while in *M. grandiflora* oil sample the main compounds were ST alcohol (E,E)-farnesol (18%) and 2-phenylethanol (10%). Both EOs were active against MDA-MB231 cell [59]. Similar action was found with EO of *Eryngium campestre* and *E. amethystinum* from central Italy, rich in ST hydrocarbons like germacrene D, allo-aromadendrene, β-elemene, spathulenol, and ledol against human breast adenocarcinoma cells [60].

In the Amazon Rain Forest, climate changes seem to influence *Iryanthera polyneura* Ducke trees. EOs obtained from leaves collected in the rainy season were more active against MCF7. STs spathulenol, α-cadinol, and τ-muurolol were identified as the main compounds [61]. Some seasonal variation was also found in EO of *Mentha* species, *M. arvensis*, *M. piperita*, *M. longifolia,* and *M. spicata*, and they all stimulated the decrease of MCF7 proliferation. Their major compounds were MTs such as menthol, menthone, piperitenone oxide, and carvone, respectively [62].

STs represent 88.57% of all the compounds detected in *Hedyosmum sprucei* EO (Chloranthaceae), collected in the Amazonian region of Pastaza, which led to cytotoxic effects on MCF7 [63]. Similar effect was observed in STs from EO of *Ballota undulata, B. saxatilis, B. nigra* [64], *Convolvulus althaeoides* [65], *Talauma gloriensis* [66], *Cedrelopsis grevei* [67] and *Feronia elephantum* Correa [68].

Pinus roxburghii Sarg. is a Nepal pine used for skin injuries. Its needle EO can inhibit up to 70% of MCF7 cells due to high concentrations of STs such as (E)-caryophyllene and α-humulene and of MT alcohols terpinen-4-ol and α-terpineol [69]. *P. sylvestris* showed cytotoxic selectivity to ER-negative BC cells MDA-MB-231 compared to ER-positive cell line (MCF7) but its chemical composition was not elucidated [70].

Volatile oil from *Saussurea lappa* root (VOSL) showed better anti-breast cancer efficacy and lower side effects than its isolated STs named costunolide (Cos) and dehydrocostuslactone (Dehy), although when combined Cos and Dehy induced apoptosis with regulation of the c-Myc/p53 and AKT/14-3-3 signaling pathways in MCF7 cells or MDA-MB-231 [71].

Thymoquinone (TQ) is a MT and the main constituent of the EO from the seed of *Nigella sativa*. It can optimize chemotherapeutic agents and reduce its toxic side effects, proving to affect the modulation of signaling pathways and molecules with important participation in oncogenic processes such as initiation, progression, invasion, metastasis, and angiogenesis [1]. TQ encapsulated in poly(D,L-lactide-co-glycolide) nanoparticles inhibits the proliferation of MDA-MB-231cells [72], and same effect was obtained with TQ loaded with liposomes in MCF7 and T47D [73]. TQ derivates decreased the growth of MCF-7/Topo [74]. TQ improved the growth inhibition of reference drug doxorubicin in multidrug-resistant MCF-7/TOPO cells, which may be a good source for a booster in the treatment [75]. TQ also showed apoptotic effect in BC cell line (T47D) in combination with gemcitabine as well as alone [76].

TQ was not only active in BC cells but also in vivo by reduction of tumor cell growth, invasion, and migration. These actions seem to be related to the activation of peroxisome proliferator-activated receptor (PPAR)-γ, which acts to inhibit cell growth and proliferation. It also increases ROS, leading to the phosphorylation of p38, a mitogen-activated protein kinase (MAPK), which leads to an antiproliferative and proapoptotic efficacy of TQ in BC [77].

In breast tumor xenograft mouse model, TQ was able to reduce the tumor growth and act synergistically with doxorubicin with antiproliferative and pro-apoptotic effects [78]. Similar result was found with mice injected with triple negative BC (MDA-MB-231 and MDA-MB-436 cells), probably due to the inhibition of eukaryotic elongation factor 2 kinase (EEF2K) signaling [79], which downregulates steps in protein synthesis and increases solid tumor size in vivo [80]. On mice transplanted with breast cancer with EMT6/P cells, the synergic action of TQ and resveratrol decreased the tumor size and led to the cure of 60% animals with no liver or kidney toxicity. The combination also induced apoptosis in EMT6/p and human epithelial BC cell lines MCF7 and T47D [81]. In the xenograft mouse model, TQ increased expression of p-p38 protein in tumors, and led to a decrease in the XIAP, survivin, Bcl-xL, and Bcl-2 antiapoptotic proteins [78].

Eugenol (Eu), an oxygenated MT, is an important volatile constituent of clove EO mainly obtained from *Syzygium*, which has promising results in vitro for the prevention of the progression of BC, with alteration in cellular energy metabolism of MCF10A-ras [82]. For MCF-7 cell, there were cytotoxicity of cinnamon, thyme, chamomile, and jasmine EOs. MT eugenol seems to play an important role in cinnamon action [83].

EO rich in MT eucalyptol from *Cinnamomum glanduliferum* from Egypt and *Nepeta menthoides* from Iran inhibited respectively, MCF7 [84] and MCF7, T47D and MDA-MB-231 [85].

The EO from *Hedychium spicatum* from different regions of western Himalaya where collected and the samples from Almora, Binsar and Uttarakhand were rich in MT and ST and showed cytotoxicity action in MCF7 [86]. Similar effect was observed with EOs obtained from mint (*Mentha spicata*), ginger (*Zingiber officinale*), lemon (*Citrus limonum*), grapefruit (*Citrus paradisi*), jasmine (*Jasminum grandiflora*), lavender (*Lavandula stoechas*), chamomile (*Anthemis nobilis*), thyme (*Thymus vulgaris*), rose (*Rosa centifolia*) and cinnamon (*C. zeylanicum*) from a commercial source in China, composed by elements such as MTs limonene and menthol [83].

Protium heptaphyllum (Aubl.) EO was collected during 3 years and did not exhibit significant cytotoxicity against MCF7 cancer cells, with no change in caspase-3 and TNF-α levels. The major compounds were MT such as terpinolene and p-cymene-8-ol, and p-cymene. However, the EO had antimutagenic activity, which might provide a chemo-preventive effect [87].

The fruit of *Angelica archangelica* L. growing in Iceland provides MT-rich α-pinene EOs differing mainly in the absence or presence of the MT β-phellandrene. However the cytotoxic activity in Crl mouse-BC-cells was independent of the quantity of their main components [88].

The method of preparation affected the composition of EO from *Pituranthos tortuosus* (Desf.) Benth and Hook (Apiaceae). The EO was rich in MT and the major components of the sample prepared by hydrodistillation (HD) were MT β-myrcene, MT sabinene, phenylpropanoids trans-iso-elemicin and MT alcohol terpinen-4-ol. The mayor components from the sample prepared by simultaneous hydrodistillation solvent (n-pentane) (DE) were MTs terpinen-4-ol, sabinene, gamma-terpinene and beta-myrcene. And the mayor components from the sample prepared by conventional-volatile-solvent extraction (SE) were MT terpinen-4-ol, phenylpropanoid dillapiole, and MT allo-ocimene. The DE sample was the most potent against MCF7 [89].

Solanum erianthum leaf volatile oil demonstrated potent inhibitory activity against Hs 578T characterized by the abundance of MT α-terpinolene (17.8%), MT α-phellandrene (17.5%), MT ρ-cymene (15.7%), and MT β-pinene (11.7%) in the leaves [90].

The EO from *Myristica fragrans* (nutmeg) was composed of MT, oxygenated MT, SQ, phenolic ether, and phenylpropanoids, while *Morinda citrifolia* (mengkudu) had mostly carboxylic acids, esters, and isothiocyanate. Both Eos decreased MCF7 cells

[91]. Similar effect was obtained with EO from leaves of *Solanium spirale* Roxb containing 48.10% of diterpene alcohol (E)-Phytol [92], with EO from leaf, stem, stem bark, and root of *Uvariodendron angustifolium* with the presence of citral (a mixture of terpenoids) [93] and with EO from *Syzygium aromaticum*, a source of TT [4].

Litsea cubeba, composed by 68.9% of MT citral, and *Cinnamomum zeylanicum*, mainly composed by (E)-cinnamaldehyde, also had inhibitory action on BC cells MCF7, T47D and MDA-MB-231 [94].

EO from *Erigeron acris* root showed higher antiproliferative activity for MCF7 and MDA-MBA-231 than *E. annuus*, which may be due to polyacetylenic compounds, matricaria and lachnophyllum ester [95], while *Waldheimia glabra* from the Himalayan Mountains, composed mainly of ST spathulenol and thujopsene, fatty alcohol 9-tetradecenol, MT α-thujone, santolina alcohol, and MT tertiary alcohols terpinen-4-ol only had mild action [96].

EO obtained from the seeds of onion *Afro styrax lepidophyllus* and garlic tree *Scorodophloeus zenkeri* are usually used as spices in Africa. It exhibited a strong inhibitory effect on MDA-MB 231. The predominant compound in both oils was the terpenoid 2,4,5,7-tetrathiaoctane [97]. EO of aerial parts, branches and leaves, of *Glandora rosmarinifolia* (Ten.) D.C. Thomas is composed mostly of aliphatic alkanes and diterpene hydrocarbons; it induces cell growth inhibition at triple negative-breast cancer-cell lines SUM 149 and MDA-MB-231 in part due to a pro-oxidant mechanism [5].

4. Optimization of the EOs' use against BC

Nanoemulsions (NEs) can work as an ally to reduce some problems associated with Eos such as sensibility and lability. That is what happened with the use of *Zataria multiflora* EO loaded into chitosan (CS) nanoparticles. This combination improved the proliferation inhibition rate of BC cells as well as apoptosis, generation of ROS, trigger of mitochondrial membrane permeabilization and DNA damage, with high selectivity to human cancer cells of breast adenocarcinoma MCF7, T47D, and MDA-MB-231 [98].

Another study made with CS and N,N,N-trimethyl chitosan (TMC) also increased the toxicity of another EO from *Ocimum gratissimum*, when loaded with TNC nanoparticles on MDA-MB-231 BC cell lines [99]. A similar result was found using *Cyperus articulatus* EO loaded with CS nanoparticles [100].

Nigella sativa L. has been used in traditional medicine for about 1400 years and it grows in countries bordering the Mediterranean Sea and India [101]. Its EO has properties such as anti-inflammatory and anticancer. *N. sativa*-EO-NE increased the apoptosis of MCF7 [102].

Mitomycin C (MTC) was solubilized in NEs of EO from ginger (EOG) and from frankincense, which was shown to increase the toxicity for MCF7 cells when compared with the use of MMC alone. EOG had the strongest apoptotic effect [103]. The same effect was seen when MTC was combined with chamomile NE oil [104].

Sandal wood EO (SEO), extracted from *Santalum* trees, was encapsulated into liposomes composed of 15% SEO, 78.5% water, 4% enzyme modified lecithin, and 2.5% polysorbate. This combination provoked DNA damage and cytotoxicity and genotoxicity against MCF7 cells [105].

5. EOs as an alternative for side effects reduction during BC treatment

Chemotherapy-induced nausea and emesis are one of the most common problems in BC patients and they can be inappropriately managed due to low

affordability of new medications [106]. Women suffering from BC received 5-day aroma therapy treatment using either ginger EO or a placebo. Nausea score was significantly lower after ginger EO inhalation but was not sustained for the overall treatment effect. Overall, the EO improved health-related quality of life [107].

Symptoms of urogenital atrophy (UA) are common in BC survivors [108]. The cause is due to systemic treatments as a side effect of endocrine therapies and topical estrogen is usually used to reduce the symptoms. Other alternatives are being sought and could be valid to improve life quality of the patients with BC. EO of *Cymbopogon martini* and *Pelargonium graveolens* affected the cell grown in hormone-dependent MCF7 and hormone-independent MDA-MB-231 cell lines with pronounced estrogenicity, but clinical trials are necessary to better understand these effects [109].

Reaction on the skin can happen in BC patients under radiotherapy treatment. Twenty four patients received an EO mixture with 32.5% of jojoba (*Simmondsia chinensis*), 30% *Aloe vera* (*Aloe barbadenisis*), 10% of Tamanu (*Calophyllum inophyllum*), 10% primrose *Oenothera biennis*), 5% frankincense (*Boswellia carteri*), 5% geranium (*Pelargonium graveolens*), 5% lavender (*Lavandula angustifolia*), and 2.5% helichrysum (*Helichrysum angustifolium*) this EO mixture had a similar result as a medication used for treating this side effect and therefore can be used as an alternative treatment [110].

6. Unsatisfactory EOs results for BC

Some EOs used in research were not able to have satisfactory in vitro anticancer effects on MCF7 as EO from *Sideritis perfoliata*, *Satureja thymbra*, *Salvia officinalis*, *Laurus nobilis*, *Pistacia palaestina* [111], *Nepeta cataria* L. [112], *Nectandra leucantha* [113], *Laurus nobilis* L, *Origanum syriacum* L, *Origanum vulgare* L, *Salvia triloba* L. [37], *Salvia officinalis* [114], grapefruit (*Citrus paradisi*), ginger (*Zingiber officinale*) [83], and *Anemopsis californica* [115].

Origanum vulgare EO, composed mostly of 4-terpineol, induced cell proliferation of MCF7, although at the concentration of 50 mg/mL opposite effect was found, but still with minor effect when compared to the result in other cancer cells [116]. *Aloysia citriodora, Boswellia sacra, Boswellia serrata, Cistus ladanifer, Citrus × aurantium, Citrus limon, Citrus sinensis, Cymbopogon citratus, Foeniculum vulgar, Illicium verum, Satureja montana, Syzygium aromaticum, Thymus capitatus, and Thymus vulgaris* presented minor effects in MCF7, T47D, and MDA-MB-231 [94].

EO of *Semenovia suffruticosa* grown in Kerman, Iran, induced cell death in MCF7, but it also had the same effect on normal cell line [117]. EO from the leaves of *Solanum macranthum* did not show anticancer properties in Hs578T [90].

This information is helpful to elucidate some effects of EOs that are used by the population. Some EOs may not have any effect for BC or can even help stimulate BC cells or have toxic action. Due to this, it is important to determinate if they are safe for common use. Furthermore, it is worth mentioning that the results show unsatisfactory effects in regard to concentrations used, which does not prevent the use of these EOs in other researches with different outcomes.

7. Conclusions

Sesquiterpenes and monoterpenes are part of the main components of essential oils, some of them already being isolated and with actions described, although it is important to establish the force of the use of multiple compounds together.

A large number of essential oils from different plants have been described in the literature with promising in vitro effect in a variety of breast cancer cells and even

with in vivo effects in murine model; it is important to continue this research and take it to the next level with clinical trials.

The articles found in the literature and their results encourage the use of essential oils. The importance of plant research and the production of these oils demonstrated the difference they can make as a supporting anticancer agent or as a reducer of the side effects of breast cancer, which shows its power in the fight against breast cancer.

Acknowledgements

The authors would like to thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Coordination for the Improvement of Higher Education Personnel), Brazil (CAPES) [Finance Code 001] and Instituto Oswaldo Cruz for financial support.

Conflict of interest

The authors declare no conflict of interest.

Author details

Isadora de Fátima Braga Magalhães[1,2], Carla Junqueira Moraga Tellis[3], Kátia da Silva Calabrese[4], Ana Lucia Abreu-Silva[1] and Fernando Almeida-Souza[1,4]*

1 State University of Maranhão, São Luís, Brazil

2 Federal Fluminense University, Niterói, Brazil

3 Instituto de Tecnologia em Fármacos Farmanguinhos, Rio de Janeiro, Brazil

4 Oswaldo Cruz Institute, Rio de Janeiro, Brazil

*Address all correspondence to: fernandoalsouza@gmail.com

References

[1] Shanmugam MK, Arfuso F, Kumar AP, Wang L, Goh BC, Ahn KS, et al. Modulation of diverse oncogenic transcription factors by thymoquinone, an essential oil compound isolated from the seeds of *Nigella sativa,* Linn. Pharmacological Research. 2018;**129**:357-364. DOI: 10.1016/j.phrs.2017.11.023

[2] Ren P, Ren X, Cheng L, Xu L. Frankincense, pine needle and geranium essential oils suppress tumor progression through the regulation of the AMPK/mTOR pathway in breast cancer. Oncology Reports. 2018;**39**:129-137. DOI: 10.3892/or.2017.6067

[3] Bagheri SM, Asl AA, Shams A, Mirghanizadeh-Bafghi SA, Hafizibarjin Z. Evaluation of cytotoxicity effects of oleo-gum-resin and its essential oil of ferula Assa-foetida and ferulic acid on 4T1 breast cancer cells. Indian Journal of Medical and Paediatric Oncology: Official Journal of Indian Society of Medical & Paediatric Oncology. 2017;**38**:116. DOI: 10.4103/ijmpo.ijmpo_60_16

[4] Kumar PS, Febriyanti RM, Sofyan FF, Luftimas DE, Abdulah R. Anticancer potential of Syzygium aromaticum L. in MCF-7 human breast cancer cell lines. Pharmacognosy Research. 2014;**6**:350-354. DOI: 10.4103/0974-8490.138291

[5] Poma P, Labbozzetta M, Notarbartolo M, Bruno M, Maggio A, Rosselli S, et al. Chemical composition, in vitro antitumor and pro-oxidant activities of Glandora rosmarinifolia (Boraginaceae) essential oil. PLoS One. 2018;**13**:e0196947. DOI: 10.1371/journal.pone.0196947

[6] Kubatka P, Uramova S, Kello M, Kajo K, Samec M, Jasek K, et al. Zubor P, Anticancer activities of Thymus vulgaris l. in experimental breast carcinoma in vivo and in vitro. International Journal of Molecular Sciences. 2019;**20**:1749. DOI: 10.3390/ijms20071749

[7] Ghasemian A, Al-Marzoqi AH, Mostafavi SK, Alghanimi YK, Teimouri M. Chemical composition and antimicrobial and cytotoxic activities of *Foeniculum vulgare* mill essential oils. Journal of Gastrointestinal Cancer. 2020;**51**:260-266. DOI: 10.1007/s12029-019-00241-w

[8] Wong RS. Apoptosis in cancer: From pathogenesis to treatment. Journal of Experimental & Clinical Cancer Research. 2011;**30**:87. DOI: 10.1186/1756-9966-30-87

[9] Akhbari M, Kord R, Jafari Nodooshan S, Hamedi S. Analysis and evaluation of the antimicrobial and anticancer activities of the essential oil isolated from *Foeniculum vulgare* from Hamedan, Iran. Natural Product Research. 2019;**33**:1629-1632. DOI: 10.1080/14786419.2017.1423310

[10] Sharopov F, Valiev A, Satyal P, Gulmurodov I, Yusufi S, Setzer WN, et al. Cytotoxicity of the essential oil of fennel (*Foeniculum vulgare*) from Tajikistan. Food. 2017;**6**:73. DOI: 10.3390/foods6090073

[11] Jamali T, Kavoosi G, Ardestani SK. In-vitro and in-vivo anti-breast cancer activity of OEO (Oliveria decumbens vent essential oil) through promoting the apoptosis and immunomodulatory effects. Journal of Ethnopharmacology. 2020;**2**(48):112313. DOI: 10.1016/j.jep.2019.112313

[12] Jamali T, Kavoosi G, Safavi M, Ardestani SK. In-vitro evaluation of apoptotic effect of OEO and thymol in 2D and 3D cell cultures and the study of their interaction mode with DNA. Scientific Reports. 2018;**8**:1-19. DOI: 10.1038/s41 598-018-34055-w

[13] Saeidi M, Asili J, Emami SA, Moshtaghi N, Malekzadeh-Shafaroudi S. Comparative volatile composition, antioxidant and cytotoxic evaluation of the essential oil of Zhumeriamajdae from south of Iran. Iranian Journal of Basic Medical Sciences. 2019;**22**:80-85. DOI: 10.22038/ijbms.2018.20829.5418

[14] Gómez CE, Carreño AA, Ishiwara DP, Martínez ES, López JM, Hernández NP, et al. *Decatropis bicolor* (Zucc.) Radlk essential oil induces apoptosis of the MDA-MB-231 breast cancer cell line. BMC Complementary and Alternative Medicine. 2016;**16**:266. DOI: 10.1186/s12906-016-1136-7

[15] Manaharan T, Thirugnanasampandan R, Jayakumar R, Kanthimathi MS, Ramya G, Ramnath MG. Purified essential oil from *Ocimum sanctum* Linn. Triggers the apoptotic mechanism in human breast cancer cells. Pharmacognos Magazine. 2016;**12**:327-331. DOI: 10.4103/0973-1296.185738

[16] Buhagiar JA, Podesta MT, Wilson AP, Micallef MJ, Ali S. The induction of apoptosis in human melanoma, breast and ovarian cancer cell lines using an essential oil extract from the conifer *Tetraclinis articulata*. Anticancer Research. 1999;**19**:5435-5443

[17] Harassi Y, Tilaoui M, Idir A, Frédéric J, Baudino S, Ajouaoi S, et al. Phytochemical analysis, cytotoxic and antioxidant activities of Myrtus communis essential oil from Morocco. Journal of Complementary and Integrative Medicine. 2019;**16**:1-8. DOI: 10.1515/jcim-2018-0100

[18] Salehi F, Behboudi H, Kavoosi G, Ardestani SK. Monitoring ZEO apoptotic potential in 2D and 3D cell cultures and associated spectroscopic evidence on mode of interaction with DNA. Scientific Reports. 2017;**7**:1-4. DOI: 10.10 38/s41598-017-02633-z

[19] Karin M, Cao Y, Greten FR, Li ZW. NF-κB in cancer: From innocent bystander to major culprit. Nature Reviews Cancer. 2002;**2**:301-310. DOI: 10.103 8/nrc780

[20] Zito P, Labbozzetta M, Notarbartolo M, Sajeva M, Poma P. Essential oil of *Cyphostemma juttae* (Vitaceae): Chemical composition and antitumor mechanism in triple negative breast cancer cells. PLoS One. 2019;**14**:e0214594. DOI: 10.1371/journal.pone.0214594

[21] Xing X, Ma JH, Fu Y, Zhao H, Ye XX, Han Z, et al. Essential oil extracted from *Erythrina corallodendron* L. leaves inhibits the proliferation, migration, and invasion of breast cancer cells. Medicine. 2019;**98**: e17009. DOI: 10.1097/MD.0000000000017009

[22] Chapuis N, Tamburini J, Green AS, Willems L, Bardet V, Park S, et al. Perspectives on inhibiting mTOR as a future treatment strategy for hematological malignancies. Leukemia. 2010;**24**:1686-1699. DOI: 10.1038/leu.2010.170

[23] Hashim YZ, Phirdaous A, Azura A. Screening of anticancer activity from agarwood essential oil. Pharmacognosy Research. 2014;**6**:191-194. DOI: 1.4103/0974 -8490.13259

[24] Suhail MM, Wu W, Cao A, Mondalek FG, Fung KM, Shih PT, et al. Boswellia sacra essential oil induces tumor cell-specific apoptosis and suppresses tumor aggressiveness in cultured human breast cancer cells. BMC Complementary and Alternative Medicine. 2011;**11**:129. DOI: 10 .1186/1472-6882-11-129

[25] Perveen S. Introductory chapter: Terpenes and terpenoids.

In: Terpenes and Terpenoids. Rijeka: IntechOpen; 2018. DOI: 10.5772/intechopen.79683

[26] Silva WMC, Andersen JL, Holanda MT, Walter MEMT, Brigido MM, Stadler PF, et al. Exploring plant sesquiterpene diversity by generating chemical networks. PRO. 2019;7:240. DOI: 10.20944/preprints201903.0015.1

[27] Li YL, Yeung CM, Chiu LC, Cen YZ, Ooi VE. Chemical composition and antiproliferative activity of essential oil from the leaves of a medicinal herb, *Schefflera heptaphylla*. Phytotherapy Research. 2009;**23**:140-142. DOI: 10.1002/ptr.2567

[28] Sibanda S, Chigwada G, Poole M, Gwebu ET, Noletto JA, Schmidt JM, et al. Composition and bioactivity of the leaf essential oil of Heteropyxis dehniae from Zimbabwe. Journal of Ethnopharmacology. 2004;**92**(1):07-111. DOI: 10.1016/j.jep.2004.02.010

[29] Bendaoud H, Romdhane M, Souchard JP, Cazaux S, Bouajila J. Chemical composition and anticancer and antioxidant activities of Schinus molle L. and *Schinus terebinthifolius* Raddi berries essential oils. Journal of Food Science. 2010;**75**:C466-C472. DOI: 10.1111/j.1750-3841.2010.01711.x

[30] Liu X, Zu Y, Fu Y, Yao L, Gu C, Wang W, et al. Antimicrobial activity and cytotoxicity towards cancer cells of *Melaleuca alternifolia* (tea tree) oil. European Food Research and Technology. 2009;**229**:247-253. DOI: 10.1007/s002 17-009-1057-5

[31] Monajemi R, Oryan S, Haeri RS, Ghanadi AR, Jafarian DA. Cytotoxic effects of essential oils of some Iranian citrus peels. Iranian Journal of Pharmaceutical Research. 2005;**4**:183-187. DOI: 10.22037/ijpr.2010.65

[32] De Sousa MH, Morgan J, Cesca K, Flach A, Moura N. Cytotoxic activity of *Cunila angustifolia* Benth essential oil. Chemistry & Biodiversity. 2020;**17**:e190, 065. DOI: 10.1002/cbdv.201900656

[33] Yousefzadi M, Riahi-Madvar A, Hadian J, Rezaee F, Rafiee R, Biniaz M. Toxicity of essential oil of *Satureja khuzistanica*: In vitro cytotoxicity and anti-microbial activity. Journal of Immunotoxicology. 2014;**11**:50-55. DOI: 10.3109/1547691X.2013.789939

[34] Sharifi-Rad J, Sharifi-Rad M, Hoseini-Alfatemi SM, Iriti M, Sharifi-Rad M, Sharifi-Rad M. Composition, cytotoxic and antimicrobial activities of *Satureja intermedia* C.A. Mey essential oil. International Journal of Molecular Sciences. 2015;**16**:17812-17825. DOI: 10.3390/ijms160817812

[35] Chabir N, Romdhane M, Valentin A, Moukarzel B, Marzoug HN, Brahim NB, et al. Chemical study and antimalarial, antioxidant, and anticancer activities of *Melaleuca armillaris* (sol ex gateau) Sm essential oil. Journal of Medicinal Food. 2011;**14**:1383-1388. DOI: 10.1089/jmf.2010.0168

[36] Bakarnga-Via I, Hzounda JB, Fokou PV, Tchokouaha LR, Gary-Bobo M, Gallud A, et al. Composition and cytotoxic activity of essential oils from *Xylopia aethiopica* (Dunal) A. rich, *Xylopia parviflora* (A. rich) Benth. and *Monodora myristica* (Gaertn) growing in Chad and Cameroon. BMC Complementary and Alternative Medicine. 2014;**14**:125. DOI: 10.1186/1472-6882-14-125

[37] Al-Kalaldeh JZ, Abu-Dahab R, Afifi FU. Volatile oil composition and antiproliferative activity of *Laurus nobilis*, *Origanum syriacum*, *Origanum vulgare*, and *Salvia triloba* against human breast adenocarcinoma cells. Nutrition Research. 2010;**30**:271-278. DOI: 10.1016/j.nutres.2010.04.001

[38] Yeo SK, Ali AY, Hayward OA, Turnham D, Jackson T, Bowen ID, et al.

β-Bisabolene, a sesquiterpene from the essential oil extract of opoponax (*Commiphora guidottii*), exhibits cytotoxicity in breast cancer cell lines. Phytotherapy Research. 2016;**30**:418-425. DOI: 10.1002/ptr.5543

[39] Scalvenzi L, Grandini A, Spagnoletti A, Tacchini M, Neill D, Ballesteros JL, et al. Myrciasplendens (Sw.) DC. (syn. M. fallax (Rich.) DC.) (Myrtaceae)essential oil from Amazonian Ecuador: A chemical characterization and bioactivity profile. Molecules. 2017;**22**:1163. DOI: 10.3390/m olecules22071163

[40] De Alencar DC, Pinheiro ML, Pereira JL, de Carvalho JE, Campos FR, Serain AF, et al. Chemical composition of the essential oil from the leaves of Anaxagorea brevipes (Annonaceae) and evaluation of its bioactivity. Natural Product Research. 2016;**30**:1088-1092. DOI: 10.1080/14786419.2015.1101103

[41] Santana JS, Sartorelli P, Guadagnin RC, Matsuo AL, Figueiredo CR, Soares MG, et al. Essential oils from *Schinus terebinthifolius* leaves—Chemical composition and in vitro cytotoxicity evaluation. Pharmaceutical Biology. 2012;**50**:1248-1253. DOI: 10.3109/13880209.2012.666880

[42] Díaz C, Quesada S, Brenes O, Aguilar G, Cicció JF. Chemical composition of Schinus molle essential oil and its cytotoxic activity on tumour cell lines. Natural Product Research. 2008;**22**:1521-1534. DOI: 10.10 80/1478641 0701848154

[43] Da Silva EB, Matsuo AL, Figueiredo CR, Chaves MH, Sartorelli P, Lago JH. Chemical constituents and cytotoxic evaluation of essential oils from leaves of *Porcelia macrocarpa* (Annonaceae). Natural Product Communications. 2013;**8**:277-279. DOI: 10.1177/1934578x1300800237

[44] Ding XF, Shen M, Xu LY, Dong JH, Chen G. 13, 14-bis (cis-3, 5-dimethyl-1-piperazinyl)-β-elemene, a novel β-elemene derivative, shows potent antitumor activities via inhibition of mTOR in human breast cancer cells. Oncology Letters. 2013;**5**:1554-1558. DOI: 10.3892/ol.2013.1213

[45] Tan WN, Lim JQ, Afiqah F, Nik Mohamed Kamal NN, Abdul Aziz FA, Tong WY, et al. Chemical composition and cytotoxic activity of *Garcinia atroviridis* Griff. ex T. Anders. essential oils in combination with tamoxifen. Natural Product Research. 2018;**32**:854-858. DOI: 10.1080/14786419.2 017.1361951

[46] Saleh AM, Al-Qudah MA, Nasr A, Rizvi SA, Borai A, Daghistani M. Comprehensive analysis of the chemical composition and in vitro cytotoxic mechanisms of *Pallines spinosa* flower and leaf essential oils against breast cancer cells. Cellular Physiology and Biochemistry. 2017;**42**:2043-2065. DOI: 10.1159/000479900

[47] Zhong Z, Yu H, Wang S, Wang Y, Cui L. Anti-cancer effects of *Rhizoma curcumae* against doxorubicin-resistant breast cancer cells. Chinese Medicine. 2018;**13**:44. DOI: 10.1186/s13020-018-0203-z

[48] Lu JJ, Dang YY, Huang M, Xu WS, Chen XP, Wang YT. Anti-cancer properties of terpenoids isolated from *Rhizoma curcumae*—A review. Jornal of Ethnopharmacology. 2012;**143**:406-411. DOI: 10.1016/j.jep.2012.07.009

[49] Zeng C, Fan D, Xu Y, Li X, Yuan J, Yang Q, et al. Curcumol enhances the sensitivity of doxorubicin in triple-negative breast cancer via regulating the miR-181b-2-3p-ABCC3 axis. Biochemical Pharmacology. 2020;**174**:113795. DOI: 10.1016/j.bcp.2020.113795

[50] Zhang P, Wang L, Rodriguez-Aguayo C, Yuan Y, Debeb BG,

Chen D, et al. miR-205 acts as a tumour radiosensitizer by targeting ZEB1 and Ubc13. Nature Communications. 2014;**5**(5):671. DOI: 10.1038/ncomms6671

[51] Furtado FB, Borges BC, Teixeira TL, Garces HG, Almeida Junior LD, Alves FC, et al. Chemical composition and bioactivity of essential oil from *Blepharocalyx salicifolius*. International Journal of Molecular Sciences. 2018;**19**:33. DOI: 10.3390/ijms19010033

[52] Tan WN, Tan ZH, Zulkifli NI, Nik Mohamed Kamal NN, Rozman NA, Tong WY, et al. Sesquiterpenes rich essential oil from *Garcinia celebica* L. and its cytotoxic and antimicrobial activities. Natural Product Research. 2019;**17**:1-5. DOI: 10.1080/14786419.2019.1569012

[53] Ding W, Liping N, Xing H, Wei Z, Zhoua Q, Nong R, et al. Essential oil extracted from leaf of Phoebe bournei (Hemsl.) yang: Chemical constituents, antitumor, antibacterial, hypoglycemic activities. Natural Product Research. 2018;**23**:1-4. DOI: 10.1080/14786419.2018.1542393

[54] Zhong ZF, Yu HB, Wang CM, Qiang WA, Wang SP, Zhang JM, et al. Furanodiene induces extrinsic and intrinsic apoptosis in doxorubicin-resistant MCF-7 breast cancer cells via NF-κB-independent mechanism. Frontiers in Pharmacology. 2017;**8**:648. DOI: 10.3389/fphar.2017.00 648

[55] Gottesman MM. Mechanisms of cancer drug resistance. Annual Review of Medicine. 2002;**53**: 615-627. DOI: 10.1146/annurev.med.53.082901.103 929

[56] Wu M, Li T, Chen L, Peng S, Liao W, Bai R, et al. Essential oils from *Inula japonica* and *Angelicae dahuricae* enhance sensitivity of MCF-7/ADR breast cancer cells to doxorubicin via multiple mechanisms. Journal of Ethnopharmacology. 2016;**180**:18-27. DOI: 10.1016/j.jep.2016.01.015

[57] Delou JMA, Vignal GM, Índio-do-Brasil V, Accioly MTS, da Silva TSL, Piranda DN, et al. Loss of constitutive ABCB1 expression in breast cancer associated with worse prognosis. Breast Cancer (Dove Med Press). 2017;**9**:415-428. DOI: 10.2147/BCT T.S131284

[58] Yu JQ, Lei JC, Zhang XQ, Yu HD, Tian DZ, Liao ZX, et al. Anticancer, antioxidant and antimicrobial activities of the essential oil of *Lycopus lucidus* Turcz. var. hirtus Regel. Food Chemistry. 2011;**126**:1593-1598. DOI: 10.1016/j.foo dchem.2010.12.027

[59] Farag MA, Al-Mahdy DA. Comparative study of the chemical composition and biological activities of *Magnolia grandiflora* and *Magnolia virginiana* flower essential oils. Natural Product Research. 2013;**27**:1091-1097. DOI: 10.1080/14 786419. 2012.696256

[60] Cianfaglione K, Blomme EE, Quassinti L, Bramucci M, Lupidi G, Dall'Acqua S, et al. Cytotoxic essential oils from Eryngium campestre and *Eryngium amethystinum* (Apiaceae) growing in central Italy. Chemistry & Biodiversity. 2017;**14**:e1700096. DOI: 10.1002/cbdv.201700096

[61] Martins ER, Díaz IE, Paciencia ML, Fana SA, Morais D, Eberlin MN, et al. Interference of seasonal variation on the antimicrobial and cytotoxic activities of the essential oils from the leaves of *Iryanthera polyneura* in the Amazon rain forest. Chemistry & Biodiversity. 2019;**16**:e19003. DOI: 10.1002/cbdv.20190037 4

[62] Hussain AI, Anwar F, Nigam PS, Ashraf M, Gilani AH. Seasonal variation in content, chemical composition and antimicrobial and cytotoxic activities of essential oils from four Mentha species. Journal of the Science of Food and

Agriculture. 2010;**90**:1827-1836. DOI: 10.1002/jsfa.4021

[63] Guerrini A, Sacchetti G, Grandini A, Spagnoletti A, Asanza M, Scalvenzi L. Cytotoxic effect and TLC bioautography-guided approach to detect health properties of amazonian *Hedyosmum sprucei* essential oil. Evidence-based Complementary and Alternative Medicine. 2016:1-8. DOI: 10.1155/2016/163834 2

[64] Rigano D, Marrelli M, Formisano C, Menichini F, Senatore F, Bruno M, et al. Phytochemical profile of three Ballota species essential oils and evaluation of the effects on human cancer cells. Natural Product Research. 2017;**31**:436-444. DOI: 10.1080/14786419.2016.1185722

[65] Hassine M, Zardi-Berguaoui A, Znati M, Flamini G, Ben Jannet H, Hamza MA. Chemical composition, antibacterial and cytotoxic activities of the essential oil from the flowers of tunisian *Convolvulus althaeoides* L. Natural Product Research. 2014;**28**:769-775. DOI: 10.1080/14786419.2013.87947

[66] Haber WA, Agius BR, Stokes SL, Setzer WN. Bioactivity and chemical composition of the leaf essential oil of *Talauma gloriensis* Pittier (Magnoliaceae) from Monteverde, Costa Rica. Records of Natural Products. 2008;**2**:1-5

[67] Afoulous S, Ferhout H, Raoelison EG, Valentin A, Moukarzel B, Couderc F, et al. Chemical composition and anticancer, antiinflammatory, antioxidant and antimalarial activities of leaves essential oil of *Cedrelopsis grevei*. Food and Chemical Toxicology. 2013;**56**:352-362. DOI: 10.1016/j.fct.2013.02.008

[68] Thirugnanasampandan R, David D. In vitro antioxidant and cytotoxic activities of essential oil of Feronia elephantum Correa. Asian Pacific Journal of Tropical Biomedicine. 2014;**4**:290-293. DOI: 10.12980/APJTB.4.2014B878

[69] Satyal P, Paudel P, Raut J, Deo A, Dosoky NS, Setzer WN. Volatile constituents of *Pinus roxburghii* from Nepal. Pharmacognosy Research. 2013;**5**:43-48. DOI: 10.4103/0974-8490.105650

[70] Hoai NT, Duc HV, Do Thi Thao AO, Raal A. Selectivity of *Pinus sylvestris* extract and essential oil to estrogen-insensitive breast cancer cells *Pinus sylvestris* against cancer cancer cells. Pharmacognosy Magazine. 2015;**11**:S290–S295. DOI: 10.4103/0973-1296.166052

[71] Peng Z, Wang Y, Fan J, Lin X, Liu C, Xu Y, et al. Costunolide and dehydrocostus lactone combination treatment inhibit breast cancer by inducing cell cycle arrest and apoptosis through c-Myc/p53 and AKT/14-3-3 pathway. Scientific Reports. 2017;**7**:41254. DOI: 10.1038/srep41254

[72] Ganea GM, Fakayode SO, Losso JN, Van Nostrum CF, Sabliov CM, Warner IM. Delivery of phytochemical thymoquinone using molecular micelle modified poly (D,L lactide-co-glycolide) (PLGA) nanoparticles. Nanotechnology. 2010;**21**:285104. DOI: 10.1088/0957-4484/21/28/285104

[73] Odeh F, Ismail SI, Abu-Dahab R, Mahmoud IS, Al BA. Thymoquinone in liposomes: A study of loading efficiency and biological activity towards breast cancer. Drug Delivery. 2012;**19**:371-377. DOI: 10.3109/10717544.2012.727500

[74] Effenberger K, Breyer S, Schobert R. Terpene conjugates of the N*igella sativa* seed-oil constituent thymoquinone with enhanced efficacy in cancer cells. Chemistry & Biodiversity. 2010;**7**:129-139. DOI: 10.1002/cbdv.200900328

[75] Effenberger-Neidnicht K, Schobert R. Combinatorial effects

of thymoquinone on the anti-cancer activity of doxorubicin. Cancer Chemotherapy and Pharmacology. 2011;**67**:867-874. DOI: 10.1007/s00280-010-1386-x

[76] Bashmail HA, Alamoudi AA, Noorwali A, Hegazy GA, AJabnoor G, Choudhry H, et al. Thymoquinone synergizes gemcitabine anti-breast cancer activity via modulating its apoptotic and autophagic activities. Scientific Reports. 2018;**8**:1-11. DOI: 10.1038/s41598-018-30046-z

[77] Woo CC, Loo SY, Gee V, Yap CW, Sethi G, Kumar AP, et al. Anticancer activity of thymoquinone in breast cancer cells: Possible involvement of PPAR-γ pathway. Biochemical Pharmacology. 2011;**82**:464-475. DOI: 10.1016/j.bcp.2011.05.030

[78] Woo CC, Hsu A, Kumar AP, Sethi G, Tan KH. Thymoquinone inhibits tumor growth and induces apoptosis in a breast cancer xenograft mouse model: The role of p38 MAPK and ROS. PloS One. 2013;**8**:e75356. DOI: 10.1371/journal.pone.0075356

[79] Kabil N, Bayraktar R, Kahraman N, Mokhlis HA, Calin GA, Lopez-Berestein G, et al. Thymoquinone inhibits cell proliferation, migration, and invasion by regulating the elongation factor 2 kinase (eEF-2K) signaling axis in triple-negative breast cancer. Breast Cancer Research and Treatment. 2018;**171**:593-605. DOI: 10.1007/s10549-018-4847-2

[80] Wang X, Xie J, Proud CG. Eukaryotic elongation factor 2 kinase (eEF2K) in cancer. Cancers (Basel). 2017;**9**:162. DOI: 10.3390/cancers9120162

[81] Alobaedi OH, Talib WH, Basheti IA. Antitumor effect of thymoquinone combined with resveratrol on mice transplanted with breast cancer. Asian Pacific Journal of Tropical Medicine. 2017;**10**:400-408. DOI: 10.1016/j.apjtm.2017.03.026

[82] Yan X, Zhang G, Bie F, Lv Y, Ma Y, Ma M, et al. Eugenol inhibits oxidative phosphorylation and fatty acid oxidation via downregulation of c-Myc/PGC-1β/ERRα signaling pathway in MCF10A-ras cells. Scientific Reports. 2017;**7**:12920. DOI: 10.1038/s41598-017-13505-x

[83] Zu Y, Yu H, Liang L, Fu Y, Efferth T, Liu X, et al. Activities of ten essential oils towards Propionibacterium acnes and PC-3, A-549 and MCF-7 cancer cells. Molecules. 2010;**15**:3200-3210. DOI: 10.3390/molecules15053200

[84] Taha AM, Eldahshan OA. Chemical characteristics, antimicrobial, and cytotoxic activities of the essential oil of egyptian *Cinnamomum glanduliferum* bark. Chemistry & Biodiversity. 2017;**14**:e1600443. DOI: 10.1002/cbdv.201600443

[85] Kahkeshani N, Hadjiakhoondi A, Navidpour L, Akbarzadeh T, Safavi M, Karimpour-Razkenari E, et al. Chemodiversity of Nepeta menthoides Boiss. & Bohse. essential oil from Iran and antimicrobial, acetylcholinesterase inhibitory and cytotoxic properties of 1,8-cineole chemotype. Natural Product Research. 2018;**32**:2745-2748. DOI: 10.1080/14786419.2017.1378202

[86] Mishra T, Pal M, Meena S, Datta D, Dixit P, Kumar A, et al. Composition and in vitro cytotoxic activities of essential oil of *Hedychium spicatum* from different geographical regions of western Himalaya by principal components analysis. Natural Product Research. 2015;**30**:1-4. DOI: 10.1080/14786419.2015.1049176

[87] De Lima EM, Cazelli DS, Pinto FE, Mazuco RA, Kalil IC, Lenz D, et al. Essential oil from the resin of *Protium heptaphyllum*: Chemical composition, cytotoxicity, antimicrobial activity,

and antimutagenicity. Pharmacognosy Magazine. 2016;**12**:S42-S46. DOI: 10.4103/0973-1296.176113

[88] Sigurdsson S, Ögmundsdóttir HM, Gudbjarnason S. The cytotoxic effect of two chemotypes of essential oils from the fruits of *Angelica archangelica* L. Anticancer Research. 2005;**25**:1877-1880

[89] Abdallah HM, Ezzat SM. Effect of the method of preparation on the composition and cytotoxic activity of the essential oil of Pituranthos tortuosus. Zeitschrift fur Naturforschung. C. Journal of Biosciences. 2011;**66**:143-148. DOI: 10.1515/znc-2011-3-408

[90] Essien EE, Ogunwande IA, Setzer WN, Ekundayo O. Chemical composition, antimicrobial, and cytotoxicity studies on *S. erianthum* and *S. macranthum* essential oils. Pharmaceutical Biology. 2012;**50**:474-480. DOI: 10.3109 /13880209.2011.614623

[91] Piaru SP, Mahmud R, Abdul Majid AM, Ismail S, Man CN. Chemical composition, antioxidant and cytotoxicity activities of the essential oils of *Myristica fragrans* and *Morinda citrifolia*. Journal of the Science of Food and Agriculture. 2012;**92**:593-597. DOI: 10.1002/jsfa.4613

[92] Keawsa-Ard S, Liawruangrath B, Liawruangrath S, Teerawutgulrag A, Pyne SG. Chemical constituents and antioxidant and biological activities of the essential oil from leaves of *Solanum spirale*. Natural Product Communications. 2012;**7**:955-958. DOI: 10.1177/1934578X1200700740

[93] Noudogbessi JP, Gary-Bobo M, Adomou A, Adjalian E, Alitonou GA, Avlessi F, et al. Comparative chemical study and cytotoxic activity of *Uvariodendron angustifolium* essential oils from Benin. Natural Product Communications. 2014;**9**:261-264. DOI: 10.1177/1934578X1400900232

[94] Najar B, Shortrede JE, Pistelli L, Buhagiar J. Chemical composition and in vitro cytotoxic screening of sixteen commercial essential oils on five cancer cell lines. Chemistry & Biodiversity. 2020;**17**:e1900478. DOI: 10.1002/ cbdv.201900478

[95] Nazaruk J, Karna E, Wieczorek P, Sacha P, Tryniszewska E. In vitro antiproliferative and antifungal activity of essential oils from *Erigeron acris* L. and *Erigeron annuus* (L.) Pers. Zeitschrift fur Naturforschung. C. Journal of Biosciences. 2010;**65**:642-646. DOI: 10.1515/znc-2010-11-1202

[96] Manzo A, Musso L, Panseri S, Iriti M, Dallavalle S, Catalano E, et al. Screening of the chemical composition and bioactivity of *Waldheimia glabra* (Decne.) Regel essential oil. Journal of the Science of Food and Agriculture. 2016;**96**:3195-3201. DOI: 10.1002/ jsfa.7499

[97] Fogang HP, Maggi F, Tapondjou LA, Womeni HM, Papa F, Quassinti L, et al. In vitro biological activities of seed essential oils from the camerooni an spices *Afrostyrax lepidophyllus* mild br. and *Scorodophloeus zenkeri* harms rich in sulfur-containing compounds. Chemistry & Biodiversity. 2014;**11**:161-169. DOI: 10.1002/cbdv.201300237

[98] Salehi F, Behboudi H, Kavoosi G, Ardestani SK. Incorporation of *Zataria multiflora* essential oil into chitosan biopolymer nanoparticles: A nanoemulsion based delivery system to improve the in-vitro efficacy, stability and anticancer activity of ZEO against breast cancer cells. International Journal of Biological Macromolecules. 2020;**143**:382-392. DOI: 10.1016/j. ijbiomac.2019.12.058

[99] Onyebuchi C, Kavaz D. Chitosan and n,n,n-trimethyl chitosan

nanoparticle encapsulation of *Ocimum gratissimum* essential oil: Optimised synthesis,in vitro release and bioactivity. International Journal of Nanomedicine. 2019;**14**:7707-7727. DOI: 10.2147/IJN.S220202

[100] Kavaz D, Idris M, Onyebuchi C. Physiochemical characterization, antioxidative, anticancer cells proliferation and food pathogens antibacterial activity of chitosan nanoparticles loaded with *Cyperus articulatus* rhizome essential oils. International Journal of Biological Macromolecules. 2019;**123**:837-845. DOI: 10.1016/j.ijbiomac.2018.11.177

[101] Gali-Muhtasib H, Roessner A, Schneider-Stock R. Thymoquinone: A promising anti-cancer drug from natural sources. The International Journal of Biochemistry & Cell Biology. 2006;**38**:1249-1253. DOI: 10.1016/j.biocel.2005.10.009

[102] Periasamy VS, Athinarayanan J, Alshatwi AA. Anticancer activity of an ultrasonic nanoemulsion formulation of *Nigella sativa* L. essential oil on human breast cancer cells. Ultrasonics Sonochemistry. 2016;**31**:449-455. DOI: 10.1016/j.ultsonch.2016.01.035

[103] Al-Otaibi WA, Alkhatib MH, Wali AN. Cytotoxicity and apoptosis enhancement in breast and cervical cancer cells upon coadministration of mitomycin C and essential oils in nanoemulsion formulations. Biomedicine & Pharmacotherapy. 2018;**106**:946-955. DOI: 10.1016/j.biopha.2018.07.041

[104] Al-Otaibi WA, Alkhatib MH, Wali AN. Evaluation of antitumor activity and hepatoprotective effect of mitomycin c solubilized in chamomile oil nanoemulsion. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents). 2019;**19**:1232-1242. DOI: 10.2174/1871520619666190408114732

[105] Ortiz C, Morales L, Sastre M, Haskins WE, Matta J. Cytotoxicity and genotoxicity assessment of sandalwood essential oil in human breast cell lines MCF-7 and MCF-10A. Evidence-based Complementary and Alternative Medicine. 2016;**2016**:3696232. DOI: 10.1155/2016/3696232

[106] Raghavendra RM, Nagarathna R, NagendrA HR, Gopinath KS, Srinath BS, Ravi BD, et al. Effects of an integrated yoga programme on chemotherapy-induced nausea and emesis in breast cancer patients. European Journal of Cancer Care. 2007;**16**:462-474. DOI: 10.1111/j.136 5-2354.2006.00739.x

[107] Lua PL, Salihah N, Mazlan N. Effects of inhaled ginger aromatherapy on chemotherapy-induced nausea and vomiting and health-related quality of life in women with breast cancer. Complementary Therapies in Medicine. 2015;**23**:396-404. DOI: 10.1016/j.ctim.2015.03.009

[108] Pruthi S, Simon JA, Early AP. Current overview of the management of urogenital atrophy in women with breast cancer. The Breast Journal. 2011;**17**:403-408. DOI: 10.1111/j.1524-4741.2011.01089.x

[109] Simões BM, Kohler B, Clarke RB, Stringer J, Novak-Frazer L, Young K, et al. Estrogenicity of essential oils is not required to relieve symptoms of urogenital atrophy in breast cancer survivors. Therapeutic Advances in Medical Oncology. 2018;**10**:1-11. DOI: 10.1177/1758835918766189

[110] Halm MA, Baker C, Harshe V. Effect of an essential oil mixture on skin reactions in women undergoing radiotherapy for breast cancer: A pilot study. Journal of Holistic Nursing. 2014;**32**:290-303. DOI: 10.1177/0898010114527184

[111] Loizzo MR, Tundis R, Menichini F, Saab AM, Statti GA, Menichini F.

Cytotoxic activity of essential oils from Labiatae and Lauraceae families against in vitro human tumor models. Anticancer Research. 2007;**27**:3293-3300

[112] Emami SA, Asili J, Hossein Nia S, Yazdian-Robati R, Sahranavard M, Tayarani-Najaran Z. Growth inhibition and apoptosis induction of essential oils and extracts of *Nepeta cataria* L. on human prostatic and breast cancer cell lines. Asian Pacific Journal of Cancer Prevention. 2016;**17**:125-130. DOI: 10.7314/APJCP.2016.17.S3.125

[113] Grecco SD, Martins EG, Girola N, de Figueiredo CR, Matsuo AL, Soares MG, et al. Chemical composition and in vitro cytotoxic effects of the essential oil from *Nectandra leucantha* leaves. Pharmaceutical Biology. 2015;**53**:133-137. DOI: 10.3109/13880209.2014.912238

[114] El Hadri A, del Rio MG, Sanz J, Coloma AG, Idaomar M, Ozonas BR, et al. Cytotoxic activity of α-humulene and transcaryophyllene from *Salvia officinalis* in animal and human tumor cells. Anales de la Real Academia Nacional de Farmacia. 2010;**76**:343-356

[115] Medina-Holguín AL, Holguín FO, Micheletto S, Goehle S, Simon JA, O'Connell MA. Chemotypic variation of essential oils in the medicinal plant, *Anemopsis californica*. Phytochemistry. 2008;**69**:919-927. DOI: 10.1016/j.phytochem.2007.11.006

[116] Begnini KR, Nedel F, Lund RG, Carvalho PH, Rodrigues MR, Beira FT, et al. Composition and antiproliferative effect of essential oil of *Origanum vulgare* against tumor cell lines. Journal of Medicinal Food. 2014;**17**(11):29-33. DOI: 10.1089/jmf.2013.0063

[117] Soltanian S, Mohamadi N, Rajaei P, Khodami M, Mohammadi M. Phytochemical composition, and cytotoxic, antioxidant, and antibacterial activity of the essential oil and methanol extract of Semenovia suffruticosa. Avicenna Journal of Phytomedicine. 2019;**9**:143-152

Chapter 5

Terpenoids as Important Bioactive Constituents of Essential Oils

Fongang Fotsing Yannick Stephane
and Bankeu Kezetas Jean Jules

Abstract

Plant and plant-derived natural products have a long and significant history in traditional medicine all over the world. Many studies in the recent past years focused on the benefic properties of essential oils (EOs) and their major components, terpenes and terpenoids (that are mostly monoterpenes and sesquiterpenes), and their biological properties. This chapter focuses on terpenoids as important bioactive constituents of EOs. It describes their uses, importance, extraction processes, and classification. The chapter provides an in-depth overview of the latest findings/research about terpenoids in EOs. It contains a well-prepared background, introduction, classification, chemical tests, bioactivities, as well as the characterization of terpenoids. It also discusses the bioactivities of EOs and that of terpenoids, with regard to their synergetic and/or their antagonistic effects.

Keywords: monoterpenoids, sesquiterpenoids, diterpenoids, terpenes, terpenoids, essential oils, bioactivities

1. Introduction

The use of plant and plant-derived natural products for medicinal, religious, and cosmetic purposes has a history dating back to the emergence of humanity. Exploring natural plant products as an option to find new chemical entities as leads is one of the fastest growing areas of research. Medicinal plants are rich sources of bioactive phytochemicals and/or bionutrients, which have shown important role in preventing chronic diseases like cancers, diabetes, and coronary heart diseases [1]. It is well documented that plants produce these chemicals to protect themselves, but they also protect plants from diseases and damages and contribute to the plant's color, aroma, and flavor [2]. The pharmaceutical properties of aromatic plants are partially attributed to essential oils (EOs), which can also be seen as an important group of plant secondary metabolites. Although the use of EOs has been primarily related to food flavorings, cosmetics, and perfumes due to their aroma, research demonstrates the high potential of the use of volatile monoterpene constituents to cure and prevent human diseases [3, 4]. During the recent years, plant EOs have come more into the focus of phytomedicine and aromatherapy; hence their widespread use has raised more interest to scientists in basic research, especially their antimicrobial, antioxidant, and anticancer activities. In general, EOs consist of chemical mixtures involving from several tens to hundreds of different types of molecules, most of them being complex natural mixture of terpene and

phenylpropanoids (benzene derivatives) which are responsible for their biological activities [5, 6]. At the first glance, terpenes and EOs can seem alike; both can come from plants and are aromatic; for many they are used for the same purpose. These similarities have led to a wide misconception that they are same, but this is not necessary the case [7].

2. Essential oils

2.1 Definition

A plethora of practical definitions of the term essential or volatile oils exist. Essential oils are concentrated aromatic hydrophobic oily volatile liquids characterized by a strong odor and produced by different plant materials such as flowers, peels, rhizomes, buds, seeds, leaves, twigs, bark, herbs or grass, wood, fruits, roots, and whole plant from one single botanic species [7–9]. However, EOs with a specific characteristic (including chemical properties and biological activities) are generally obtained from a single botanical source when the age of the plant, the climate, and the edaphic and harvest period are relatively identical [10]. They are called "essential oils" because they contain the "essence" of the plant material. A few are produced by animals and microorganisms [11]. Mosses, liverworts, seaweeds, and fungi have also been shown to contain EOs. EOs are limpid, rarely colored, and soluble in nonpolar or weakly polar organic solvents and of lower density (lighter) than water, with very few exceptions [12]. They are usually colorless particularly when fresh, but few may also be pale yellow (yellow mandarin), blue (*Matricaria chamomilla* well known as chamomile), orange (sweet orange, *Citrus sinensis*), and green (bergamot, *Citrus bergamia*) [13]. Nevertheless, they may be readily oxidizable with age by light, heat, or air, which resulting to the dark color [14]. Therefore, they need to be stored in a cool and dry place, preferably in amber glass containers. The primary difference between terpenes and EOs is that they contain terpenes and a variety of other compounds as well.

2.2 Distribution, uses, and importance

The quality and the quantity of EOs in plant material depends on the climate, the soil type, the age and vegetable cycle stage, the preparation method, chemotypes, as well as the plant organ [8]. An estimated 3000 EOs, from about 2000 plants, are of great value and are used in a very large variety of fields [15, 16]. All plants possess principally the ability to produce volatile compounds, quite often, however, only in traces. Those plants that can produce an EO of commercial interest are called essential oils plants [17]. EOs occur specially in higher plants (with about 17,500 known species) but are distributed in good amount in a limited number of families including Myrtaceae, Myristicaceae, Oleaceae, Rosaceae, Acoraceae, Cupressaceae, Lauraceae, Compositae, Rutaceae, Lamiaceae, Asteraceae, Umbelliferae, Apiaceae, Poaceae, Zingiberaceae, etc. [18–21].

In most cases, the biological function of EOs remains obscure. They are nowadays subject of intensive scientific research and also attract attention of diverse industries due to their potentials as active pharmacological compounds or natural preservatives [22]. Their ecological role is however well studied and described. The most known are plant interactions (allelopathic agents, germination inhibitors) and plant–animal interactions for protection against predators (insects, fungi, herbivores) and attraction of pollinating insect to their host [23]. Industries have always

had special interest on the microbial safety of cosmetics, as microbial spoilage can lead to product degradation and cause a risk for customers' health. EOs and drugs containing them are of great importance in pharmacy, perfumery (heal, perfume, incense, household cleaning products), food technology (favor for food, drinks, spices, preservative), agriculture (insecticide), and aromatherapy. Their importance is nowadays known and appreciated in plant chemotaxonomy [24, 25].

2.3 Extraction and analysis

2.3.1 Extraction of essential oils

The world production and consumption of EOs and perfumes are increasing very fast. Production technology of EOs is an essential element to improve their overall yield. They are obtained from raw material by several extraction techniques such as water or steam distillation, solvent extraction, expression under pressure, microwave-assisted extraction, supercritical fluid, or subcritical water extractions [22, 26–28]. The best extraction method to use depends on the ease of evaporating (volatility) and the hydrophilicity or hydrophobicity (polarity) of the desired components. The extraction method chosen greatly affects the chemical composition of EOs.

2.3.1.1 Classical and conventional methods

They are the most frequently used method for the extraction of EOs from plants.

2.3.1.1.1 Hydrodistillation

It is the oldest and easiest conventional method of extraction of EOs [11, 29–31]. The principle is based on the isotropic distillation. The plant material soaks up water during the boiling process, and the oil contained in the oil cells diffuses through the cell walls by means of osmosis. The distillation time depends on the plants material being processed (**Figure 1**).

2.3.1.1.2 Steam distillation

The principle of this technique is that the combined vapor pressure equals the ambient pressure at about 100°C so that the volatile components with the

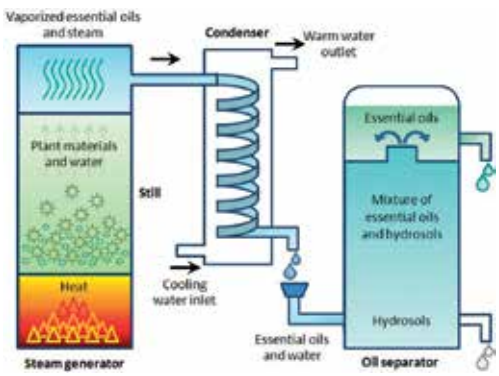

Figure 1.
Diagrammatic illustration of hydrodistillation (HD) method [32].

boiling points ranging from 150 to 300°C can be evaporated at a temperature close to that of water. The steam distillation takes advantage of the volatility of a compound to evaporate when heated with steam and the hydrophobicity of the compound to separate into an oil phase during the condensation process (**Figure 2**) [33].

2.3.1.1.3 Solvent extraction

Also known as liquid–liquid partitioning, its principle is based on the solubility in an organic solvent non-mixable to water. This technique is used on delicate plants to produce higher amounts of EOs at a lower cost. The method is limited by the compound solubility in the specific solvent used, long extraction time, relatively high solvent consumption and often unsatisfactory reproducibility and purity (**Figure 3**) [33].

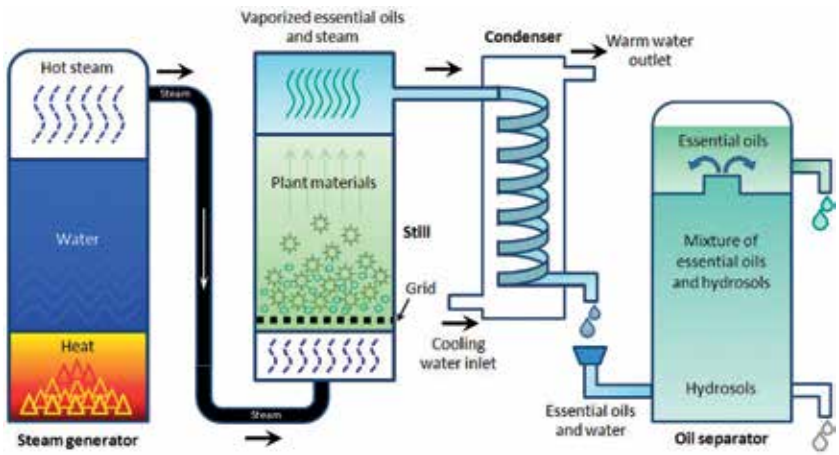

Figure 2.
Diagrammatic illustration of steam distillation method [32].

Figure 3.
Illustration of liquid–liquid extraction method.

2.3.1.1.4 Soxhlet extraction

Typically, it is a solid–liquid extraction used when the desired compound has a limited solubility in a solvent and the impurity is insoluble in that solvent. There are several advantages of using this technique. These advantages include:

- Low solvent consumption for a larger amount of raw material,
- Repeatedly brought into contact with fresh portions of the solvent, this prevents the possibility of the solvent to become saturated with extractable material and enhances the removal of analyte from the matrix. Moreover, the temperature of the system is close to the boiling point of the solvent. This helps to increase the extraction kinetic of the system.

As disadvantages, it requires several hours or days to be performed; moreover, the sample is diluted in a large volume of solvent.

Due to heating, the thermal degradation and volatilization of components have been observed, and hydrolysis of esters to yield alcohols and carboxylic acids can occur (**Figure 4**) [34].

2.3.1.1.5 Cool pressing method

Also kwon as scarification method, this is one of the best methods to extract EOs. The term cool pressed theoretically means that the oil is expeller-pressed at low temperature and pressure. This process insures that the resulting oil is 100% pure and retains all the properties of the plant. Here the heat is reduced and minimized throughout the batching of the raw material. EOs are then separated from the material by centrifugation [36].

Since economy, competitiveness, eco-friendly, sustainability, operation costs, high efficiency, and good quality become keywords of the modern industrial production, the development of EO extraction techniques has never been interrupted. The most relevant disadvantage of conventional techniques are time and solvent consumption and also related to the thermolability of EOs components which undergo chemical alteration (hydrolyze, isomerization, oxidation) due to the high

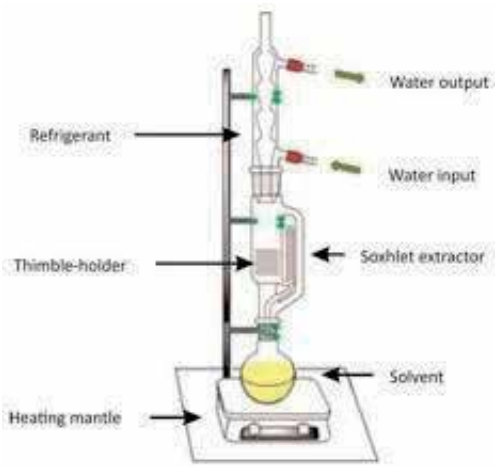

Figure 4.
Soxhlet equipment [35].

applied temperatures [37]. The quality of the obtained oil is damaged, particularly if the extraction time is long. It is important that the extraction method maintain the chemical composition and the natural proportion at its original state. Strictly speaking, conventional methods are not the only way for the removal of EOs. Novel techniques known as innovative have been developed for this purpose but may not necessarily be widely used for commercial production due to the high cost of production of oils without any alteration of their thermosensitive components (**Figure 5**).

2.3.1.2 Innovative techniques of extraction (nonconventional)

2.3.1.2.1 Supercritical fluid extraction

It is a process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. In practice, more than 90% of all analytical supercritical fluid extraction (SFE) is performed with carbon dioxide (CO_2) as the most used fluid. The CO_2 is chosen for several reasons including the following: relatively low critical pressure (74 bars) and temperature (32°C), inertness, non-toxic, nonflammable, high soluble, non-corrosive, safe, available in high purity at relatively low cost, perfect conditions for thermosensitive compounds extraction, selectivity for desired compounds, and easy removal from the extract. At lower temperatures, to avoid potential damage of desired components of EOs, supercritical CO_2 extraction technique is highly recommended [39, 40]. Extraction of EOs by SFs, particularly with CO_2, provides products free of toxic waste, having a higher quality (especially it reserves the thermal instability of compounds) than EOs obtained by conventional methods (**Figure 6**) [40–42].

2.3.1.2.2 SFE assisted by cold pressing (SFEAP)

SFEAP is a novel technique of extraction recently developed by Johner and collaborators [43]. It integrated both the cold-pressed extraction method and the SFE technique. Here, the solid raw material is loaded inside the extraction vessel, and

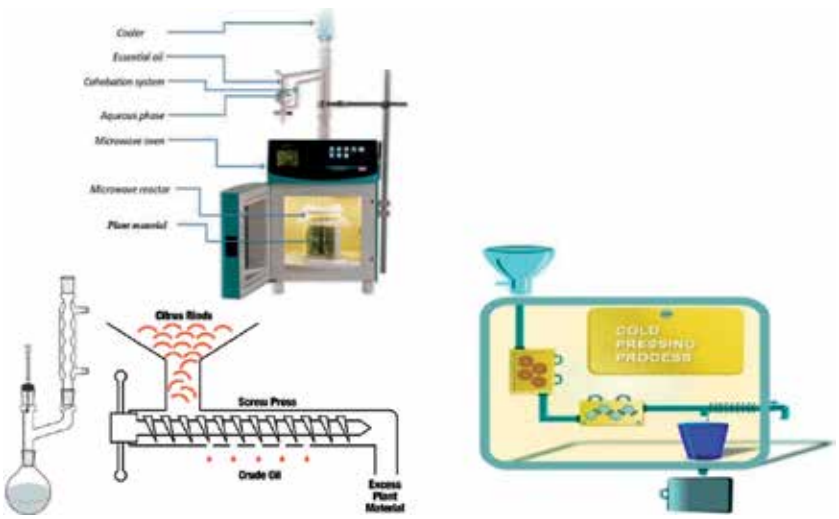

Figure 5.
Cold pressing apparatus and procedure distillation method [34, 38].

a cold pressing is provided by contracting an under pressure piston with the raw material. SFEAP has been shown to offer faster extraction rate at 333 K and 40 MPa with the best yield [44]. Its advantages include gain of extraction time and solvent consumption. This technique has been used to extract EOs from *Foeniculum vulgare*, *Caryocar brasiliense*, and clove (**Figure 7**) [43–45].

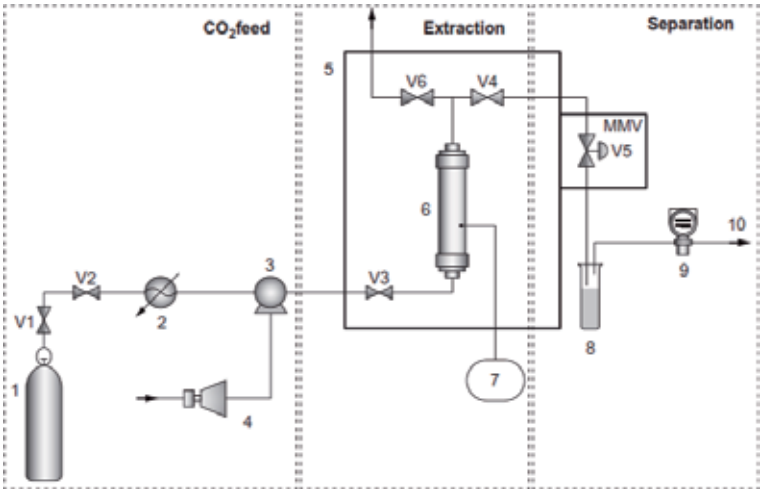

Figure 6.
Flow diagram of SC-CO_2 extraction [40]. (1) CO_2 cylinder, (2) cooling bath, (3) pump, (4) compressor, (5) oven, (6) extractor vessel, (7) monitor, (8) collecting bottle, (9) flowmeter, and (10) CO_2 outlet. V1–V_6 flow control valves.

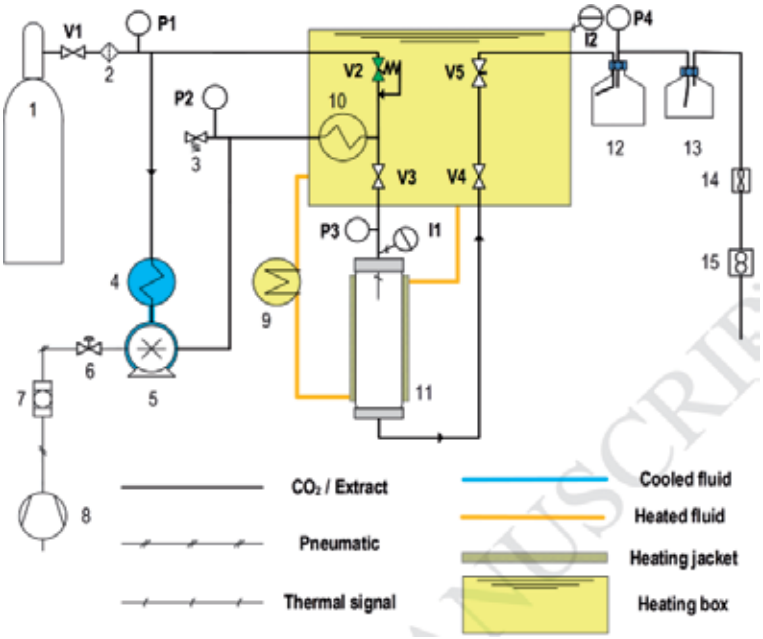

Figure 7.
Schematic diagram of SFEAP apparatus [43]. (1) CO_2 reservoir; (2) CO_2 filter; (3) safety valve; (4) cooling bath; (5) air-driven CO_2 pump; (6) control (air flow); (7) air filter; (8) air compressor; (9) heating bath; (10) serpentine tube; (11) extraction cell; (12) 1° extract collecting vessel; (13) 2° extract collecting vessel; (14) flowmeter (15) flow totalizer, V2 back pressure; V5 micrometering valve; V(1,3,4) blocking valve; P(1,2,3,4) pressure gauge; I1 temperature indicator; I2 temperature indicator.

2.3.1.2.3 Microwave-assisted hydrodistillation

The principle of the microwave-assisted hydrodistillation (MAHD) is based upon its direct impact with polar materials/solvents and is governed by two phenomena: ionic conduction and dipole rotation, which in most cases occurs simultaneously [46]. MAHD has been shown to reduce both extraction time and volume of solvent required, minimizing environmental impact by emitting less CO_2 in atmosphere [47–49]. Some recently reported studies have successfully utilized a microwave oven for the extraction of volatile active components from plants [50]. It has been regarded as an important alternative in conventional extraction techniques because of its advantages which mainly are a reduction of extraction time, solvents, selectivity, volumetric heating, and controllable heating process (**Figure 8**) [51].

2.3.1.2.4 Ultrasound-assisted extraction

The basic principle of ultrasound-assisted extraction (UAE) to extract EOs from plant raw material consist of generating sound waves (ultrasound frequency about 20 KHz), which create cavitation bubbles in the solution and produce enough energy to break the structure containing the oil in order to release it. Moreover, UAE can act as an emulsifier dispersing lipophilic molecules in water, this facilitating the subsequent separation and purification of EOs [54, 55]. This technique was developed in 1950 [56]. It has been used to extract many EOs especially from flowers, leaves, or seeds [32, 55]. As known disadvantages, it requires filtration steps, and possible degradation of compounds at high frequencies occurs (**Figure 9**) [57].

2.3.1.2.5 The microwave-assisted extraction

Microwave-assisted extraction (MAE) is a process of using microwave energy to heat the solvent in contact with a sample in order to partition analytes from the sample into the solvent. The ability to rapidly heat the sample solvent mixture is inherent to MAE and is the main advantage of this technique [59]. It is a recent green technology broadly used to extract various EOs from plant. It has been established as an alternative method to conventional heating because it allows gain of time, volume of solvent used, and amount of biomass needed while increasing

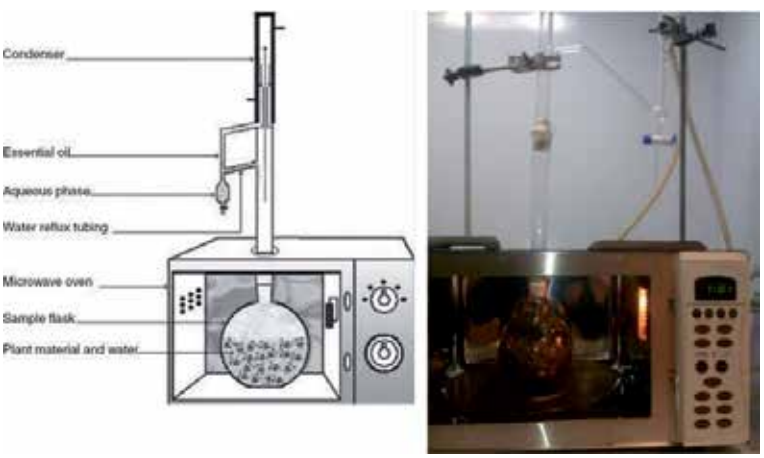

Figure 8.
Schematic and picture of MAHD apparatus [52, 53].

the extraction yield [28]. In most cases, recoveries of analytes and reproducibility are improved compared to conventional techniques (**Figure 10**) [59].

2.3.1.2.6 Solvent-free microwave extraction

Solvent-free microwave extraction (SFME) is proposed as a method for "green" extraction of edible EOs from fresh plant material, at atmospheric pressure without addition of water or organic solvent [61]. The SFME apparatus (**Figure 3**) is an original combination of microwave heating and dry distillation at atmospheric pressure. Based on a relatively simple principle, this method involves placing the plant material in a microwave reactor, without adding any solvent or water. The internal heating of the in situ water within the fresh plant material distends the plant cells and leads to the rupture of the glands and oleiferous receptacles. This process thus free EO which is evaporated by in situ water of the plant material. A cooling system outside the microwave oven condensed the distillate continuously. The excess of water is refluxed to the extraction vessel in order to restore in situ water to the plant material. At the end, EO is removed from the aqueous extract by simple decantation. SFME is neither a modified microwave-assisted extraction (MAE) which uses organic solvents nor a modified hydrodistillation process which uses a large amount of water; it can be consider as a dry distillation process, with water coming from the fresh plant material [62–64]. As advantages, the SFME

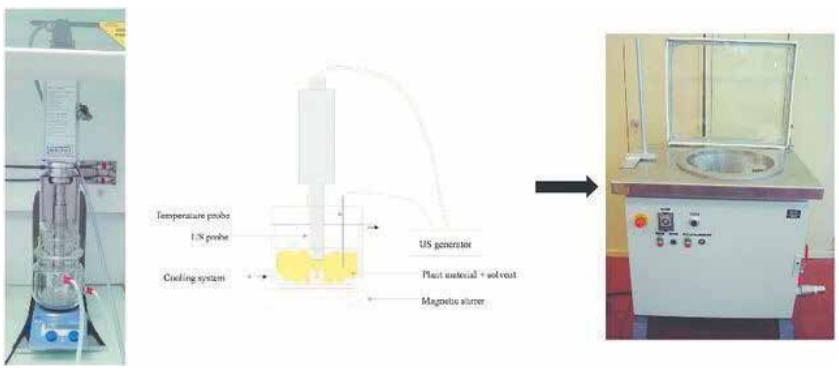

Figure 9.
Ultrasound-assisted extraction (UAE): from laboratory (a) to pilot scale (b) [58].

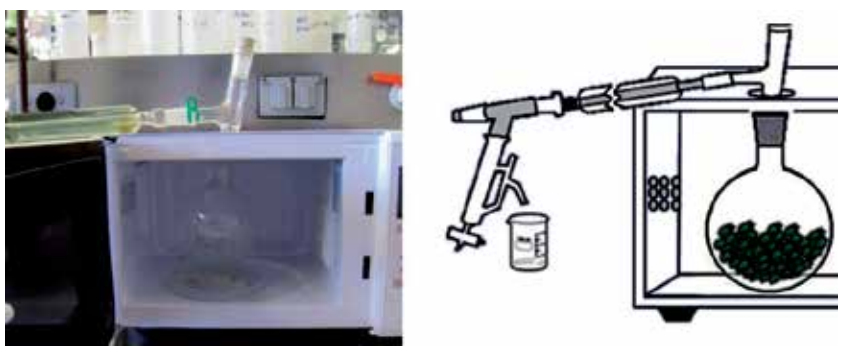

Figure 10.
Picture and schematic diagram of the microwave oven adaptation to perform MAE [60].

method increases the EO yield, ameliorate the EO composition, eliminate the waste of water treatment, and also contributes to limited time, and lower an energy consumption (**Figure 11**) [62].

2.3.1.2.7 Microwave hydrodiffusion and gravity

Microwave hydrodiffusion and gravity (MHG) is a new green extraction technique of EOs developed by Vian and collaborators in 2008. This green extraction technique is an original "upside down" microwave alembic combining microwave heating and earth gravity at atmospheric pressure [65]. MHG has become not only an economic and efficient but also an environmental- and eco-friendly, not require water or solvent and as it does require less energy (**Figure 12**) [65, 66].

2.3.2 Analysis of essential oils

As the consumption of EOs is growing up annually, their world production by different companies to satisfy the market demand has been increasing every year. The quality control of produced EOs has become then necessary to ensure the

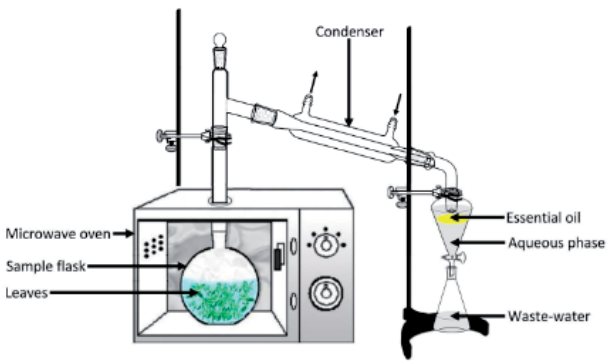

Figure 11.
Schematic representation of the solvent-free microwave extraction apparatus [63].

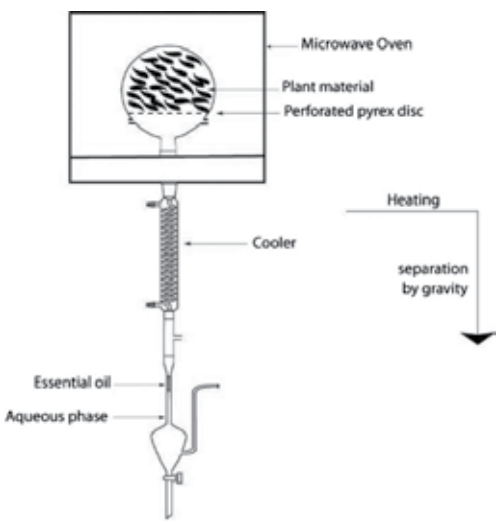

Figure 12.
Schematic representation of the microwave hydrodiffusion and gravity [65].

genuineness of the product, the shelf life, and the storage conditions [67]. The EO composition can sometimes be falsified by adding cheaper oils; it is often necessary to characterize small differences between oils that correspond to variation in geographic or genetic origin of the plant material. EOs analysis can be summarized in few points: the qualitative composition, the quantitative determination (major and/or minor constituents), and the detection of alteration of true EOs. With regard to the quality aspect of the EO, the identity and the purity are always investigated. Their physical properties are commonly assessed by specific gravity, the relative density, the optical rotation, the refractive index, etc.

Most of the methods applied in the analysis of EOs rely on chromatographic procedures, which enable component separation and identification. These include gas chromatography–mass spectrometry (GC–MS), liquid chromatography-mass spectrometry (LC–MS), gas chromatography-Fourier transform infrared spectrometry (GC-FT-IR), gas chromatography-Fourier transform infrared spectrometry-mass spectrometry (GC-FT-IR-MS), gas chromatography-atomic emission detector (GC-AED), gas chromatography-isotope ratio mass spectrometry (GC-IR-MS), on-line coupled liquid chromatography-gas chromatography (LC-GC), and multidimensional gas chromatography (MDGC) [68–78].

2.4 Bioactivities and toxicity of essential oils

A considerable large number of studies on EOs to evaluate their pharmacological properties and toxicity in order to find possible alternative medicine have become active in recent years [79]. EOs are known to exhibit a large range of biological activities.

2.4.1 Antioxidant activity

It is one of the most intensively studied properties of EOs. This could be explained by the damages of various biological substances by oxidation which subsequently causes many degenerative and/or metabolic diseases such as cancer, diabetes, arthritis, inflammation, and Parkinson's and Alzheimer's disease just to name a few [80–84]. EOs are known as rich sources of potential antioxidants that can be investigated to prevent oxidative damage [85]. Antioxidants comprise substances that, in low concentrations, significantly delay or inhibit the oxidation of the substrate [86]. Volatile compounds in EO, beside their protective antioxidant activity, can also act as prooxidant, by affecting the cellular redox status and damage cellular biomolecules, in the first instance proteins and DNA [15]. All these must be taken into account when antioxidant properties of EOs are considered.

Although phenolic compounds are recognized as being responsible for the antioxidant ability, recent studies showed that volatile components could also individually and/or in mixture (essential oil) contribute to the whole antioxidant ability. EO of lemon balm (*Melissa officinalis* L.) was reported to exhibit the highest antioxidant activity than BHT. Its GC–MS analysis showed that the main compounds were citronellal, neral, and geranial with a percentage yield of 13.7, 16.5, and 23.4%, respectively [87].

2.4.2 Anticancer activity

Cancer is a worldwide public health concern with 18.1 million people been diagnosed with the disease annually. It is the second largest single leading cause of death claiming in excess of 9.6 million lives in the world in 2018, with approximately 70% of deaths occurring in low- and middle-income countries [88]. Current

valuable drugs used in the treatment include vinblastine, vincristine, camptothecin, and Taxol [89]. Many studies pointed out the anticancer properties of plants. Over 500 research papers are published on the anticancer activity of EOs [90–93], even though, till date, there are no scientific studies showing that aromatherapy can cure or prevent cancer. Most promising research results obtained from in vitro studies revealed that EOs were found to affect cancer cell lines in petri dishes. EOs are well known for their anti-inflammatory activity; hence it appeared that EOs could also have anticancer effects as there is a relationship between the production of reactive oxygen species to the origin of oxidation and inflammation that can lead to cancer. More than 100 EOs from more than 20 families of plants have been tested on more than 20 different types of cancers in the past 10 years [94]. Bourgou and collaborators showed that the EO from seeds of black cumin (*Nigella sativa* L.) significantly inhibits the growth of A-549 and DLD-1 cancer cell lines with IC_{50} values of 43.0 and 46.0 μg/mL, respectively [95]. In 2012, Wang and collaborators reported the toxicology potential of EO of *Rosmarinus officinalis* L. and its three main components (including α-pinene, β-pinene, and 1,8-ceneole) toward three human cancer cell lines: the EO showed a strong cytotoxicity toward the three cancer cells with IC_{50} values of 0.025, 0.076, and 0.13‰ (v/v) on SK-OV-3, HO-8910, and Bel-7402, respectively [96].

2.4.3 Antimicrobial activity

EOs are well-kwon as antimicrobial agents and are well documented in numerous research works. Their antimicrobial activity depends not only on the presence of the main active compounds but also on the interaction between different components which can have synergistic or antagonistic actions. It also depends on the content, concentration, interaction between main active components, and susceptibility of microorganisms [97, 98]. The inactive compounds might influence resorption, the rate of the reactions, as well as biological activities of active compounds. The combination of both major and minor components can thus modify the activity to exert significant synergistic or antagonistic effect [99, 100]. EOs extracted from cinnamon, oregano, and thyme showed significant antibacterial activities against *Escherichia coli*, *Bacillus thermosphacta*, *Listeria monocytogenes*, and *Pseudomonas fluorescens* [101].

In general, EOs in decreasing order of antimicrobial activities are reportedly as follows: oregano (*Origanum vulgare*) > clove (*Syzygium aromaticum*) > coriander (*Coriandrum sativum*) > cinnamon (*Cinnamomum cassia*) > thyme (*Thymus vulgaris*) > mint (*Mentha*) > rosemary (*Salvia rosmarinus*) > mustard (*Sinapis alba*) > sage (*Salvia officinalis*) [102].

2.4.3.1 Antibacterial and antifungal activities

Antibiotic resistance is one of the most serious health burdens worldwide due to the continuous appearance of antibiotic-resistant bacterial strains. The bacteria that cause the most major clinical problems are *Klebsiella* and *Enterobacter* species, *Staphylococcus aureus*, *Enterococcus faecium*, *Clostridium difficile*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Escherichia coli* [103]. Generally, EOs are more active on gram-positive bacteria due to the presence of peptidoglycan layer, which lies outside the outer membrane. In gram-negative bacteria, the outer membrane is composed of a double layer of phospholipids, which is linked to the inner membrane by lipopolysaccharide [104]. Several studies on the bioactivity of EOs have revealed their antibacterial and antifungal potential on different pathogen microorganisms [105–108]. Previous studies revealed that the EOs from *Piper guineense*

fruit and *P. caldense* roots were active against the gram-negative bacteria *E. coli* and *P. aeruginosa* [109, 110]. EOs have been reported to possess potent antimicrobial activity, exhibiting bacteriostatic and bactericidal effects against tested pathogens.

2.4.3.2 Antiviral activity

New agents that are effective against common pathogens are needed particularly for those resistant to conventional antiviral agents. The ability of viruses to persist in fresh products, as well as their low infectious dose, could lead to serious food-borne problems [111]. Plants and plant-derived natural products provide unlimited opportunities for new antiviral drugs. Many EOs have been investigated in recent years toward their antiviral activity. As conclusion of their work, Reichling and collaborators reported that particular free viruses are very sensitive to EOs [112].

2.4.4 Anti-inflammatory activity

Most of EOs have been firstly identified and used for the treatment of inflammatory and oxidative diseases. *Cymbopogon citratus* (Lemongrass) is a popular herb used as analgesic and anti-inflammatory agent. It has been reported that its EO suppresses COX-2 expression promoter activity; citral was identified as the major component responsible for suppressing COX-2 expression and for activating PPARα and γ [113].

2.4.5 Miscellaneous activities

The insect repellent activity of EOs is well studied and many research papers have been published. The EOs of *Hyptis spicigera* Lamarck and *Hyptis suaveolens* (L) Poitier and *Lavandula angustifolia* (Miller) showed repellent activity on *Sitophilus zeamais* adults [114].

EOs of the leaves of *Endlicheria bracteolate* was tested against *Leishmania amazonensis* by Rottini and collaborators. The antileishmanial activity was evaluated against promastigotes and intracellular amastigotes, and cytotoxicity was performed with J774.G8, which were incubated with different concentrations of *E. bracteolate*. Promastigote forms showed *E. bracteolate* EO IC_{50} value of 7.945 μg/mL (24 h). The IC_{50} value was 15.14 μg/mL showing that *E. bracteolate* EO is less toxic to macrophages than to parasites [115].

2.5 Composition of essentials oils

EOs are generally very complex mixture (60–300) of nonpolar and semipolar lipophilic constituents of low molecular weight, at different concentrations with two or three appearing to be major ones [116, 117]:

- Terpenoids
- Straight-chain compounds not containing any side chain
- Aromatic and phenolic components
- Sulfured derivatives

The variation in odor and taste of EO depends on the plants variety, the harvesting seasons, the geographical location, the drying methods, and the extraction

techniques [102, 118–120]. The major volatile constituents may be classified into two main categories: terpenoids and polypropanoids [121–123]. We will focus our investigation on terpenoids.

3. Terpenes and terpenoids

Terpenes are defined as secondary metabolites with molecular structures containing carbon backbones of isoprene (2-methylbuta-1,3-diene) units [124]. Terpenes are synthetized in the cytoplasm of plant cells through the mevalonic acid pathway. Biochemical modification such as oxidation or rearrangement of terpenes produces the related terpenoids. Terpenoids are then oxygenated derivatives of hydrocarbon terpenes such as aldehydes, ketones, alcohols, acids, ethers, and esters [34]. Terpenoids are the largest classes of plants' natural products accounting for more than 40,000 individual compounds of both primary and secondary metabolisms been identified; to date, new terpenoids are being discovered every year [12, 124].

In general, terpenoids can be divided into at least four groups of compounds that include true terpenes, steroids, saponins, and cardiac glycosides.

These types of natural lipids can be found in every class of living things, mainly in plants as constituents of EOs, and are therefore considered as the largest and structurally diverse group of natural products [125]. In general, only the hemiterpenoids, the monoterpenoids, and sesquiterpenoids are sufficiently volatile to be components of EOs. As widely acknowledged, the composition of EOs is mainly represented by mono-, sesqui-, and even diterpene hydrocarbons and their respective oxygenated derivatives [30, 126–128].

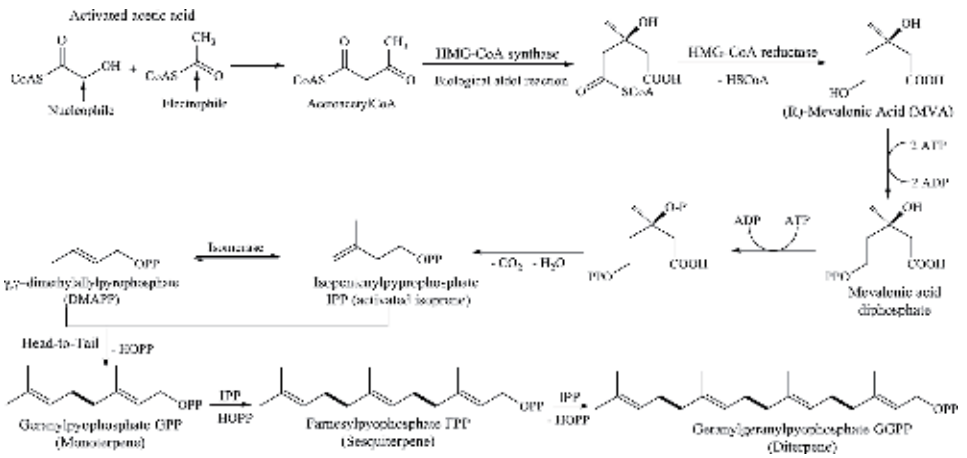

Figure 13.
Biosynthesis pathways of monoterpenes, sesquiterpenes, and diterpenes.

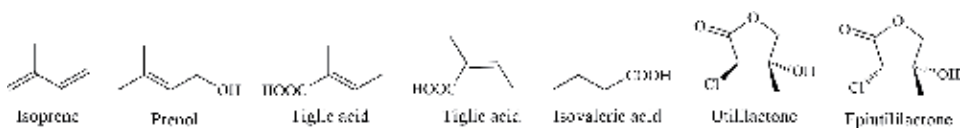

Figure 14.
Structure of few isolated hemiterpenes and hemiterpenoids.

3.1 Classification

Structurally, EO constituents typically have low molecular weights, which contribute to their high volatility. Terpenes are the most common constituents found in EOs [128]. They are made from isoprene units (several five carbon base units). Each group of terpenes arises from the head-to-tail condensation of a variable number of isoprene units. Variations in the number of isoprene unit repetitions, cyclisation reactions, and rearrangements are primarily responsible for their chemical and structural diversity. EOs consist of mainly monoterpenes (C_{10}) and sesquiterpenes (C_{15}) but also have diterpenes (C_{20}), triterpenes (C_{30}), and tetraterpenes (C_{40}) at very low concentration with their oxygenated derivatives, respectively (**Figure 13**) [15, 102, 130].

3.1.1 Hemiterpenes

Hemiterpenes are part of minor terpenes of EOs. They are usually alcohols, aldehydes, and esters, with a 2-methylbutane skeleton [131]. The number of

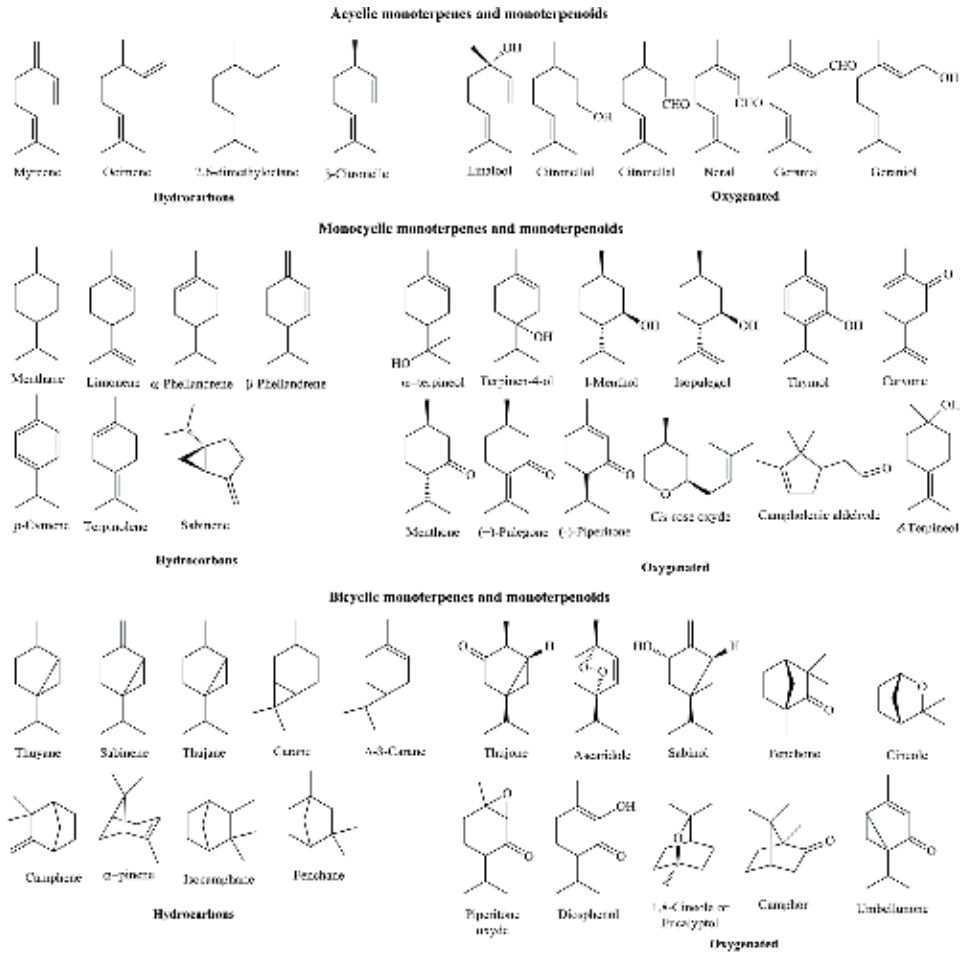

Figure 15.
Structures of some monoterpenes and monoterpenoids.

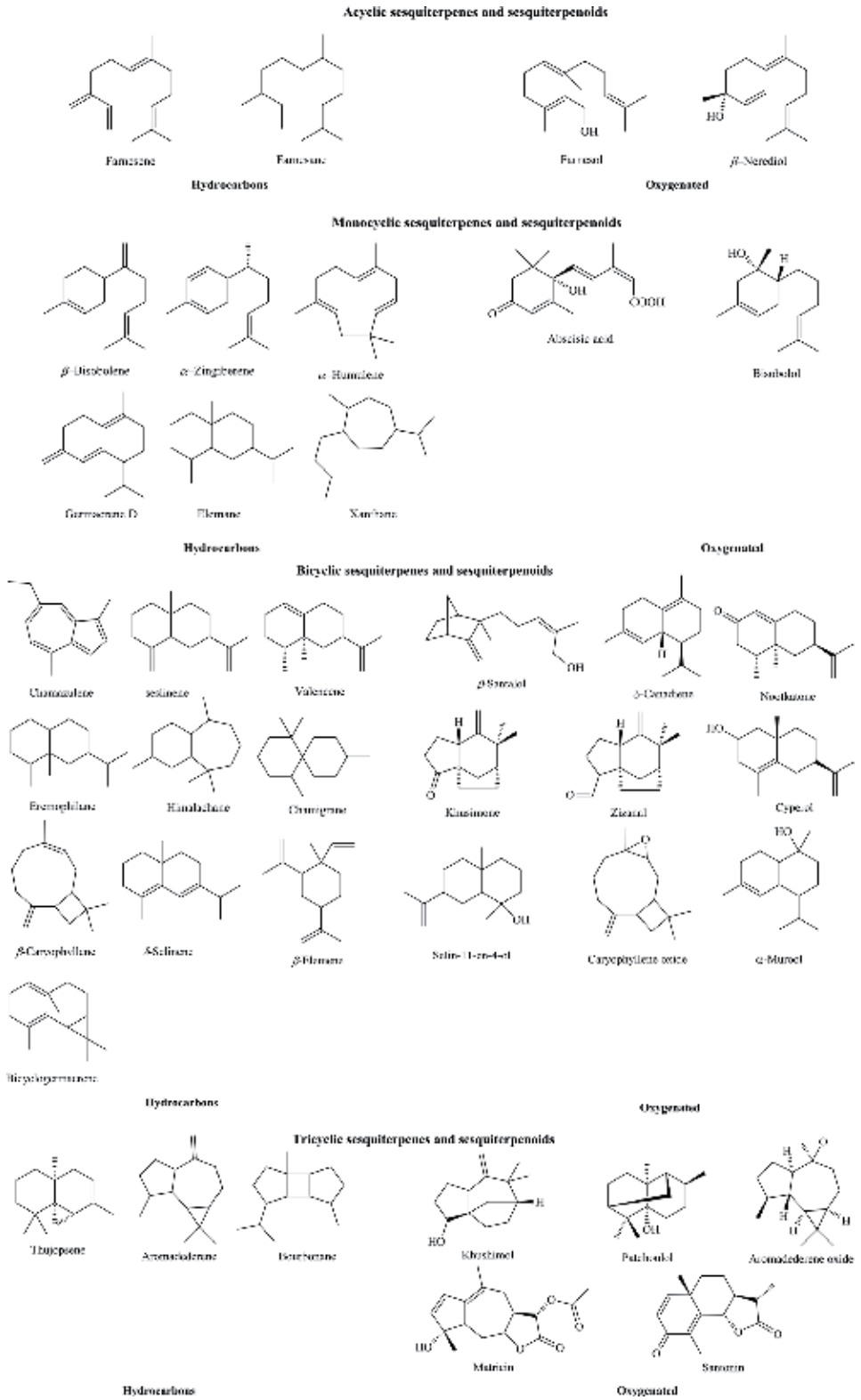

Figure 16.
Structures of some sesquiterpenes and sesquiterpenoids.

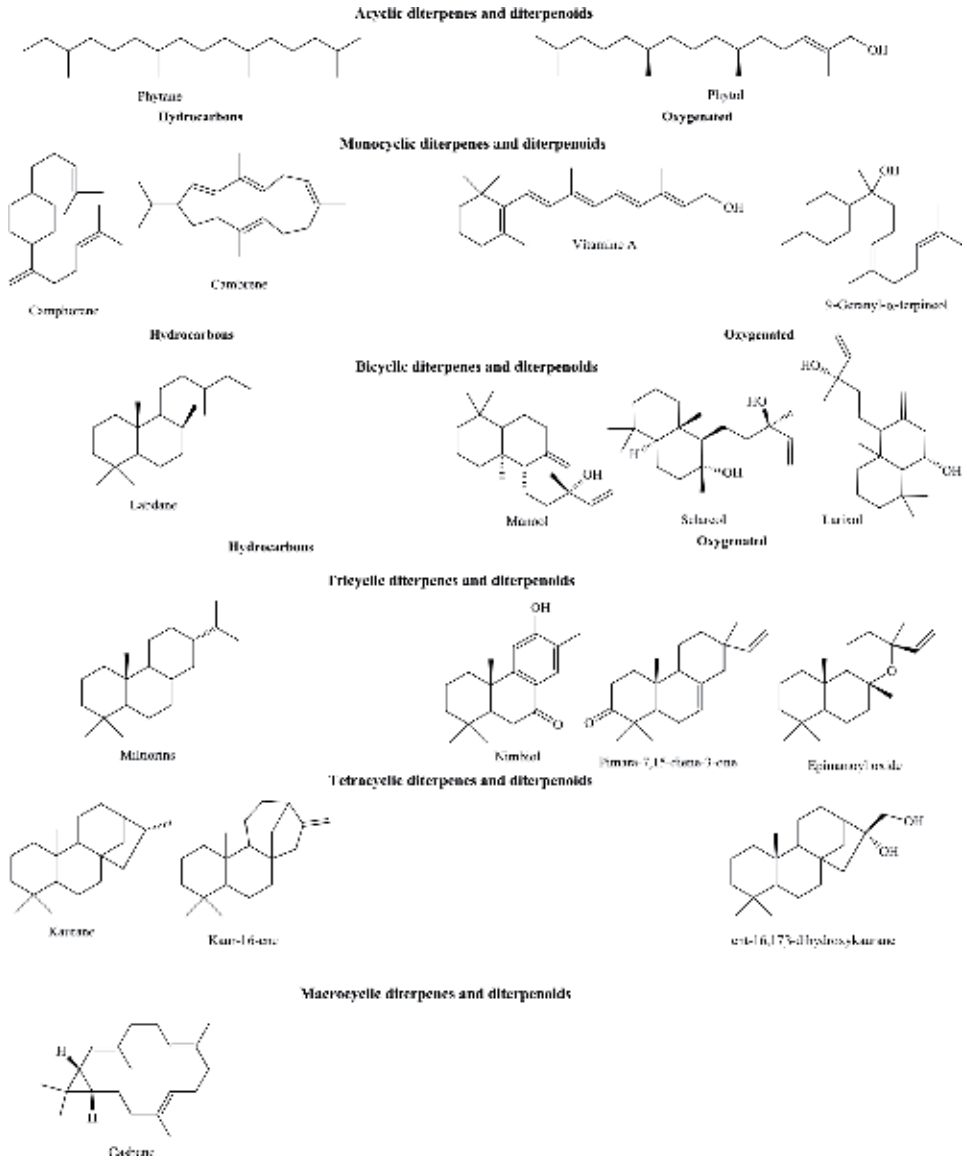

Figure 17.
Structures of some diterpenes and diterpenoids.

hemiterpene aglycone is less than 100 [132]. Chlorinated hemiterpenes were recently isolated from the leaves of *Prinsepia utilis* (**Figure 14**) [133].

3.1.2 Monoterpenes

Regular monoterpenes are made from the combination of two isoprene units (C_{10}) linked by the head-to-tail binding. They are the major molecules consisting of 90% of (some) EOs; thereby, they contribute to the specific smell of plants [134, 135]. Monoterpenes are found in nearly all EOs and usually possess one double bond in their structures. In nature, they are mostly involved in plant–animal and plant–plant interactions such as pollination, seed and fruit dissemination, and allelopathic agents. Monoterpenes occur in more than 30 known

skeletons and can be divided into 3 subgroups: acyclic, monocyclic, and bicyclic. A number of monoterpenes are oxygenated (**Figure 15**).

3.1.3 Sesquiterpenes

Sesquiterpenes are other major EO components and are less volatile than monoterpenes. They are derived from three isoprene units and exist in a wide variety of forms, including linear, monocyclic, bicyclic, and tricyclic frameworks. Sesquiterpenes are the most diverse group of terpenoids (**Figure 16**).

3.1.4 Diterpenes

They are chemically complex and are usually components of plants resins but are sometimes encountered as by-products in the isolation of EOs. Diterpenes are less volatile because of their high molecular weights and less numerous than the mono- and sesquiterpenes. Consequently, they are difficult to extract by steam distillation and then appear rarely in distilled EOs. When present, they are found in EOs in very low amounts. However, traditional extraction using distillation allows separation and identification of diterpenes present in EOs [136]. Generally, molecules with molecular masses higher than 300 uma can be seen as sign of improper extraction conditions or adulteration. Diterpenes that are usually found in EOs include camphorene, cafestol, kahweol, cambrene, and taxideme (**Figure 17**).

3.2 Bioactivities of terpenoids

Some sesquiterpenoids are very toxic, but some are antifungals, carminatives, and insecticides.

Being complex mixtures of constituents, overall activities of EOs cannot therefore be attributed only to their major components (terpenoids) [137]. Many aroma components of EOs, such as terpenes and terpenoids, were proposed to contribute to their antioxidant activity; that include β-terpene and β-terpinolene in *Melaleuca alternifolia*, 1,8-cineole in *Mentha aquatic*, and linalool in black cumin. Less volatile but strongly bitter-tasting or toxic terpenes also protect some plant from being eaten by animals. Some terpenes are potent drugs against diseases such as heart disease, malaria, and cancer [34].

4. Importance of terpenes terpenoids found in essentials oils

Terpenoids are, by far, the most important group (numerous and structurally diverse) of natural products as far as EOs are concerned. Reports on the level of terpenoids in EOs vary considerably. Many terpenes have biological activities and are used for medical purposes. For example, the antimalarial drug artemisinin and the anticancer drug Taxol (paclitaxel) are two of a few terpenes with established medical applications [26].

Monoterpenes are well known as main constituents of EOs, floral, and scents. Monoterpenes and monoterpenoids have antioxidant, anticonvulsant, antiulcer, anti-inflammatory, antiseptic, antitumor, antiviral, analgesic, antihypertensive, antibacterial, and therapeutic antidiabetic properties [26, 138]. The general mechanism of action of monoterpenes, such as their antimicrobial and antitussive activity, is mainly related to their volatility. Their hydrophobicity, as well as the EOs as a whole, determines their effect on bacterial cell structures with a subsequent antimicrobial effect [139]. α-Terpineol is used to enhance skin penetration and

also has insecticidal properties [140]. Monoterpenes have been shown to exert chemopreventive as well as chemotherapeutic activities in mammary tumor models and thus may represent a new class of therapeutic agents [138]. The EO of *Melissa officinalis* L. can inhibit the replication of HSV-2, due to the presence of citral and citronellal [141]. Linalool is an unsaturated alcohol monoterpene found as principal constituent in many EOs known to exhibit various biological activities that include antibacterial, antiplasmodial, and antinociceptive effects in different animal models [142–144]. Linalool also plays an important role in nature as a key compound in the complex pollination biology of various plant species to ensure reproduction and survival. It is also a key compound for the industrial production of a variety of fragrance chemicals such as geraniol, nerol, citral and its derivatives, as well as a lead compound in the synthesis of vitamins A and E. Its repellent properties on various crop-destroying insects are well studied and documented, hence accentuating the application of linalool in eco-friendly pest management [145]. In Malaysia, linalool is reported to be the major component of EOs of different aromatic species of the Lauraceae family; hence it may be classified as a taxon of this family [146]. Limonene is among the most abundant monoterpene constituents found in nature, and it occurs in a variety of trees and herbs that include *Citrus* species. It has been an interesting target molecule for chemists and biologists. Limonene inhibits LPS-induced NO and PGE2 production that included dose-dependent decreases in the expression of iNOS and COX-2 proteins [147]. Some in vitro and in vivo studies have revealed the effects of monoterpenes on diabetes, insulin resistance, and obesity. The role of inflammation as a link between diabetes and obesity has been established. Many monoterpenes exhibit ameliorative effects in inflammatory conditions associated with diabetes [148]. The analgesic effect of many plant EOs rich in monoterpenes has been established experimentally [149].

Some bicyclic monoterpenoids are known to suppress the acetylcholinesterase activity, which is increased in patient with Alzheimer's disease. In a study of 17 monoterpenes and monoterpenoids, (+)- and (−)-α-pinene and (+)-3-carene appeared as potent inhibitors of the enzyme AChE, while the bicyclic ketones and alcohol inhibitions were weak [150].

In recent years, a considerable large number of research studies have been carried out on the chemical constituents of EOs as source of bioactive natural products against cancer. Piaru and collaborators showed that EO of *Myristica fragrans* exhibited good cytotoxic activity, possibly due to the presence of some potential anticancer substances such as limonene, terpinen-4-ol, eugenol, and myristicin [151]. Similarly, EO from *Vepris macrophylla* demonstrated a strong cytotoxic effect, which may be attributed to the presence of specific components like citral, citronellol, and myrcene [152, 153].

Many EO components possess enantiomers that can be sometime present in an oil. It is important to note that there is a close relationship between the chirality of organic compounds and their biological properties. For a given optically active substance, the activity is not identical for both enantiomers [153]. Linalool, for example, has two enantiomers: (3*S*)-(+)-linalool known as coryandrol and (3*R*)-(−)-linalool known as licareol. Both have distinct properties. It was reported that although (*S*)-(+)- and (*R*)-(−)- have similar activity profiles, the effect of (*R*)-(−)-linalool is more intense [154]. Similarly, De Sousa and coworkers showed that regarding the anticonvulsant activity, (*R*)-(−)-linalool and the racemate form were more active than the (*S*)-(+)- enantiomer, which had effects compatible with diazepam and phenytoin, known as anticonvulsant agents [148, 153].

Geraniol, an acyclic aldehyde monoterpene present in various EOs from many aromatic plants, has in vitro and in vivo antitumor activity against several cancer cell lines. In fact, geraniol alters several metabolic pathways of HepG2 cells such

as the mevalonate pathway and the phosphatidylcholine biosynthesis, which results in cell growth inhibition, cell cycle arrest occurring at the G0/G1 interphase, and increased apoptosis [155]. Antibacterial and antifungal activities of oils with high levels of sesquiterpenes as cadinene, spathulenol, and selinene were described [156].

Cristiani and coworkers have reported the antimicrobial activity of four monoterpenes (*p*-cymene, *γ*-terpinene, carvacrol, and thymol) against the Gram-positive bacterium *S. aureus* and the Gram-negative bacterium *E. coli*. They concluded that thymol was considerably more toxic against *S. aureus* than the other three terpenes, while carvacrol and *p*-cymene were the most active against *E. coli* [157]. Germacrene D with its three double bonds as electron-rich centers demonstrated good ability to scavenge superoxide radical anions [156]. However, linalool and nerolidol may also display pro-oxidant activity. Carvacrol and thymol are reported to be the main constituents of volatile oils from *Origanum* species in general [158, 159]. With limonene, citronellol, myrtenol, linalool, and carvacrol are among monoterpenes showing in vitro and in vivo cardiovascular effects in both humans and animals [159].

Monoterpenes, sesquiterpenes, and oxygenated derivatives extracted from EOs have shown strong inhibitory activities against pathogenic bacteria, hence suggesting their use as flavoring and antioxidant agents [104].

Alzheimer's disease is by far the most prevalent of all known forms of dementia. Wojtunik-Kulesza and collaborators showed that three monocyclic monoterpenes (carvone, pulegone, and γ-terpene) possess acetylcholinesterase (AchE) inhibitory activity. Among the investigated terpenes, the three later were recognized as compounds with promising activities in the development of multi-target directed ligands [160]. The lipophilic character of terpene skeleton combined with the hydrophobic character of the functional group is essential for activity. Thus, a rank of activity has been proposed as follows: aldehydes > ketones > alcohols > esters > hydrocarbons [156].

In 2010, Conti and coworkers measured the insect repellent activity of three EOs. They found that at lowest dose (0.001%), the OE of *Hyptis suaveolens* exhibited a significant higher repellent effect compare to *Hyptis spicigera* and *Lavandula angustifolia*. After chemical analyses of the OE of *H. suaveolens*, monoterpene hydrocarbons were the most represented class of volatiles (64.1%), followed by sesquiterpene hydrocarbons (24.0%) [114].

In EOs, the components found in higher concentrations and related to antimicrobial activity are phenolic compounds such as linalool, sabinene, menthol, myrcene, and camphene [161].

Sesquiterpenes have anti-inflammatory and anti-allergic properties. The anti-inflammatory activities of some medicinal plants are due to the presence of one or more sesquiterpene lactones [26]. Above all, terpenes are responsible for the smell and flavor typical of the different varieties of *Cannabis sativa*, whereas phytocannabinoids are odorless [162].

5. Conclusion

Terpenes represent one of the largest and most diverse classes of natural products. They have numerous roles ranging from defense repellents against herbivores or pathogens through animal attract hormones to agents designed to help disperse seeds and pollen. Monoterpenoids and sesquiterpenoids are obviously the major constituents of EOs, while in some oils the occurrence of diterpenoids was observed as quite minor constituents when present. In an ecological context, mono- and

sesquiterpenes play an important role in the relations between organisms, for example, as attractants of pollinators or deterrents of herbivores. The enormous diversity of terpenoids and wide spectrum of biological activities make them attractive for many industries, and new areas of application still have not been discovered. Despite their rich and complex composition, the use of EOs remains limited to the cosmetics and perfumery domains. It is worthy to develop a better understanding of their chemistry and biological properties as well as that of their individual components for new and valuable applications in human health.

Despite their well-recognized bioactivities, EOs have been misused with regard to their level of toxicity. Some EOs or their major constituents have been recorded to be much toxic with bad side effects including convulsions, irritation, and photodermatosis. Literature review of the available data shows that serious accidents, most of which involve young children, are due to a small number of EOs, ingested in large amount. The development and the expansion of therapies using EOs and the evaluation of their acute toxicity have become more important to avoid their abusive use. The most common adverse events are eye, mucous membrane, and skin irritation and sensitization particularly to oils containing aldehydes and phenols. Despite all, no well-defined studies have proved that these EOs are harmful, but this deserves more detailed studies.

Author details

Fongang Fotsing Yannick Stephane[1*] and Bankeu Kezetas Jean Jules[2]

1 Department of Chemistry, Higher Teachers Training College, University of Maroua, Maroua, Cameroon

2 Department of Chemistry, Faculty of Science, University of Bamenda, Bambili, Cameroon

*Address all correspondence to: fongangfys@yahoo.fr

IntechOpen

References

[1] Saxena M, Saxena J, Nema R, Singh D, Gupta A. Phytochemistry of medicinal plants. Journal of Pharmacognosy and Phytochemistry. 2013;**1**(6):168-182

[2] Rao N. Bioactives phytochemicals in Indian foods and their potential in health promotion and disease prevention. Asia Pacific Journal of Clinical Nutrition. 2003;**12**(1):9-22

[3] Caputi L, Aprea E. Use of terpenoids as natural flavouring compounds in food industry. Recent Patents on Food, Nutrition & Agriculture. 2011;**24**:9-16

[4] Djilani A, Dicko A. The therapeutic benefits of essential oils. In: Bouayed J, Bohn T, editors. Nutrition, Well-being and Health. Vol. 7. Rijeka: IntechOpen; 2012. pp. 155-179

[5] Astani A, Reichling J, Schniztler P. Comparative study on the antiviral activity of selected monoterpenes derived from essential oils. Phytotherapy Research. 2010;**24**:673-679

[6] Almeida RN, Fatima AM, Souto MF, Sousa DP. Essential oils and their constituents: Anticonvulsant activity. Molecules. 2011;**16**:2726-2742

[7] Kumari S, Pundhir S, Priya P, Jeena G, Punetha A, Chawla K, et al. EssoilDB: A database of essential oils reflecting terpene composition and variability in the plant kingdom. Database. 2014;**14**:1-12

[8] Palazzolo E, Laudicina VA, Germanà MA. Current and potential use of *Citrus* essential oils. Current Organic Chemistry. 2013;**17**:3042-3049

[9] Costa MA, Zia ZQ, Davin LB, Lewis NG. Chapter four: Toward engineering the metabolic pathways of cancer-preventing lignans in cereal grains and other crops. In: Romeo JT, editor. Recent Advances in Phytochemistry, Phytochemicals in Human Health Protection, Nutrition, and Plant Defense. Vol. 33. New York: Springer Science+Business Media Kluwer Academic/Plenum Publishers; 1999. pp. 67-87

[10] Sirousmehr A, Arbabi J, Asgharipour MR. Effect of drought stress levels and organic manures on yield, essential oil content and some morphological characteristics of sweet basil (*Ocimum basilicum*). Biology. 2014;**8**(4):880-885

[11] Surburg H, Panten J. Common Fragrance and Flavor Materials. Preparation, Properties and Uses. 6th ed. Germany: Wiley-VCH Verlag GmbH Co; 2016. pp. 84-85

[12] Gupta V, Mittal P, Bansal P, Khokra SL, Kaushik D. Pharmacological potential of *Matricaria reticula*. International Journal of Pharmaceutical and Drug Research. 2010;**2**:67-71

[13] Martin A, Varona S, Navarette A, Cocero MJ. Encapsulation and co-precipitation processes with supercritical fluids: Applications with essential oils. The Open Chemical Engineering Journal. 2010;**4**:31-41

[14] Skold M, Karlberg AT, Matura M, Borje A. The fragrance chemical β-caryophyllene-air oxidation and skin sensitization. Food and Chemical Toxicology. 2006;**44**:538-545

[15] Bakkali F, Averbeck S, Averdeck D, Idaomar M. Microbial effects of essential oils—A review. Food and Chemical Toxicology. 2008;**46**:446-475

[16] Raut JS, Karuppayil SM. A status review on the medicinal properties

of essential oils. Industrial Crops. 2014;**62**:250-264

[17] Chlodwin F, Novak J. Sources of essential oils. In: KHC B, Buchbauer G, editors. Handbook of Essential Oils: Science, Technology, and Applications. London, UK: Taylor and Francis Group; 2010

[18] Ebadollahi A. Essential oils from Myrtaceae family as natural insecticides. Annual Review & Research in Biology. 2013;**3**(3):148-175

[19] Butnariu M, Sarac I. Essential oils from plants. Journal of Biotechnology and Biomedical Science. 2018;**1**(4):35-43

[20] Nieto G. Biological activities of three essential oils of the Lamiaceae family. Medecines. 2017;**4**:63-72

[21] Ur-Rahman A. Frontiers in Clinical Drug Research—Antiinfectives. Vol. 3. Saif Zone, Saif, Sharjah, United Arab Emirates: Bentham eBooks; 2016. p. 141

[22] Dreger M, Wielgus K. Application of essential oils as natural cosmetic preservatives. Herba Polonica. 2013;**59**:142-156

[23] Harrewijn P, Oosten VAM, Piron PGM. Natural Terpenoids as Messengers. Dordrecht, The Netherlands: Kluwer Academic Publishers; 2001

[24] Vetter S, Franz C, Glasl S, Kastner U, Saukel J, Jurenitsch J. Inheritance of sesquiterpene lactone types within the *Achillea millefolium complex* (Compositae). Plant Breeding. 1997;**116**:79-82

[25] de CN G, Quintero A, Orellana RC. Chemotaxonomic value of essential oil compounds in *Citrus* species. Acta Horticulrae. 2002;**576**:49-55

[26] Wang L, Well CL. Recent advances in extraction of nutraceuticals from plants. Trends in Food and Sciences technology. 2006;**17**:300-312

[27] Dick AJ, Starmans HHN. Extraction of secondary metabolites from plant material: A review. Trends in Food Science and Technology. 1996;**7**(6):191-197

[28] Guiliana MVV, Barros DA, Oliviera MS, Aquino GLB, Santos DM, Paulo JR, et al. A green protocol for microwave-assisted extraction of volatile oil terpenes from *Pterodon emarginatus* Vogel. (Fabaceae). Molecules. 2018;**23**:651-663

[29] Morsy NFS. A short extraction time of high quality hydrodistillation cardamom (*Elettaria cardamomum* L. Maton) essential oil using ultrasound as a pretreatment. Industrial Crops and Products. 2015;**65**:287-292

[30] Bowles EJ. The Chemistry of Aromatherapeutic Oils. 3rd ed. Crows Nest: Allen and Unwin; 2003. p. 236

[31] Kahriman N, Tosuni G, Genc H, Yayli N. Comparative essential oil analysis of *Geranium sylvaticum* extracted by hydrodistillation and microwave distillation. Turkish Journal of Chemistry. 2014;**34**:969-976

[32] Phakawat T, Soottawat B. Essential oils: Extraction, bioactivities, and their uses for food preservation. Journal of Food Science. 2014;**79**(7):1231-1249

[33] Prado JM, Vardanega R, Debien ICN, Meireles MAA, Gerschenson LN, Sowbhagya HB, et al. Chapter 6: Conventional extraction. In: Galanakis CM, editor. Food Waste Recovery. Processing Technologies and Industrial Techniques. 1st ed. Academic Press; 2015. pp. 127-148. 412 p. DOI: 10.1016/C2013-0-16046-1

[34] Rassem HHA, Nour AH, Yunus RM. Techniques for extraction of essential oils from plants: A review. Australian

Journal of Basic and Applied Sciences. 2016;**10**(16):117-127

[35] Guntero VA, Mancini PM, Kneeteman MN. Introducing organic chemistry students to the extraction of natural products found in vegetal species. World Journal of Chemical Education. 2017;**5**(4):142-147

[36] Geramitcioski T, Mitrevski V, Mijakovski V. Design of a small press for extracting essential oil according VDI 2221. In: IOP Conference Series: Materials Science and Engineering, Novi Sad, Serbia. Vol. 393. 2018. pp. 1-8. DOI: 10.1088/1757-899X/393/1/012131

[37] Mandal V, Mohan Y, Hemalatha S. Microwave-assisted extraction-an innovative and promising extraction tool for medicinal plant research. Pharmacognosy Reviews. 2007;**1**(1):7-18

[38] Ferhat MA, Boukhatem MN, Hazzit M, Meklati BY, Chemat F. Cold pressing, hydrodistillation and microwave dry distillation of *Citrus* essential oil from Algeria: A comparative study. Electronic Journal of Biology. 2016;**S1**:30-41

[39] Yousefi M, Rahimi-Nasrabadi M, Pourmorttazavi SM, Wysokowski M, Jesionowski T, Ehrlich H, et al. Supercritical fluid extraction of essential oils. Trends in Analytical Chemistry. 2019;**118**:182-193

[40] Bezerra FWF, da Costa WA, de Oliviera MS, de Aguiar AEH, de Carvalho JRN. Transesterification of palm pressed-fibers (*Elaeis guineensis* Jacq.) oil by supercritical fluid carbon dioxide with entrainer ethanol. Journal of Supercritical Fluids. 2018;**136**:136-143

[41] Bezerra FWF, de Oliviera MS, Bezerra PN, Cunha VMB, Silva MP, da Costa WA, et al. Extraction of bioactives compounds. In: Green Sustainable Process for Chemical and Environmental Engineering and Science. 1st ed., Kindle ed. Elsevier; 2020. pp. 149-167

[42] Wu H, Li J, Jia Y, Xiao Z, Li P, Xie Y, et al. Essential oil extracted from *Cymbopogon citronella* leaves by supercritical carbon dioxide: Antioxidant and antimicrobial activities. Journal of Analytical Methods in Chemistry. 2019;**2019**:1-10

[43] Johner JCF, Hatami T, Meireles MAA. Developing a supercritical fluid extraction method assisted by cold pressing: A novel extraction technique with promising performance applied to Pequi (*Caryocar brasiliense*). The Journal of Supercritical Fluids. 2018;**137**:34-39

[44] Hatami T. Supercritical fluid extraction assisted by cold pressing from clove buds: Extraction performance, volatile oil composition, and economic evaluation. The Journal of Supercritical Fluids. 2019;**144**:39-47

[45] Hatami T, Johner JCF, Meireles MAA. Extraction and fractionation of fennel using supercritical fluid extraction assisted by cold pressing. Industrial Crops and Products. 2018;**123**:661-666

[46] Letellier M, Budzinski H, Charrier L, Capes S, Dorthe AM. Optimization by factorial design of focused microwave-assisted extraction of polycyclic aromatic hydrocarbons from marine sediment. Journal of Analytical Chemistry. 1999;**364**:228-237

[47] Farhat A, Ginies C, Romdhane M, Chemat F. Eco-friendly and cleaner process for isolation of essential oil using microwave energy: Experimental and theoretical study. Journal of Chromatography A. 2009;**1216**(26):5077-5085

[48] Ferhat M, Meklati B, Smadja J, Chemat F. An improved microwave Clevenger apparatus for distillation of essential oils from orange peel. Journal of Chromatography A. 2006;**1112**(1-2):121-126

[49] Lucchesi ME, Chemat F, Smadja J. Solvent-free microwave extraction of essential oil from aromatic herbs: Comparison with conventional hydro-distillation. Journal of Chromatography A. 2004;**1043**:323-327

[50] Golmakani MT, Rezaei K. Microwave-assisted hydrodistillation of essential oil from *Zataria multiflora* Boiss. European Journal of Lipid Science and Technology. 2008;**110**(5):448-454

[51] Branchet A, Christen P, Veuthey JL. Focused microwave-assisted extraction of cocaine and benzoylecgonine from *coca* leaves. Phytochemistry. 2002;**13**:162-169

[52] Jeyaratnam N, Nour AH, Akindoyo JO. The potential of microwave assisted hydrodistillation in extraction of essential oil from *Cinnamomum cassia* (cinnamon). ARPN Journal of Engineering and Applied Sciences. 2016;**11**(4):2179-2183

[53] Moradi S, Fazlali A, Hamedi H. Microwave-assisted hydro-distillation of essential oil from rosemary: Comparison with traditional distillation. Avicenna Journal of Medical Biotechnology. 2018;**10**(1):22-28

[54] Rosello-Soto E, Galanakis CM, Berncic M, Orlien V, Trujillo FJ, Mamson R, et al. Clean recovery of antioxidant compounds from plant foods, by-products and algae assisted by ultrasounds processing. Modeling approaches to optimize processing conditions. Trends in Food Science & Technology. 2015;**42**(2):134-149

[55] Sereshti H, Rohanifar A, Bakhtiari S, Samadi S. Bifunctional ultrasound assisted extraction and determination of *Elettaria cardamomum* Maton essential oil. Journal of Chromatography A. 2012;**1238**:46-53

[56] Vinatoru M. An overview of the ultrasonically assisted extraction of bioactive principles from herbs. Ultrasonic Sonochemistry. 2001;**8**:303-313

[57] Medina-Torres N, Ayora-Talavera T, Espinosa-Andrews H, Sanchez-Contreras A, Pacheco N. Ultrasound assisted extraction for the recovery of phenolic compounds from vegetable sources. Agronomy. 2017;**7**:47-66

[58] Jaconet-Navarro M, Rombaut N, Deslis S, Fabiano-Tixier AS, Pierre FX, Bily A, et al. Toward a "dry" bio-refinery without solvents or added water using microwaves and ultrasound for total valorization of fruit and vegetable by-products. Green Chemistry. 2016;**18**(10):3106-3115

[59] Eskilsson CS, Björklund E. Analytical-scale microwave-assisted extraction. Journal of Chromatography A. 2000;**902**:227-250

[60] Cardoso-Ugarte GA, Juarez-Bacerra GP, Sosa-Morales ME, Lopez-Malo A. Microwave-assisted extraction of essential oil from herbs. The Journal of Microwave Power and Electromagnetic Energy. 2013;**47**(1):63-72

[61] Filly A, Fernandez X, Minuti M, Visioni F, Cravotto G, Chemat F. Solvent-free microwave extraction of essential oil from aromatic herbs: From laboratory to pilot and industrial scale. Food Chemistry. 2014;**150**:193-198

[62] Lucchesi ME, Chemat F, Smadja J. Original solvent-free

microwave extraction of essential oils from species. Flavor and Fragrance Journal. 2004;**19**:134-138

[63] Kusama H, Putri D, Dewi I, Mahfud M. Solvent-free microwave extraction as the useful tool for extraction of edible essential oils. Chemistry & Chemical Technology. 2016;**2**:213-218

[64] Kusama H, Mahfud M. Preliminary study: Kinetics of oil extraction from sandalwood by microwave-assisted hydrodistillation. ASEAN Journal of Chemical Engineering. 2015;**16**:62-69

[65] Vian MA, Fernandez X, Visinoni F, Chemat F. Mcrowave hydrodiffusion and gravity, a new technique for extraction of essential oils. Journal of Chromatography A. 2008;**1190**(1-2):14-17

[66] Chemat F, Lucchesi ME, Smadja, J. Extraction sans solvant assistée par micro-ondes de produits naturels [PhD thesis]. Université de la Reunion; 2005

[67] Smelcerovic A, Djordjevic A, Lazarevic J, Stojanovic G. Recent advances in analysis of essential oils. Current Analytical Chemistry. 2013;**9**:61-70

[68] Yukawa Y, Ito S. Spectral Atlas of Terpenes and the Related Compounds. Tokyo: Hirokawa Publi. Co.; 1973

[69] Jennings W. Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography. New York: Academic Press; 1980

[70] James AT, Martin AJP. Gas-liquid partition chromatography: The separation and micro-estimation of volatile fatty acids from formic acid to dodecanoic acid. Biochemical Journal. 1952;**50**:679-690

[71] Vekey K. Mass spectrometry and mass-selective detection in gas chromatography. Journal of Chromatography A. 2001;**921**:227-236

[72] Bicchi C, Brunelli C, Cordero C, Rubiolo P, Galli M, Sironi A. High-speed gas chromatography with direct resistively-heated column (ultra-fast module-GC)-separation measure (*S*) and other chromatographic parameters under different analysis conditions for samples of different complexities and volatilities. Journal of Chromatography A. 2005;**1071**(1-2):3-12

[73] Nowotny HP, Schmalzing D, Wistuba D, Schurig V. Extending the scope of enantiomer separation on diluted methylated β-cyclodextrin derivatives by high-resolution gas chromatography. Journal of High Resolution Chromatography. 1989;**12**:383-393

[74] Giacomo DA, Mincione B. Gli Olii Essenziali Agrumari in Italia, Chap. 3. Reggio Calabria: Baruffa editore; 1994

[75] Deans DR. A new technique for heart cutting in gas chromatography. Chromatographia. 1968;**1**(1-2):18-22

[76] Mondello L, Catalfamo M, Proteggente AR, Bonaccorsi I, Dugo G. Multidimensional capillary GC-CG for the analysis of real complex samples. 3. Enantiomeric distribution of monoterpene hydrocarbons and monoterpene alcohols of mandarin oils. Journal of Agricultural Food Chemistry. 1998;**46**:54-61

[77] Dugo P, Fernandez MD, Cotroneo A, Dugo G, Mondello L. Optimization of a comprehensive two-dimensional normal-phase and reversed-phase liquid chromatography system. Journal of Chromatographic Science. 2006;**44**(9):561-565

[78] Mondello L, Dugo P, Bartle KD, Cotroneo A. Automated HPLC-HRCG: A powerful method for essential oils analysis. Part V. Identification of

terpene hydrocarbons of bergamot, lemon, mandarin, sweet orange, bitter orange, grapefruit, clementine and Mexican lime oils by coupled HPLC-HRGC-MS(ITD). Flavour and Fragrance Journal. 1995;**10**:33-42

[79] Shabaan HAE, El-Ghorab AH, Shibamoto T. Bioactivity of essential oils and their volatile aroma components: Review. The Journal of Essential Oil Research. 2012;**24**(2):203-212

[80] Moreira P, Smith MA, Zhu X, Honda K, Lee HG, Aliev G, et al. Since oxidative damage is a key phenomenon in Alzheimer's disease, treatment with antioxidants seems to be a promising approach for slowing disease profession. Oxidative damage and Alzheimer's disease: Are antioxidant therapies useful? Drug News & Perspectives. 2005;**18**:13

[81] Naito Y, Uchiyama K, Yoshikama T. Oxidative stress involvement in diabetic nephropathy and its prevention by astaxanthin. Oxidative Stress Disease. 2006;**21**:235-242

[82] Lui J, Mori A. Oxidative damage hypothesis of stress associated aging acceleration: Neuroprotective effects of natural and nutritional antioxidants. Research Communication in Biology Psychology, Psychiatry and Neuroscience. 2005;**30-31**:1-16

[83] Beal MF. Mitochondrial, oxidative damage, and inflammation in Parkinson's disease. Annals of the New York Academy of Sciences. 2003;**991**:120-131

[84] Mimica-Dukic N, Bozin B, Sokovic M, Simin N. Antimicrobial and antioxidant activities of *Melissa officinalis* L. (Lamiaceae) essential oil. Journal of Agriculture and Food Chemistry. 2004;**52**:2485-2489

[85] Yanishlieva-Maslarova N. Sources of natural antioxidants: Vegetables, fruits, herbs, spices and teas. In: Yanishlieva N, Pokorny J, Gordor M, editors. Antioxidant in Food: Practical Applications. Cambridge: Woodhead Publishing Ltd; 2001. pp. 201-249

[86] Halliwell B. The antioxidant paradox. Lancet. 2000;**355**(9210):1179-1180

[87] Mimica-Dukic N, Orcic D, Lesjak M, Sibul F. Essential oils as powerful antioxidants: Misconception or scientific fact? In: Medicinal and Aromatic Crops: Production, phytochemistry, and Utilization. Washington, DC, USA: Ed ACS; 2016. pp. 187-208

[88] International Agency for Research on Cancer (IARC). World Health Organization, 2018. Press release No. 263. 3 p

[89] Wall ME, Wani MC. Camptothecin and Taxol: From discovery to clinic. Journal of Ethnopharmacology. 1996;**51**:239-254

[90] Bhalla Y, Gupta VK, Jaitak V. Anticancer activity of essential oils: A review. Journal of the Science of Food and Agriculture. 2013;**93**:3643-3653

[91] Magalhaes HIF, De Sousa EBV. Antitumor essential oils. In: de Sousa DP, editor, Bioactive Essential Oils and Cancer. Switzerland: Springer International Publishing; 2015. pp 135-175.

[92] Prashar A, Locke IC, Evans CS. Cytotoxicity of lavender oil and its major components to human skin cells. Cell Proliferation. 2004;**37**:221-229

[93] Legault J, Pichette A. Potentiating effect of beta-caryophyllene on anticancer activity of alpha-humulene, isocaryophyllene and paclitaxel. Journal of Pharmacy and Pharmacology. 2007;**59**:1643-1647

[94] Bayala B, Bassole IHN, Scifo R, Gnoula C, Morel L, Lobaccaro JM, et al. Anticancer activity of essential oils and their chemical components- a review. American Journal of Cancer Research. 2014;**4**(6):591-607

[95] Bourgou S, Pichette A, Marzouk B, Legault J. Bioactivities of black cumin essential oil and its main terpenes from Tunisia. South African Journal of Botany. 2010;**76**:210-216

[96] Wang W, Li N, Luo M, Zu Y, Efferth T. Antimicrobial activity and anticancer activity of *Rosmarinus officinalis* L. essential oils compared to that of its main components. Molecules. 2012;**17**:2704-2713

[97] Basollé IHN, Juliani HR. Essential oils in combination and their antimicrobial properties. Molecules. 2012;**17**:3989-4006

[98] Gallucci MN, Olivia M, Casero C, Dambolena J, Luna A, Zygadlo J, et al. Antimicrobial combined action of terpenes against the food-borne microorganisms *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus*. Flavour and Fragrance Journal. 2009;**24**(6):348-354

[99] Pandey AK, Singh P, Tripathi NN. Chemistry and bioactivities of essential oils of some *Ocimum* species: An overview. Asian Pacific Journal of Tropical Biomedicine. 2014;**4**:682-694

[100] Nascimiento MNG, Junqueira JGM, Terezan AP, Severino RP, Silva TS, Martins CHG, et al. Chemical composition and antimicrobial activity of essential oils from *Xylopia aromatic* (Annonaceae) flowers and leaves. Revista Virtual de Quimica. 2018;**10**(5):1578-1590

[101] Mith H, Dure R, Delcenserie V, Zhiri A, Daube A, Clinquart. Antimicrobial activities of commercial essential oils and their components against food-borne pathogens and food spoilage bacteria. Food Science Nutrition 2014; 4: 403-416.

[102] Burt S. Essential oils: Their antibacterial properties and potential applications in foods—A review. International Journal of Food Microbiology. 2004;**94**:223-253

[103] Espinoza J, Urzua A, Sanhueza WM, Fincheira P, Muñoz ML, Wilkens M. Essential oil, extracts, and sesquiterpenes obtained from the heartwood of *Pilgerodendron uviferum* act as potential inhibitors of the *Staphylococcus aureus* NorA multidrug efflux pump. Frontiers in Microbiology. 2019;**40**:337-351

[104] Bhavaniramya S, Vanajothi R, Vishnupriya S, Al-AboodyMS,VijayakumarR,BaskaranD. Computational characterization of deleterious SNPs in Toll-like receptor gene that potentially cause mastitis in dairy cattle. Biocatalysis and Agricultural Biotechnology. 2019;**19**:101151

[105] Hammer KA, Carson CF, Dunstan JA, Hale J, Lehmann H, Robinson CJ, et al. Antimicrobial an anti-inflammatory activity of five *Taxandria fragrans* oils *in vitro*. Microbiology and Immunology. 2008;**52**:522-530

[106] Assiri AMA, Elbanna K, Al-Thubiani A, Ramadan MF. Cold-pressed oregano (*Orangium vulgare*) oil: A rich source of bioactive lipids with novel antioxidant and microbial properties. European Food Research Technology. 2016;**242**:1013-1023

[107] Rahman A, Al-Reza SM, Kang SC. Antifungal activity of essential oil and extracts of *Piper chaba* hunter against phytopathogenic fungi. Journal of the American Oil Chemists' Society. 2011;**88**:573-579

[108] Zore GB, Thakre AD, Jadhav S, Karuppayil SM. Terpenoids inhibit *Candida albicans* growth by affecting membrane integrity and arrest of cell cycle. Phytomedicine. 2011;**18**:1181-1190

[109] Oyedeji OA, Adeniyi BA, Ayayi O, König WA. Essential oil composition of Piper guineense and its antimicrobial activity. Another chemotype from Nigeria. Phytotherapy Research. 2005;**19**:362-364

[110] Rocha DS, Silva JM, Navarro DM, Camara CAG, Lira CS, Ramos CS. Potential antimicrobial and chemical composition of essential oils from *Pipper caldense* tissues. Journal of the Mexican Chemical Society. 2016;**60**(3):148-151

[111] Birmpa A, Constantinou P, Dedes C, Bellou M, Sazakli E, Leotsinidis M, et al. Antibacterial and antiviral effect of essential oils combined with non-thermal disinfection technologies for ready-to-eat romaine lettuce. Journal of Nutrition, Food Research and Technology. 2018;**1**(1):24-32

[112] Reichling J, Schnitzler P, Suschke U, Saller R. Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties—An overview. Forschende Komplementärmedizin. 2009;**16**:79-90

[113] Katsukawa M, Nakata R, Takizawa Y, Hori K, Takahashi S, Inoue H. Citral, a component of lemongrass oil, activates PPARα and γ and suppresses COX-2 expression. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids. 2010;**1801**(11):1214-1220. DOI: 10.1016/j.bbalip.2010.07.004

[114] Conti B, Cnale A, Cioni PL, Flamini G. Repellence of essential oils from tropical and Mediterranean Lamiaceae against *Sitophilus zeamais*. Bulletin of Insectology. 2010;**63**(2):197-202

[115] Rottini MM, Amaral ACF, Ferreira JLP, Oliviera ESC, Siva JR, Taniwaki NN, et al. *Endlicheria bracteolate* (Meisn.) essential oil as a weapon against *Leishmania amazonensis*: *In vitro* assay. Molecules. 2019;**24**:2525-2538

[116] Masango P. Cleaner production of essential oils by steam distillation. Journal of Cleaner Production. 2005;**13**:833-839

[117] Sell CS. The Chemistry of Fragrance. From Perfumer to Consumer. 2nd ed. Cambridge, UK: The Royal Society of Chemistry. p. 329

[118] Hussaina AI, Anwar F, Sherazi STH, Przybylski R. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. Food Chemistry. 2008;**108**:986-995

[119] Taiz L, Zeiger E. Plant Physiology. 5th ed. MA, USA: Sinauer Associates Inc., Publishers Sunderland; 2010. p. 782

[120] Dima C, Dima S. Essential oils in foods: Extraction, stabilization and toxicity. Current Opinion in Food Science. 2015;**5**:29-35

[121] Andrade EHA, Alves CN, Guimaraes EF, Carreira LMM, Maia JGS. Variability in essential oil composition of *Piper dilatatum* LC rich. Biochemical Systematics and Ecology. 2011;**39**:669-675

[122] Griffin SG, Wyllie SG, Markam JL, Leach DN. The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. Flavour and Fragrance Journal. 1999;**14**:322-332

[123] Sangwan NS, Farooqi AHA, Shabih F, Sangwan RS. Regulation of essential oil production in plants. Plant Growth Regulation. 2001;**34**:3-21

[124] Lichtfouse E. Suistanable Agriculture Reviews. Vol. 12. France: Springer Nature; 2013. p. 233. DOI: 978-94-007-5961-9

[125] Harborne JB, Tomas-Bardenan FA. Ecological Chemistry and Biochemistry of Plant Terpenoids. Oxford: Clarendon; 1991

[126] Surburg H, Panten J. Common Fragrance and Flavor Materials. Preparation, Properties and Uses. 5th ed. Weinheim: Wiley-VCH; 2006

[127] Reineccius GA. Flavour-isolation of essential oils. In: Berger RG, editor. Flavour and Fragrances: Chemistry, Bioprocessing and Sustainability. Springer-Verlag: Heidelberg; 2007. pp. 409-426

[128] Baharum SN, Bunawan H, Ghani MA, Mustapha WA, Noor NM. Analysis of the chemical composition of the essential oil of *Polygonum minus* Huds. Using two-dimensional gas chromatography-time-of-fligth mass spectrometry (GC-TOF MS). Molecules. 2010;**15**:7006-7015

[129] Chizolla R. Regular monoterpenes and sesquiterpenes (essential oils). In: Ramawat KG, Merillon JM, editors. Naturals Products. Berlin Heidelberg: Pringer-Verlag; 2013. pp. 2973-3008

[130] Ruberto G, Baratta MT. Antioxidant activity of selected essential oils components in two lipid model systems. Food Chemistry. 2000;**69**:167-174

[131] Sell C. Chemistry of essential oils. In: KHC B, Buchbauer G, editors. Handbook of Essential Oils: Sciences, Technology, and Applications. Boca Raton, Florida: CRC Press/Taylor & Francis Press; 2010. p. 131

[132] Ludwiczuk A, Skalicka-Wozniak K, Georgiev MI. Chapter 11 - Terpenoids. In: Badal S, Delgoda R, editors. Pharmacognosy. Boston, MA, USA: Academic Press; 2017. pp. 233-266

[133] Ying-Qian X, Zhi Y, Jun-Yi H, Jie T, Yoshihisa T, Hong-Quan D. Immunosuppressive terpenes from *Prinsepia utilis*. Journal of Asian Natural Products Research. 2007;**7**:637-642

[134] Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils: A review. Food Chemistry and Toxicology. 2008;**46**:446-475

[135] Loza-Ravera H. Monoterpenes in essential oils: Biosynthesis and properties. Advance in Experimental Medicine and Biology. 1999;**464**:49-62

[136] Mansoor K, Lockwood GB. Chromatography/terpenoids. In: Encyclopedia of Separation Science. Academic Press; 2007

[137] Isman MB, Wilson JA, Bradbury R. Insecticidal activities of commercial rosemary oils (*Rosemarinus officinalis*) against larvae *of Pseudaletia unipuncta* and *Trichoplusia ni*. in relation to their chemical compositions. Pharmaceutical Biology. 2008;**46**:82-87

[138] Shuaib A, Rohit A, Piyush M. A review article on essential oils. Journal of Medicinal Plants Studies. 2016;**4**(3):237-240

[139] Dragomanova S, Tancheva L, Georgieva M. A review: Biological activity of myrtenal and some myrtenal-containing medicinal plant essential oils. Scripta Scientifica Pharmaceutica. 2018;**5**(2):22-33

[140] Khaleel C, Tabanca N, Buchbauer G. α-Terpineol, a natural monoterpene: A review of its biological properties. Open Chemistry. 2018;**16**:349-361

[141] Allahverdiyev A, Duran N, Ozguven M, Koltas S. Antiviral activity of the volatile oils of *Melissa officinalis* L. against Herpes simplex virus type-2. Phytomedicine. 2004;**11**:657-661

[142] Van Zyl RL, Seatlholo ST, Van Vuuren SF, Viljoen AM. The biological activities of 20 nature identical essential oil. Journal of Essential Oil Research. 2006;**18**:129-133

[143] Peana AT, Marzocco S, Popolo A, Pinto A. (-)-Linalool inhibits *in vitro* NO formation: Probable involvement in the antinociceptive activity of this monoterpene compound. Life Sciences. 2006;**78**:719-723

[144] Peana AT, Rubattu P, Piga GG, Fumagalli S, Boatto G, Pippia P, et al. Involvement of adenosine A1 and A2A receptors in (-)-linalool-induced antinociception. Life Sciences. 2006;**78**:2471-2474

[145] Kamatou GPP, Viljoen AM. Linalool—A review of a biologically active compound of commercial importance. Natural Product Communications. 2008;**3**(7):1183-1192

[146] Salleh WM, Ahmad F, Yen KH, Zulkifi RM. Essential oil composition of Malaysian Lauraceae: A mini review. Pharmaceutical Sciences. 2016;**22**:60-67

[147] Yoon WJ, Lee NH, Hyun CG. Lomonene suppresses lipopolysaccharide-induced production of nitric oxide, prostaglandin E2, and pro-inflammatory cytokines in RAW 264.7 macrophages. Journal of Oleo Science. 2010;**59**:415-421

[148] Kong P, Chi R, Zhang L, Wang N, Lu Y. Effects of paeoniflorin on tumor necrosis factor-α-induced insulin resistance and changes of adipokines in3T3-L1 adipocytes. Fitoterapia. 2013;**91**:44-50

[149] De Sousa DP. Analgesic-like activity of essential oils constituents. Molecules. 2011;**16**(3):44-50

[150] Miyazawa M, Yamafuji C. Inhibition of acetylcholinesterase activity by bicyclic monoterpenoids. Journal of Food Chemistry. 2005;**53**(3):1765-1768

[151] Piaru SP, Mahmud R, Majid AM, Ismail S, Man CN. Chemical composition, antioxidant and cytotoxicity activities of the essential oils of *Myristica fragrans* and *Morinda citrofolia*. Journal of the Science of Food and Agriculture. 2012;**92**:593-597

[152] Sobral MV, Xavier AL, Lima TC, Sousa DP. Antitumor activity of monoterpenes found in essential oils. The Scientific World Journal. 2014;**2014**:1-35

[153] Damasceno CSB, Higaki NTF, Dias JFG, Miguel MD, Miguel OG. Chemical composition and biological activities of essential oils in the family Lauraceae: A systematic review of the literature. Planta Medica. 2019;**85**(13):1054-1072

[154] Aprotosoaie AC, Hancianu M, Costache II, Miron A. Linalool: A review on a key odorant molecule with valuable biological properties. Flavour and Fragance Journal. 2014;**29**:193-219

[155] Crespo R, Montero VS, Abba MC, De Bravo MG, Polo MP. Transcriptional and post-transcriptional inhibition of HMGCR and PC biosynthesis by geraniol in 2 Hep-G2 cell proliferation linked pathways. Biochemistry and Cell Biology. 2013;**91**:131-139

[156] Korosh AR, Juliani HR, Zygaldo JA. Bioactivity of essential oils and their components. In: Berger RG, editor. Flavours and Fragrances. Chemistry, Bioprocessing and Sustainability. Berlin: Springer; 2005

[157] Cristiani MT, D'Arrigo M, Mandalari G, Castelli F, Sarpietro MG, Castelli F, et al. Interaction of four monoterpenes contained in essential oils with model membranes: Implications for their antibacterial activity. Journal of Agricultural and Food Chemistry. 2007;**55**:6300-6308

[158] Letswaart JH. A Taxonomic Revision of the Genius *Origanum* (Labiatae). Springer Netherland: Dordrecht, The Netherlands; 1980. ISBN 978-90-6021-463-3.

[159] Spyridopoulou K, Fitsiou E, Bouloukosta E, Tiptiri-Kourpeti A, Vamvakias M, Oreopoulou A, et al. Extraction, chemical composition, and anticancer potential of *Origanum onites* L. essential oil. Molecules. 2019;**24**(14):2612-2637

[160] Wojtunik-Kulesza KA, Targowska-Duda K, Katarzyna K, Ginalska G, Jozwiak K, Waksmundzka-Hajnos M, et al. Volatile terpenoids as potential drug leads in Alzheimer's disease. Open Chemistry. 2017;**15**:332-343

[161] Gouvea FDS, Rosenthal A, Ferreira EHR. Plant extract and essential oils added as antimicrobials to cheeses: A review. Ciencia Rural, Santa Maria. 2017;**47**:1-9

[162] Vuerich M, Ferfuia C, Zuliani F, Piani B, Sepulcri A, Baldini M. Yield and quality of essential oils in Hemp varieties in different environments. Agronomy. 2019;**9**:356-373

Section 2

Volatile Compounds and Applications

Chapter 6

Aromatherapy as Complementary Medicine

Amira Ahmed Kamal El-din El-Anssary

Abstract

Aromatherapy is the practice of using the natural oils extracted from bark, flowers, stems, roots, leaves, or other parts of a plant to enhance psychological and physical well-being. It is a type of complementary medicine that uses volatile oils and other aromatic compounds with the aim of changing a person's mind and mood. Volatile oils are hydrophobic in nature. Essential oils are extracted by different methods as steam distillation. Some evidence exists that volatile oils may have therapeutic potential. Volatile oils are often absorbed through the skin, where they travel through the bloodstream and might promote whole-body healing. Essential oils are showing a spread of applications, including pain treatments, enhancement of mood, and increased cognitive function. Essential oils are available in a large number, each with its own healing properties.

Keywords: aromatherapy, complementary medicine, essential oils, therapeutic benefits, ketones, ancient civilization, biological activities, distribution

1. Introduction

For a long time, essential oils were well-known for their therapeutic importance. They were used as perfumes and flavors for foods and beverages or to heal both the body and mind for many years [1–4]. They were used in ancient civilizations as Chinese, Indian, and ancient Egyptian and show their uses in many treatments in different forms. The ancient Chinese were the first culture to use aromatherapy in folk medicine, and then the ancient Egyptians created undeveloped distillation machine that is used for the crude extraction. Greece learned a large deal from the ancient Egyptians, and they also learned the therapeutic and aromatic advantages of the aromatic plants [5–8].

Volatile oils consist of very small aromatic molecules that are easily absorbed through the skin and respiratory system. These medicinal compounds next enter the bloodstream and then spread all over the whole body where they can create their useful curing powers. As they are too concentrated, even a small amount of volatile oil is effective. Nowadays aromatherapy is one of the most popular complementary therapies, offering a highly effective treatment to both the acute and chronic diseases. In addition, the continuous use of aromatherapy and home-use products helps our immune system [9].

2. Definition and localization of essential oils

Volatile oils are aromatic compounds which occur only in 10% of the plant kingdom and are stored in plants in specific secretory cells such as glands, hairs, ducts, cavities, or resin ducts [10–13]. Essential oils are hydrophobic in nature; they can be dissolved by polar solvent like alcohols and nonpolar solvents, waxes, and oils. Most of them are pale yellow or with no color with the exception of the blue volatile oil of *Matricaria chamomilla* L., and most are liquid and of low density than water except the essential oil of *Cinnamomum verum* Blume. and *Syzygium aromaticum* L. [14, 15].

Volatile oils are easily oxidizable by light, heat, and air due to the presence of olefenic double bonds and functional groups such as hydroxyl, aldehyde, and ester [16, 17].

3. Extraction of volatile oils

The oils contained within the plant cells are liberated through heat and compression from different organs of the plant, for example, the leaves, flowers, fruit, bark, and gums. The extraction of the oils from different plant organs is achieved by different methods, such as hydro-distillation, which is the most common method of extraction [18, 19]. Essential oils are composed of a mixture of volatile components and consist of about 20–60 individual compounds, and some may contain more than 100 components as jasmine, lemon, and cinnamon volatile oils [20–23].

4. Factors affecting chemical composition of volatile oils

The fragrance and chemical composition of the oils can vary according to different factors as the geo-climatic location and growing conditions (soil type, climate, altitude, and amount of water available), season, and time of day when harvesting is done. Therefore, these factors influence the biochemical synthesis of the oils in a plant, so that the same species of the plant make the same volatile oil but maybe of different chemical compounds, which will affect their therapeutic activities. These different chemical compositions led to different chemotypes. Chemotype is in general a different population of the same species of plant which produces many chemical profiles for a particular class of secondary metabolites. Examples of some chemotypes are shown in **Table 1** [24–27].

5. Distribution of the volatile oils in the plant kingdom

Although only 100 species are widely known for their volatile oils, there are over 2000 plant species widespread over 60 families such as Lamiaceae, which is also

Plant name	Chemotype 1	Chemotype 2	Chemotype 3
Thyme (*Thymus vulgaris* L.)	Thymol	Thujanol	Linalool
Peppermint (*Mentha piperita* L.)	Menthol	Carvone	Limonene
Rosemary (*Rosmarinus officinalis* L.)	1,8 Camphor	Cineole	Verbenone
Dill (*Anethum graveolens* L.)	Carvone	Limonene	Phellandrene
Lavender (*Lavandula angustifolia* Mill.)	Linalool	Linalyl acetate	β-Caryophyllene

Table 1.
Examples of different chemotypes.

Class of compounds	Example	Bioactivities	References
Ketones	Carvone, menthone, pulegone, fenchone, camphor	Mucolytic, cell regenerating, sedative, antiviral, neurotoxic, analgesic, spasmolytic	[38–40]
Aldehydes	Citral, myrtenal, cuminaldehyde, citronellal, cinnamaldehyde, benzaldehyde	Antiviral, antimicrobial, tonic, vasodilators, hypotensive, calming, antipyretic, sedative	[26, 41]
Phenols	Thymol, eugenol, carvacrol, chavicol	Antimicrobial, spasmolytic, immune stimulating	[26, 40]
Alcohols	Linalool, menthol, borneol, santalol, nerol, citronellol, geraniol	Antimicrobial, antiseptic, tonifying, spasmolytic	[26, 40]
Lactones	Nepetalactone, bergaptene	Antimicrobial antiviral, antipyretic, sedative, hypotensive, analgesic	[26, 40]
Hydrocarbons	Limonene, myrcene, pinene, sabinene, cymene, myrcene, phellandrene	Stimulant, antiviral, antitumor, decongestant, antibacterial, hepatoprotective	[26, 40]
Esters	Linalyl acetate, geraniol acetate, eugenol acetate, bornyl acetate	Spasmolytic, sedative, antifungal, anti-inflammatory	[26, 40]
Oxides	Bisabolone oxide, linalool oxide, sclareol oxide	Anti-inflammatory, expectorant, stimulant	[26, 40]

Table 2.
Different classes of volatile oils and their biological activities.

called the mint family. It is one of most important plant families in the plant kingdom. This family is rich in essential oils, especially menthol thyme, Rosemary, and Oregano. Apiaceae or Umbelliferae is a family of mostly aromatic flowering pla nts, which contains economically important plants as caraway, coriander, cumin, and fennel [28–31]. Volatile oils contribute in a lot of industries as food products, drinks, perfumes, pharmaceuticals, and cosmetics [32–34]. The production and consumption of essential oils increase rapidly all over the world [35]. Regardless of the high costs because of the large amounts of plant material needed, volatile oil production has been increasing. The expected world production of the oils ranges from 40,000 to 60,000 tons/year and represents a market of approximately 700 million US$ [36, 37]. Examples of some classes of essential oils their medical uses and structures are illustrated in (**Table 2**), (**Figures 1** and **2**) [26, 38–41].

6. Therapeutic benefits of essential oils

Many plant essential oils are used as medicine for hundreds of years and have demonstrated several health benefits, including effects on infectious, chronic, and acute diseases. The medical preparations made with plant essential oils as well as their single constituents applied in the therapy of human infectious diseases are well documented. However, the selection of suitable safe oil and the determination of the best efficient dose should be taken into consideration to avoid any side effects when they are applied [41]. The action of volatile oils begins by entering the human body through three possible ways including direct absorption through inhalation, ingestion, or diffusion through the skin tissue.

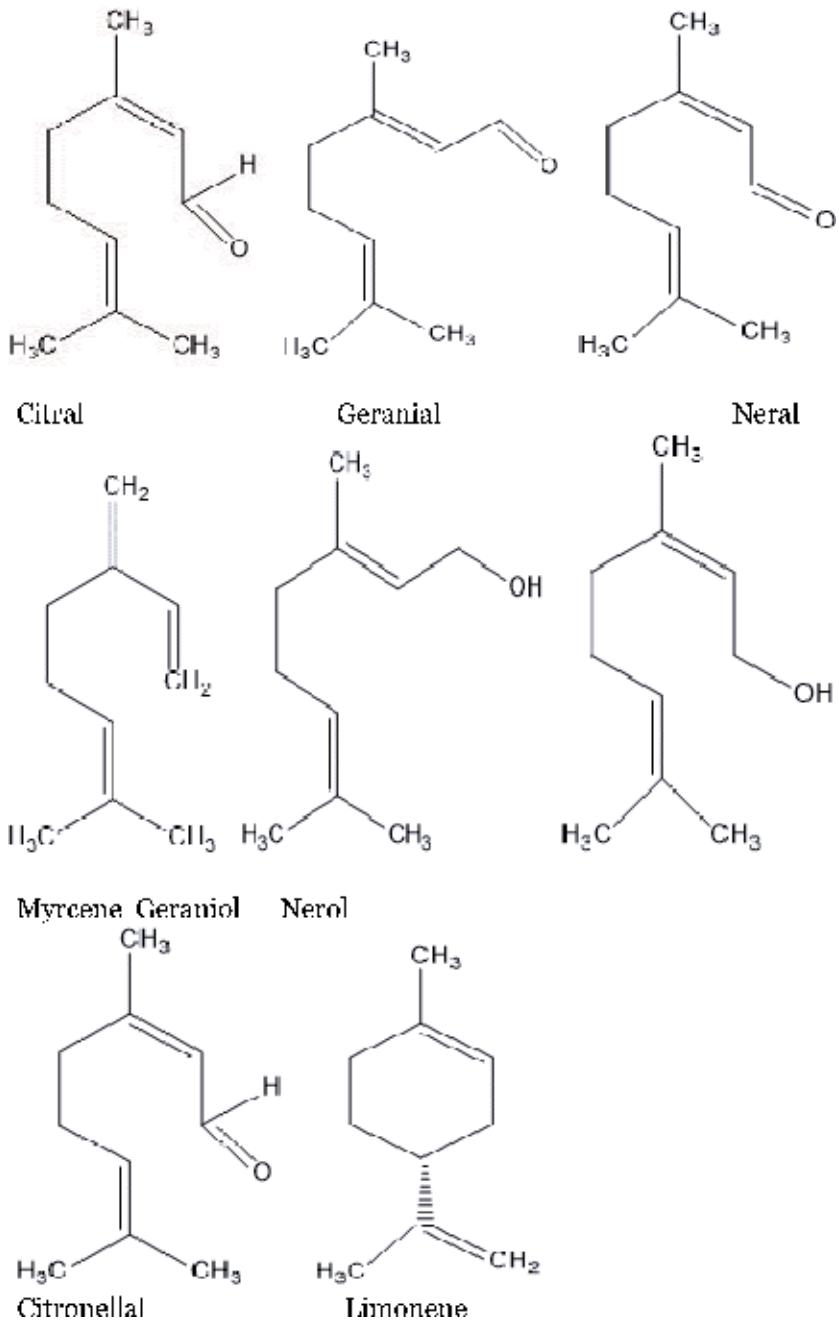

Figure 1.
Different classes of volatile oil.

6.1 Absorption through the skin

Volatile oil components are lipid soluble, so they have the ability to penetrate the membranes of the skin before being captured by the micro-circulation and drained into the systemic circulation, reaching all target organs [42, 43]. An example of this are the inflammatory disorders which are associated with pain, redness, and swelling, leading to loss of vital functions. Tea tree oil has been shown to increase monocytic differentiation in vitro and reduce inflammation, therefore assisting the healing of chronic wounds [44].

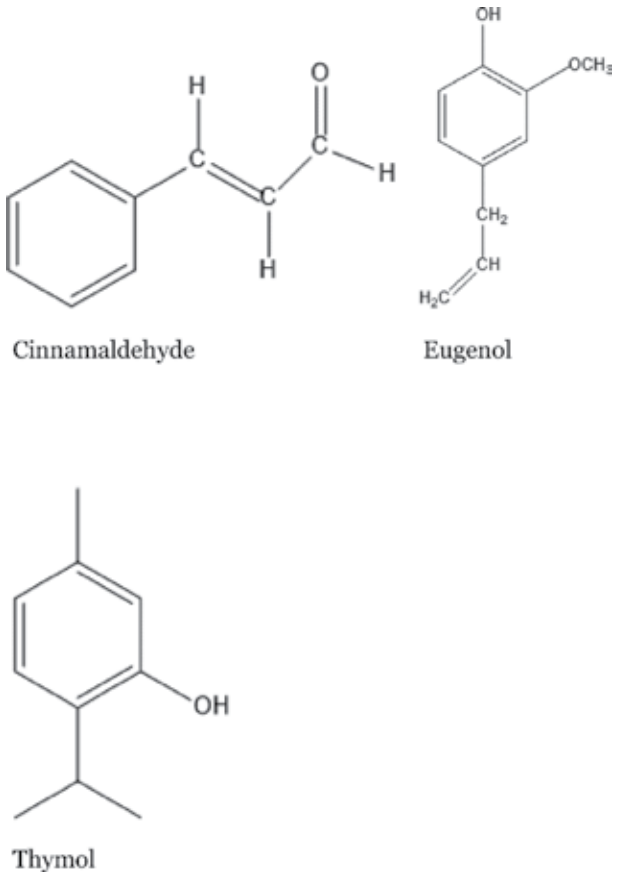

Figure 2.
Different classes of volatile oil.

6.2 Inhalation

Volatile oils enter the body through the respiratory system. Due to their volatile ability, they can be inhaled easily through the upper respiratory tract and enter the lungs, by which it can be spread to the blood stream. In general, the respiratory tract is considered to be the most easiest way of entry, followed by the dermal pathway [45]. Inhalation of essential oils has given rise to olfactory aromatherapy, where simple inhalation has resulted in enhanced emotional wellness, calmness, relaxation, or rejuvenation of the human body. The release of stress is welded with pleasurable scents which unlock odor memories. Essential oils are complemented to medical treatment and can never be taken as a replacement for it [46–48].

6.3 Ingestion

Oral ingestion of essential oils needs to be done carefully due to the possible toxicity of some oils. Ingested volatile oil compounds and/or their metabolites may then be absorbed and delivered to the rest of the body and then distributed to different organs. Once volatile oil are entered in to the body, they create their therapeutic effect through physiological functions (**Table 3**). For example, *Roman chamomile* is extensively used to relieve pain from physical conditions, menstrual cramps, and tension with its application on the lower abdomen [49–52].

No.	Name	Active compounds	Ref.	Biological activities	Ref.
1.	Chamomile essential oil (*Matricaria chamomilla* L.)	Bisabolol and chamazulene	[53, 54]	Anti-inflammatory Anti-allergic Anti-pruritic Decongestive Antispasmodic	[55–57]
2.	Anise essential oil (*Pimpinella anisum* L.)	Anethole	[58]	Antispasmodic 2-Emmenagogue Stomachic Carminative Diuretic	[59, 60]
3.	Nutmeg essential oil (*Myristica fragrans* Houtt.)	Sabinene, 4-terpineol, myristicin	[61]	Antimicrobial Pesticidal activity General tonic Antioxidant	[62, 63]
4.	Cedar essential oil (*Cedrus libani* (A. Rich.))	Limonene	[64]	Larvicidal Lymphotonic Powerful diuretic Regenerative blood Astringent Scalp tonic Antifungal	[65, 66]
5.	Garlic essential oil (*Allium sativum* L.)	Diallyl disulfide	[67, 68]	Protects and maintains the cardiovascular system Hypoglycemic Regulates blood pressure Antimicrobial	[67, 69, 70]
6.	Clove essential oil (*Syzygium aromaticus* L.)	Eugenol, eugenyl acetate	[71, 72]	Antiviral Antimicrobial Antifungal Aphrodisiac	[73, 74]
7.	Cinnamon essential oil (*Cinnamomum cassia* (Blume))	Cinnamaldehyde	[75, 76]	Powerful antibacterial Antiviral Antifungal	[77, 78]
8.	Eucalyptus essential oil *(Eucalyptus globulus* Labill.)	1,8-Cineole	[79, 80]	Anticatarrhal Expectorant and mucolytic Antimicrobial and antiviral	[81, 82]
9.	Peppermint essential oil *(Mentha piperita* L.*)*	Menthol and menthone	[83, 84]	Tonic and stimulant Decongestant Anesthetic and analgesic Antipruritic Expectorant	[85, 86]
10.	Lavender essential oil *(Lavandula officinalis* Chaix)	Linalool and linalyl acetate	[87, 88]	Antispasmodic Sedative Relaxing Analgesic and anti-inflammatory	[89, 90]
11.	Tea tree essential oil (*Melaleuca alternifolia* Cheel)	Terpinene-1-ol-4	[91]	Antimicrobial Antiviral Antiasthenic Neurotonic	[92–94]

No.	Name	Active compounds	Ref.	Biological activities	Ref.
				Decongestant Radioprotective	
12.	Lemon essential oil (*Citrus limonum*) (L.) Osbeck	Limonene	[95, 96]	Strengthen natural immunity Tonic nervous system Antimicrobial and antiviral	[97–99]

Table 3.
Therapeutic properties of some essential oils.

7. Conclusion

There is a significant and growing interest to find safe and effective methods of treatment. Aromatherapy is one of the most usable methods across the world. It has gained popularity due to its safety, easy accessibility, and effective effects. From previous data we can notice that essential oils have a lot of pharmacological effects and can help in the treatment of many diseases.

Author details

Amira Ahmed Kamal El-din El-Anssary
Pharmacognosy Department, Pharmaceutical Science Division, National Research Center, Giza, Egypt

*Address all correspondence to: amiraelanssary@yahoo.com

References

[1] Baris O, Güllüce M, Sahin F, Ozer H, Kılıc H, Ozkan H, et al. Biological activities of the volatile oil and methanol extract of *Achillea biebersteini* Afan. (Asteraceae). Turkish Journal of Biology. 2006;**30**:65-73

[2] Margaris N, Koedam A, Vokou D. Aromatic Plants: Basic and Applied Aspects. The Hague, London, Boston: Martinus Nijhoff Publishers; 1982

[3] Tisserand RB. In the Art of Aromatherapy. Rochester, VT: Healing Arts Press; 1997

[4] Shibamoto K, Mochizuki M, Kusuhara M. Aroma therapy in anti-aging medicine. Anti-Aging Medicine. 2010;**7**:55-59

[5] Burt S. Volatile oils: Their antibacterial properties and potential applications in foods. International Journal of Food Microbiology. 2004;**94**: 223-253

[6] Suaib L, Dwivedi GR, Darokar MP, Kaira A, Khanuja SPS. Potential of rosemary oil to be used in drug-resistant infection. Alternative Therapies. 2007; **13**:54-59

[7] Shirley Price's Aromatherapy Workbook. London, UK: Thorsons; 1993

[8] Lawless J. The Illustrated Encyclopedia of Essential Oils. Rockport, MA: Element Books, Inc.; 1995

[9] Saeidi K, Moosavi M, Lorigooini Z, Maggi F. Chemical characterization of the essential oil compositions and antioxidant activity from Iranian populations of *Achillea wilhelmsii* K. Koch. Industrial Crops and Products. 2018;**112**:274-280

[10] Ahmadi L, Mirza M, Shahmir F. The volatile constituents of Artemisia marschaliana Sprengel and its secretory elements. Flavour and Fragrance Journal. 2002;**17**:141-143

[11] Bezić N, Šamanić I, Dunkić V, Besendorfer V, Puizina J. Volatile oil composition and internal transcribed spacer (ITS) sequence variability of four south-Croatian *Satureja* species (Lamiaceae). Molecules. 2009;**14**:925-938

[12] Ciccarelli D, Garbari F, Pagni AM. The flower of *Myrtus communis* (Myrtaceae): Secretory structures, unicellular papillae, and their ecological role. Flora. 2008;**203**:85-93

[13] Gershenzon J. Metabolic costs of terpenoid accumulation in higher plants. Journal of Chemical Ecology. 1994;**20**:1281-1328

[14] Gupta V, Mittal P, Bansal P, Khokra SL, Kaushik D. Pharmacological potential of *Matricaria recutita*. International Journal of Pharmaceutical Sciences and Drug Research. 2010;**2**: 12-16

[15] Martín A, Varona S, Navarrete A, Cocero MJ. Encapsulation and co precipitation processes with supercritical fluids: Applications with volatile oils. The Open Chemical Engineering Journal. 2010;**4**:31-41

[16] Skold M, Karlberg AT, Matura M, Borje A. The fragrance chemical caryophyllene air oxidation and skin sensitization. Food and Chemical Toxicology. 2006;**44**:538-545

[17] Skold M, Hagvall L, Karlberg AT. Autoxidation of linalyl acetate, the main compound of lavender oil, creates potent contact allergens. Contact Dermatitis. 2008;**58**:9-14

[18] Bowles EJ. The Chemistry of Aromatherapeutic Oils. 3rd ed. Griffin: Press; 2003

[19] Surburg H, Panten J. In: Svoboda K, Hampson J, Hunter T, editors. Common Fragrance and Flavor Materials. Preparation, Properties and Uses. 5th ed. Weinheim: Wiley-VCH; 1999. p. 2006

[20] Miguel MG. Antioxidant and anti-inflammatory activities of volatile oils. Molecules. 2010;**15**:9252-9287

[21] Sell CS. The Chemistry of Fragrance. From Perfumer to Consumer. 2nd ed. Cambridge: The Royal Society of Chemistry; 2006. p. 329

[22] Skaltsa HD, Demetzos C, Lazari D, Sokovic M. Volatile oil analysis and antimicrobial activity of eight *Stachys* species from Greece. Phytochemistry. 2003;**64**:743-752

[23] Thormar H. Lipids and Volatile Oils as Antimicrobial Agents. Chichester: John Wiley and Sons; 2011

[24] Andrade EHA, Alves CN, Guimarães EF, Carreira LMM, Maia JGS. Variability in volatile oil composition of *Piper dilatatum* L.C. Rich. Biochemical Systematics and Ecology. 2011;**39**:669-675

[25] Zehra Küçükbay F, Kuyumcu E, Çelen S, Azaz AD, Arabac T. Chemical composition of the essential oils of three Thymus Taxa from Turkey with antimicrobial and antioxidant activities. Records of Natural Products. 2014;**8**: 110-120

[26] Pengelly A. The Constituents of Medicinal Plants: An Introduction of the Chemistry and Therapeutics of Herbal Medicine. Australia, Sydney: Allen and Unwin; 2004

[27] Sangwan NS, Farooqi AHA, Shabih F, Sangwan RS. Regulation of volatile oil production in plants. Plant Growth Regulation. 2001;**34**:3-21

[28] Baylac S, Racine P. Inhibition of 5-lipoxygenase by volatile oils and other natural fragrant extracts. International Journal of Aromatherapy. 2003;**13**: 138-142

[29] Delamare APL, Moschen-Pistorello IT, Artico L, Atti-Serafini L, Echeverrigaray S. Antibacterial activity of the volatile oils of *Salthrough officinalis* L. and *Salthrough triloba* L. cultivated in South Brazil. Food Chemistry. 2007;**100**:603-608

[30] Sivropoulou A, Nikolau C, Papanikolau E, Kokkini S, Lanaras T, Arsenakis M. Antimicrobial, cytotoxic, and antiviral activities of *Salthrough fruticosa* volatile oil. Journal of Agricultural and Food Chemistry. 1997; **45**:3197-3201

[31] Sivropoulou A, Papanikolaou E, Nikolau C, Kokkini S, Lanaras T, Arsenakis M. Antimicrobial and cytotoxic activities of origanum volatile oils. Journal of Agricultural and Food Chemistry. 1996;**44**:1202-1205

[32] Anwar F, Hussain AI, Sherazi STH, Bhanger MI. Changes in composition and antioxidant and antimicrobial activities of volatile oil of fennel (*Foeniculum vulgare* mill.) fruit at several, stages of maturity. Journal of Herbs, Spices and Medicinals Plants. 2009;**15**:1-16

[33] Celiktas OY, Kocabas EEH, Bedir E, Sukan FV, Ozek T, Baser KHC. Antimicrobial activities of methanol extracts and volatile oils of *Rosmarinus officinalis*, depending on location and seasonal variations. Food Chemistry. 2007;**100**:553-559

[34] Hammer KA, Carson CF, Dunstan JA, Hale J, Lehmann H, Robinson CJ, et al. Antimicrobial and anti-inflammatory activity of five *Taxandria fragrans* oils in vitro. Microbiology and Immunology. 2008; **52**:522-530

[35] Lawless J. The Illustrated Encyclopedia of Volatile Oils: The Complete Illustrated Guide to the Use of Oils in Aromatherapy and Herbalism. Shaftesbury, Dorset, UK: Element; 1995

[36] Verlet N. Huiles essentielles: Production mondiale, échanges internationaux et évolution des prix. Res. Mediterranea Magazine. 1994;**1**:4-9

[37] Hunter M. Volatile Oils: Art, Agriculture, Science, Industry and Entrepreneurship. New York: Nova Science Publishers, Inc.; 2009

[38] Gali-Muhtasib H, Hilan C, Khater C. Traditional uses of *Salthrough libanotica* (East Mediterranean sage) and the effects of its volatile oils. Journal of Ethnopharmacology. 2000;**71**:513-520

[39] De Sousa DP, Júnior GA, Andrade LN, Calasans FR, Nunes XP, Barbosa-Filho JM, et al. Structure and spasmolytic activity relationships of analogues found in many aromatic plants. Zeitschrift für Naturforschung. Section C. 2008;**63**:808-812

[40] De Sousa DP, Júnir GAS, Andrade LN, Batista JS. Spasmolytic activity of chiral monoterpene esters. Records of Natural Products. 2011;**5**: 117-122

[41] Elshafie HS, Camele I. An overview of the biological effects of some mediterranean essential oils on human health. Biomed Research International. (Review article). 2017:14. Article ID: 9268468. DOI: 10.1155/2017/9268468

[42] Adorjan B, Buchbauer G. Biological properties of volatile oils: An updated review. Flavour and Fragrance Journal. 2010;**25**:407-426

[43] Baser KHC, Buchbauer G. Handbook of Volatile Oils: Science, Technology, and Applications. NW: CRC Press; 2010

[44] Mart-nez-Pérez EF, Juárez ZN, Hernández LR, Bach H. Natural antispasmodics: Source, stereochemical configuration, and biological activity. (Review article). BioMed Research International. 2018:32. Article ID: 3819714. DOI: 10.1155/2018/3819714

[45] Moss M, Cook J, Wesnes K, Duckett P. Aromas of rosemary and lavender volatile oils several, ially affect cognition and mood in healthy adults. International Journal of Neuroscience. 2003;**113**:15-38

[46] Maxwell-Hudson C. Aromatherapy Massage Book Dorling. London: Kindersley; 1995

[47] Price S. Aromatherapy for Common Ailments. London: Fireside; 1991

[48] Price S. The Aromatherapy Workbook. London: Thorsons; 1993

[49] Buchbauer G. Molecular interaction: Biological effects and modes of action of volatile oils. International Journal of Aromatherapy. 1993;**5**:11-14

[50] Johnson AJ. Cognitive facilitation following intentional odor exposure. Sensors. 2011;**11**:5469-5488

[51] Wei A, Shibamoto T. Antioxidant/ lipoxygenase inhibitory activities and chemical compositions of selected volatile oil. Journal of Agricultural and Food Chemistry. 2010;**58**:7218-7225

[52] Lawless J. The Illustrated Encyclopedia of Essential Oils: The Complete Guide to the Use of Oils in Aromatherapy & Herbalism. Rockport: Element Books Ltd.; 1995

[53] Cemek M, Kaga S, Simsek N, Buyukokuroglu ME, Konuk M. Antihyperglycemic and antioxidative potential of *Matricaria chamomilla* L. in streptozotocin-induced diabetic rats. Journal of Natural Medicines. 2008;**62**: 284-293

[54] Kamatou GPP, Viljoen AM. A review of the application and pharmacological properties of -bisabolol and -bisabolol-rich oils. Journal of the American Oil Chemists' Society. 2010; **87**:1-7

[55] Jarić S, Kostić O, Mataruga Z, Pavlović D, Pavlović P. Traditional wound-healing plants used in theBalkan region (Southeast Europe). Journal of Ethnopharmacology. 2018;**211**:311-328

[56] Tolouee M, Alinezhad S, Saberi R, Eslamifar A, Zad SJ, Jaimand K, et al. Effect of *Matricaria chamomilla* L. flower volatile oil on the growth and ultrastructure of *Aspergillus nigervan* Tieghem. International Journal of Food Microbiology. 2010;**139**:127-133

[57] Shoara R, Hashempur MH, Ashraf A, Salehi A, Dehshahri S, Habibagahi Z. Efficacy and safety of topical *Matricaria chamomilla* L. (chamomile) oil for knee osteoarthritis: A randomized controlled clinical trial. Complementary Therapies in Clinical Practice. 2015;**21**:181-187

[58] El-Salam MA, Ammar NM, Yassin N, Ezzeldin N, Ziki E, El-Anssary AK, et al. A clinico-pharmacological assessment of a herbal preparation for treatment of bronchial asthma. World Journal of Medical Sciences. 2015;**12**: 115-124

[59] Mosaffa-Jahromi M, Lankarani KB, Pasalar M, Afsharypuor S. Efficacy and safety of enteric coated capsules of anise oil to treat irritable bowel syndrome. Journal of Ethnopharmacology. 2016; **194**:937-946

[60] Iannarelli R, Marinelli O, Morelli MB, Santoni G, Maggi F. Aniseed (*Pimpinella anisum* L.) essential oils reduces pro-inflammatory cytokines and stimulates mucus secretion in primary airway bronchial and tracheal epithelial cell lines. Industrial Crops and Products. 2018;**114**:81-86

[61] Muchtaridi, Subarnas A, Apriyantono A, Mustarichien R. Identification of compounds in the volatile oil of nutmeg seeds (*Myristica fragrans* Houtt.) that inhibit locomotor activity in mice. International Journal of Molecular Sciences. 2010;**11**: 4771-4781

[62] Tomaino A, Cimino F, Zimbalatti V, Venuti V, Sulfaro V, De Pasquale A, et al. Influence of heating on antioxidant activity and the chemical composition of some spice volatile oils. Food Chemistry. 2005;**89**:549-554

[63] Piaru SP, Mahmud R, Majid AMSA, Nassar ZDM. Antioxidant and antiangiogenic activities of the essential oils of *Myristica fragrans* and *Morinda citrifolia*. Asian Pacific Journal of Tropical Medicine. 2012;**5**:294-298

[64] Cetin H, Kurt Y, Isik K, Yanikoglu A. Larvicidal effect of *Cedrus libani* seed oils on mosquito *Culex pipiens*. Pharmaceutical Biology. 2009; **47**:665-668

[65] Dharmagadda VSS, Naik SN, Mittal PK, Vasudevan P. Larvicidal activity of *Tagetes patula* volatile oil against three mosquito species. Bioresource Technology. 2005;**96**: 1235-1240

[66] Kizil M, Kizil G, Yavuz M, Aytekin C. Antimicrobial activity of resins obtained from the roots and stems of *Cedrus libani* and *Abies Cilicia*. Applied Biochemistry and Microbiology. 2002;**38**:144-146

[67] Behbahani BAZ, Fooladi AAI. Evaluation of phytochemical analysis and antimicrobial activities *Allium* essential oil against the growth of some microbial pathogens. Microbial Pathogenesis. 2018;**114**:299-303

[68] Thomson M, Ali M. Garlic (*Allium sativum*): A review of its potential use as

an anti-cancer agent. Current Cancer Drug Targets. 2003;**3**:67-81

[69] Klevenhusen F, Zeitz JO, Duval S, Kreuzer M, Soliva CR. Garlic oil and its principal component diallyl disulfide fail to mitigate methane, but improve digestibility in sheep. Animal Feed Science and Technology. 2011;**166-167**: 356-363

[70] Bading Taika B, Bouckandou M, Souza A, Bourobou Bourobou HP, MacKenzie LS, Lione L. An overview of anti-diabetic plants used in Gabon: Pharmacology and toxicology. Journal of Ethnopharmacology. 2018;**216**:203-228

[71] Silva NCC, Fernandes JA. Biological properties of medicinal plants: A review of their antimicrobial activity. The Journal of Venomous Animals and Toxins Including Tropical Diseases. 2010;**16**:402-413

[72] Fichi G, Flamini G, Giovanelli F, Otranto D, Perrucci S. Efficacy of an volatile oil of *Eugenia caryophyllata* against *Psoroptes cuniculi*. Experimental Parasitology. 2007;**115**:168-172

[73] Koba K, Nenonene AY, Raynaud C, Chaumont JP, Sanda K. Antibacterial activities of the buds volatile oil of *Syzygium aromaticum* (L.) Merr. and Perry from Togo. Journal of Biologically Active Products from Nature. 2011;**1**: 42-51

[74] Machado M, Dinis AM, Salgueiro L, Custódio JBA, Cavaleiro C, Sousa MC. Anti-Giardia activity of *Syzygium aromaticum* volatile oil and eugenol: Effects on growth, throughbility, adherence and ultrastructure. Experimental Parasitology. 2011;**127**: 732-739

[75] Hseini S, Kahouadji A. Étude ethnobotanique de la flore médicinale dans la région de Rabat (Maroc occidental). Lazaroa. 2007;**28**: 79-93

[76] Vyawahare NS, Deshmukh VV, Gadkari MR, Kagathara VG. Plants with antiulcer activity. Pharmacognosy Reviews. 2009;**3**:118-125

[77] Geng S, Cui Z, Huang X, Chen Y, Xu D, Xiong P. Variations in volatile oil yield and composition during *Cinnamomum cassia* bark growth. Industrial Crops and Products. 2011;**33**: 248-252

[78] Cheng SS, Liu JY, Tsai KH, Chen WJ, Chang ST. Chemical composition and mosquito larvicidal activity of volatile oils from leaves of several, *Cinnamomum osmophloeum* provenances. Journal of Agricultural and Food Chemistry. 2004;**52**: 4395-4400

[79] Nerio LS, Olivero-Verbel J, Stashenko EE. Repellent activity of volatile oils from seven aromatic plants grown in Colombia against *Sitophilus zeamais* Motschulsky (Coleoptera). Journal of Stored Products Research. 2009;**45**:212-214

[80] Vilela GR, de Almeida GS, Moraes MHD, Brito JD, Da Silva MF, Silva SC, et al. Activity of volatile oil and its major compound, 1,8-cineole, from *Eucalyptus globulus* Labill., against the storage fungi *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare. Journal of Stored Products Research. 2009;**45**: 108-111

[81] Ben-Arye E, Dudai N, Eini A, Torem M, Schiff E, Rakover Y. Treatment of upper respiratory tract infections in primary care: A randomized study using aromatic herbs. Evidence-Based Complementary and Alternative Medicine. 2011:7. Article ID 690346

[82] Caballero-Gallardo K, Olivero-Verbel J, Stashenko EE. Repellent activity of volatile oils and some of their individual constituents against *Tribolium castaneum* Herbst. Journal of

Agricultural and Food Chemistry. 2011; **59**:1690-1696

[83] Alexopoulos A, Kimbaris AC, Plessas S, Mantzourani I, Theodoridou I, Stavropoulou E, et al. Antibacterial activities of volatile oils from eight Greek aromatic plants against clinical isolates of *Staphylococcus aureus*. Anaerobe. 2011;**17**(6):399-402

[84] Sala H. Aromatherapy: Current and emerging applications. Alternative and Complementary Therapies. 2011;**17**:26-31

[85] Sabzghabaee AM, Nili F, Ghannadi A, Eizadi-Mood N, Maryam AM. Role of menthol in treatment of candidial napkin dermatitis. World Journal of Pediatrics. 2011;**7**:167-170

[86] Kumar P, Mishra S, Malik A, Satya S. Insecticidal properties of *Mentha* species. Industrial Crops and Products. 2011;**34**:802-817

[87] Lee YL, Wu Y, Tsang WH, Leung AY, Cheung WM. A systematic review on the anxiolytic effects of aromatherapy in people with anxiety symptoms. The Journal of Alternative and Complementary Medicine. 2011;**17**: 101-108

[88] Hajhashemi V, Ghannadi A, Sharif B. Anti-inflammatory and analgesic properties of the leaf extracts and volatile oil of *Lavandula angustifolia* Mill. Journal of Ethnopharmacology. 2003;**89**:67-71

[89] Woronuk G, Demissie Z, Rheault M, Mahmoud S. Biosynthesis and therapeutic properties of *Lavandula* volatile oil constituents. Planta Medica. 2011;**77**:7-15

[90] Zuzarte M, Gonçalves MJ, Cavaleiro C, Canhoto J, Vale-Silva L, Silva MJ, et al. Chemical composition and antifungal activity of the volatile oils of *Lavandula viridis* L'Hér. Journal of Medical Microbiology. 2011;**60**: 612-618

[91] Van Vuuren SF, Suliman S, Viljoen AM. The antimicrobial activity of four commercial volatile oils in combination with conventional antimicrobials. Letters in Applied Microbiology. 2009;**48**:440-446

[92] Garozzo A, Timpanaro R, Bisignano B, Furneri PM, Bisignano G, Castro A. In vitro antiviral activity of *Melaleuca alternifolia*. Letters in Applied Microbiology. 2009;**49**:806-808

[93] Lobo R, Prabhu K, Shirwaikar A, Shirwaikar A, Ballal M. Formulation and evaluation of antiseptic activity of the herbal cream containing *Curcuma longa* and tea tree oil. Journal of Biologically Active Products from Nature. 2011;**1**:27-32

[94] Mickienė R, Bakutis B, Baliu Konienė V. Antimicrobial activity of two volatile oils. Annals of Agricultural and Environmental Medicine. 2011;**18**: 139-144

[95] Bacanlı PM, Başaran AA, Başaran N. The antioxidant and antigenotoxic properties of citrus phenolics limonene and naringin. Food and Chemical Toxicology. 2015;**81**:160-170

[96] Fisher K, Phillips C. Potential antimicrobial uses of volatile oils in food: Is citrus the answer. Trends in Food Science and Technology. 2008;**19**: 156-164

[97] Koul O, Walia S, Dhaliwal GS. Volatile oils as green pesticides: Potential and constraints. Biopesticides International. 2008;**4**:63-84

[98] Pavela R. Insecticidal properties of several volatile oils on the house fly (*Musca domestica* L.). Phytotherapy Research. 2008;**22**:274-278

[99] Pavela R. Insecticidal activity of some volatile oils against larvae of *Spodoptera littoralis*. Fitoterapia. 2005; **76**:691-696

Chapter 7

Volatile Compounds, Chemical Composition and Biological Activities of *Apis mellifera* Bee Propolis

Jorddy Neves Cruz, Adriane Gomes da Silva, Wanessa Almeida da Costa, Ely Simone Cajueiro Gurgel, Willison Eduardo Oliveira Campos, Renan Campos e Silva, Marcos Ene Chaves Oliveira, Antônio Pedro da Silva Souza Filho, Daniel Santiago Pereira, Sebastião Gomes Silva, Eloisa Helena de Aguiar Andrade and Mozaniel Santana de Oliveira

Abstract

Propolis is a wax-like resin collected by bees from tree shoots and/or other botanical sources that is used as glue to seal cracks or open spaces in the hive. Its color varies from green to brown and reddish, depending on its botanical origin. Among the substances that can be found in propolis, low molecular weight compounds, such as monoterpenes and sesquiterpenes are the most common. Several biological activities are attributed to these classes of substances, such as antifungal, antibacterial, and others. The objective of this work was to evaluate the chemical composition of volatile compounds present in propolis samples and to analyze their correlation with biological activities.

Keywords: essential oils, Africanized bees, bioactive compounds

1. Introduction

Propolis is formed by vegetable oils and resins, mixed with salivary secretions from bees, and may be in the form of isolated accumulations or combined with waxes. It is constituted by a complex mixture of various compounds and looks similar to a resinous wax collected by bees from tree shoots or other botanical sources. It is also used as glue to seal cracks or open spaces in the hive. Its color varies from green to brown and reddish, depending on its botanical origin and chemical composition. Bees can also use it to prevent diseases and parasites in the hive. In terms of chemical composition, it is generally composed of resin, wax, essential oils, phenolic acids, flavonoids, terpenes, aldehydes, alcohols, fatty acids, and phytosterols [1–4].

In this sense, propolis may represent a natural alternative in the search for bioactive compounds [5], since the use of secondary metabolites is increasing and represents a very broad field of research that can still be explored [6]. In addition, the wide variety of natural substances that can be found in organic matrices can provide key substances for the treatment of various pathologies [7]. The main substances present in propolis are low molecular weight, nonpolar, and volatile compounds [8].

The chemical composition of volatile substances present in propolis is very varied. Several compounds can be found, such as: nerolidol, α-pinene, β-pinene, cedrol, 3-methyl-2-buten-1-ol, octane, tricyclene, β-caryophyllene, spatulenol, δ-cadinene, selina-3,7(11)diene, nerolidol, benzenepropanoic acid, allyl benzyl ether, 1,8-epoxy-p-menth-2-ene, γ-terpinene, mentha-3(8),6-diene, cis-sabinol, 2,3-dehydro-1,8-cineole, α-copaene, p-ethylguaiacol, β-copaene, junipene, γ-cadinene, (3e)-6-phenyl-3-hexen-2-one, p-mentha-1(7),2-dien-8-ol, 4-terpineol, β-fenchyl alcohol, sabinene, δ-3-carene, limonene, α-thujene, α-terpinene, α-terpinolene, trans-verbenol, camphene, verbenene, o-cymene, and α-phellandrene. Moreover, geographical origin and seasonality may influence this composition [9, 10].

Authors have been studying volatile compounds and their applications [11–13] and have seen how these secondary metabolites can be promising in treating various diseases, such as neurodegenerative syndromes [14, 15] and infections caused by microorganisms [16, 17]. Considering the importance of the search for volatile substances present in propolis that may be beneficial for the maintenance of human health, this work aims to perform a literature review in order to address the main biological activities of these metabolites.

2. Main methods of essential oil (EO) extraction

EOs can be extracted from different plant parts and by different methodologies, which generally depend on the botanical material used, and may have a direct relation to the quality of the extracted oil. Therefore, choosing an inappropriate procedure can cause changes in its composition [18, 19]. EO extraction methods are divided into two categories: conventional methods and innovative methods. Traditional methods include hydrodistillation and steam distillation, and among the innovative ones, supercritical fluid extraction [20].

2.1 Hydrodistillation

Hydrodistillation (HD) is the most traditional, simple, and versatile technique used in the extraction of EOs [21, 22]. The basic principle of this type of extraction is azeotropic distillation (substances behave as if they were pure in relation to the boiling point), and to occur, a heating source, a container to place the vegetal biomass (for example, a volumetric flask), a condenser, and a decanter for collecting the oil and water mixture are necessary. HD is considered a multilateral method and, although simple, can be used in small or large industries because of its selectivity and low installation cost [20, 23, 24]. Hydrodistillation process is originated in alembics, however, since the third edition of the European pharmacopeia, its use along with the modified Clevenger system has been recommended, as this system enables the condensate recycling [20].

In HD, plant material, which can be any plant organ, is immersed in boiling water [19, 25]. In summary, the hydrodistillation system (**Figure 1**) consists of a container, usually a volumetric flask, which is connected to a Clevenger-type apparatus attached to a refrigeration system, with temperature ranging from 10 to

Figure 1.
Hydrodistillation system.

15°C. The solid-liquid mixture is heated, at atmospheric pressure, until it reaches water boiling temperature, allowing the odorous molecules to evaporate along with the water, forming an azeotropic mixture, which is drawn into the condenser, where it liquefies and is collected at the end of the extraction. Due to its hydrophobic character, the oil does not mix with water, so it can be separated by decantation. After separation, the oil is completely dehydrated using anhydrous Na_2SO_4 [19, 26, 27].

Hydrodistillation has some drawbacks that can qualitatively and quantitatively interfere in EOs, such as prolonged extraction time and chemical changes in terpene molecules, caused by hydrolysis and cyclization reactions. These are due to excessive contact time with water and loss of some polar molecules [20, 26].

2.2 Steam distillation

Steam distillation (SD) is another traditional method for EO extraction widely used for commercialization and can be used on laboratory and/or industrial scale for its simplicity and low cost, compared to other more sophisticated methods [28, 29]. The fundamental difference between steam distillation and hydrodistillation lies in the fact that, in SD, plant material is not in direct contact with water [20].

Steam distillation is divided into two basic types: direct (or wet) steam distillation and indirect steam distillation. In direct steam distillation (**Figure 2A**), the plant material is placed in a grid above the hot water, and steam passes through it. The leaves should be carefully distributed on the grid to allow uniform extraction and vaporization. Indirect steam distillation (**Figure 2B**) is the most common method for extraction of essential oils. In this process, no water is poured into the distillation tank. Instead, steam is directed to the tank from an external source. Volatiles are released from plant material when steam breaks the glands containing the oil molecules. From this stage, condensation and separation processes are the same [30].

Generally, the time in steam distillation extraction is reduced, which, together with the lack of contact between plant biomass and water, minimizes the chemical changes in EO's constituent molecules [20]. In addition, this technique is appreciated for generating high oil yield and being energy efficient [31].

2.3 Supercritical fluid extraction

Although traditional methods are still widely used in EO extraction, supercritical fluid extraction has become widespread as an alternative to conventional

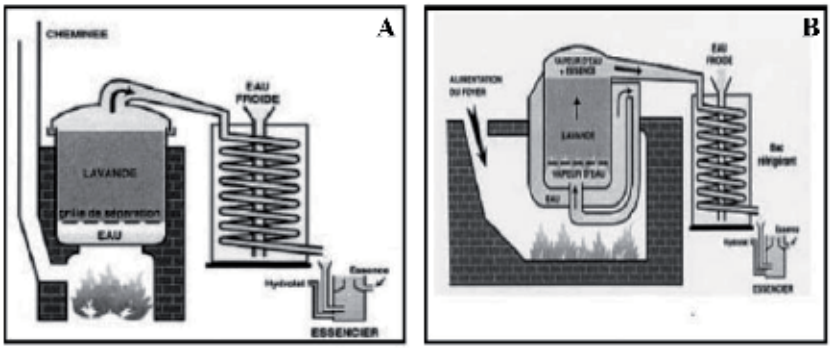

Figure 2.
Representative schemes of direct (wet) steam distillation (A) and indirect steam distillation (B) [20].

extraction methods [32, 33]. Supercritical fluid extraction arose from the need of new techniques that could minimize chemical changes and optimize extraction time [34]. This technique is considered an innovative "green" separation process to obtain natural products, such as EOs, and presents a prominent role in food and pharmaceutical industries [35, 36]. Among many possible supercritical fluids, CO_2 is the most widely used. Its critical point is reached at pressure of 72.9 atm and temperature of 31.2°C, which makes it not harmful to EO thermolabile molecules, thus preventing the chemical changes that occur in classic extraction processes [20, 36]. In addition, CO_2 is an inert gas, which means it is not reactive and can be eliminated simply by pressure decrease at the extractor outlet [20].

Carbon dioxide has characteristics that justify its use as supercritical fluid, such as low viscosity, high diffusivity, and density close to that of liquids [20]. Other factors related to CO_2 also help to understand the importance of using this gas as a supercritical fluid: non-toxicity, non-flammability, insipidity [20, 37], non-corrosivity, non-explosivity [35], great availability [36], and selectiveness [38]. It is also noteworthy that supercritical fluid extraction provides the purest EOs, as no trace of solvent remains after the end of the process, and no external substances are present in the extracted material [39].

In addition to providing a purer product, extraction using supercritical CO_2 is also more advantageous in relation to extraction time, as it is faster than conventional methods [35]. The low viscosity and high diffusivity of the supercritical fluid increase its penetration power based on the high mass transfer rate of solutes, allowing efficient extraction of compounds in the plant material. In addition, low viscosity contributes to lower fluid transport costs [36]. The efficiency of supercritical carbon dioxide extraction is due to the fact that it is a nonpolar solvent, similar to EO's constituents [35].

Despite being a very sophisticated, advantageous, and efficient method for the production of EOs, supercritical fluid extraction has some disadvantages regarding installation costs and equipment maintenance [20], besides high energy consumption to set pressure and temperature [26].

The supercritical fluid extraction system (**Figure 3**) is basically constituted by a carbon dioxide cylinder, cooling bath, high-pressure pump, oven, extraction container, vial, air compressor, flow meter, and flux control valves [35, 40, 41].

The process begins when the CO_2 contained in the cylinder is pumped into the cooling bath, in which it is liquefied and then pressurized by the high-pressure pump. The compressed CO_2 is then transferred to the main extraction cell, maintaining the required process temperature. These processes guarantee the ideal

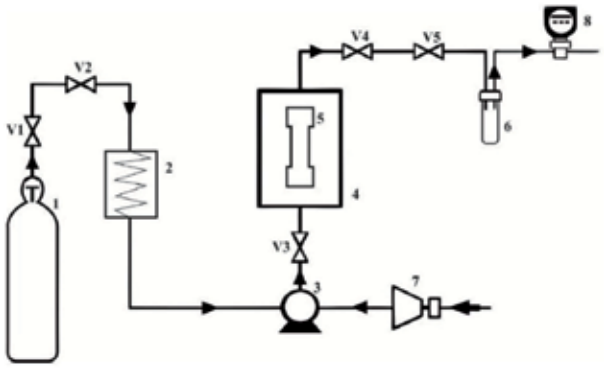

Figure 3.
Apparatus of supercritical fluid extraction of essential oils [40] (1) CO_2 tank; (2) cooling bath; (3) pump; (4) oven; (5) extraction container; (6) vial; (7) air compressor; (8) flow meter; V1-V5. Flux control valves.

thermodynamic conditions of the fluid that will pass through the material, thus extracting the essential oil.

In supercritical fluid extraction, there are two periods: the first is called static, at which the valve V4 is closed for about 30 minutes; then, the dynamic conditions are adjusted and V4 is opened, thus initiating the dynamic period and the extraction itself, because at this point, the essential oil begins to be poured into the collecting vial [35].

2.4 Factors that influence EO composition

Essential oils biosynthesized by aromatic plants can be directly influenced by multiple factors such as genetic, anthropic action, environmental conditions, geographical origin, circadian regime, seasonality, stage of development, and others [42–49].

Variability in content and composition of the EOs and other secondary metabolites is a way that the plant finds to better adapt to the exposed conditions, since the metabolic activity of the plants is a chemical interconnection between the plant and the environment it's inserted [47, 50].

The composition of EOs in plants of the same species that are living in the same place, but that have different chemical profiles, may be influenced by different genetic factors [51]. Other factors that significantly influence both quantitative and qualitative chemical variability of EOs are seasonal and circadian variations, which are related, respectively, to different periods of the year, and to day and night variations [52]. The chemical composition of the EO constituents obtained from the same plant organ may vary according to the species and extraction method used.

3. Chemical composition of *Apis mellifera* essential oil

Apis mellifera bees produce propolis by chewing resins collected from trees by adding salivary enzymes to them. The wax produced is used to cover hive failures, besides having antibacterial, antioxidant, antifungal, and antiviral activities, thus helping to protect the bees themselves.

Due to these properties, the extraction of propolis essential oil has gained prominence in the research field, being reported the presence of compounds such as terpenoids, alcohols, aldehydes, hydrocarbons, and aliphatic ketones in its chemical composition [53, 54]. And due to geographic factors, bee types, and

trees, volatile compounds of propolis essential oil have variable chemical composition [55, 56].

The volatile constituents of propolis are responsible for the pleasant aroma and contribute to its biological activity. These constituents may also play an important role as olfactory cues during resin collection by bees.

The chemical composition of propolis essential oil has already been studied, especially in Brazil. In the work of Albuquerque et al. [57], the chemical composition of propolis essential oil produced by *Apis mellifera* bees in Minas Gerais state was determined. Oliveira et al. [8], Kasumoto et al. [58], and Bancova et al. [10] also studied the chemical composition of propolis essential oil obtained in Brazil, in different regions, as can be seen in **Table 1**. The identification of each compound was performed by comparison with mass spectra and retention indices (RI).

In conducting the first study on propolis essential oil, Janas and Bumba [59] identified few constituents, such as benzoic acid, benzylic acid, vanillin, and eugenol. But later studies [60] show that the constituents of propolis essential oil are quite diverse, with variations in their polar constituents such as flavonoids, phenolic compounds, and phenolic acids, for example.

Frederica Pellati et al. [61] collected nine samples of propolis from *Apis mellifera* in different locations in Italy, extracted their essential oil through hydrodistillation, and identified them by gas chromatography coupled to mass spectroscopy and *headspace*. Then, 99 chemical components were identified.

Major compounds	Reference
(E)-nerolidol	[57]
β-caryophyllene	
Petrolatum 3.7 (11)-diene	
2,2-Dimethyl-8-renyl-6-vinylchromene	[54]
2,6-Diprenyl-4-vinylphenol	
Acetophenone	
Linalool	

Table 1.
Major volatile constituents of propolis in Brazil.

Country	Main compounds	Biological activity	Reference
Bulgaria	β-eudesmol (8.8%), δ-cadinene (5.3%), sesquiterpene alcohol (15.5%)	Antibacterial and antifungal	[62]
Turkey	Ethyl phenyl alcohol (7.7%), benzyl alcohol (7.4%), decanal (6.7%), ethyl benzoate (6.5%), nonanal (5%), cedrol (4.1%)	Antibacterial	[63]
Tunisia	α-Pinene (45.22%), cedrol (8.23%)	Antifungal	[10]
Brazil	Acetophenone (15.2%), nerolidol (13.3%), spatulenol (11.6%)	Antioxidant	[64]
India	Tricosane (13,6%), hexacosane (11.5%), palmitic acid (8.5%), linalool (6.7%), methyleugenol (6.0%)	Repellent activity against bees	[65]
Brazil	Longipinene (24.9%), α-eudesmol (6.9%)	Therapeutic effect	[66]

Table 2.
Main compounds and their biological activities in propolis.

Table 2 shows some important chemical constituents and their respective biological activities.

Geographic differences influence the chemical composition of essential oils extracted around the world, and as a result, these differences contribute significantly to the chemical properties and biological activities of all types of propolis. Its collection period also influences its oil composition, as it can be mixed with hive resins and wax.

In Venezuela [67], propolis essential oil produced by *Apis mellifera* had three main constituents: D-germacrene (26.5%), β-caryophyllene (10.2%), and β-elemene (8.1%), thus being similar to the chemical constituents of Brazilian propolis [64].

4. Biological activities of *Apis mellifera*

The main chemical compounds isolated from *Apis mellifera* are aliphatic acids and esters, aromatic acids and esters, sugars, alcohols, aldehydes, fatty acids, amino acids, steroids, ketones, chalcones and dihydrochalcones, flavonoids (flavones, flavonols, and flavonones), terpenoids, proteins, vitamins B1, B2, B6, C, E, as well as various minerals. Although flavonoids are the most studied components, they are not the only responsible for its pharmacological properties. Several other compounds have been related to the medicinal properties of *Apis mellifera* [68, 69].

There are reports attributing to *A. mellifera* the most varied applications in folk and veterinary medicine, which corroborates its great therapeutic potential, especially in relation to anti-inflammatory, antimicrobial, antineoplastic, antidiabetic, and antioxidant activities [70].

4.1 Anti-inflammatory activity

Amaral et al. [69] evaluated the anti-inflammatory potential of *Apis mellifera* against stomach inflammation induced in healthy adult female Wistar rats infected with *Helicobacter pylori*. This bacterium may cause chronic irritation and increase the risk of developing gastric ulcers. They concluded that the administration of solutions of *Apis mellifera* increases the endogen prostacyclin in rats mucosa, incrementing cytoprotection, and reducing pathogen population. In addition, the high contents of phenolic compounds and flavonoids aid in the protection of the mucin producing cells of the stomach, also contributing to its therapeutic potential.

4.2 Antimicrobial activity

Han et al. [71] evaluated the response of *Apis mellifera* venom (BV) against *acne vulgaris,* in order to prove its antimicrobial potential. *Acne vulgaris* is a chronic inflammatory disorder of the sebaceous follicles. The authors incubated *P. acnes,* clindamycin-resistant *P. acnes*, *Staphylococcus epidermidis*, and *Streptococcus pyogenes*. In their results, BV proved to be bacteriostatic and exhibited low cytotoxicity at 10 μg/ml in human epidermal keratinocytes and monocytes. The authors state that BV can be an alternative for the treatment of *acne vulgaris*.

4.3 Antineoplastic activity

There are several studies that report the antineoplastic activity of *Apis mellifera*. Lee et al. [71] evaluated the anticancer potential of *Apis mellifera* venom (BV), which showed cytotoxicity in HL-60 cells and normal human lymphocytes. Hamzaoglu et al. [71] implanted cancer cells into mice wounds. A significant decrease in the tumors was observed in mice that were treated with *Apis mellifera*

coating before and after surgery. This property may be due to its hypertonicity, acceleration of epithelization, low pH, and the presence of inhibin and catalase.

4.4 Antioxidant activity

In the work of Souza et al. [72], *A. mellifera* extracts, obtained by hydrodistillation, exhibited high antioxidant activity evaluated by free radical DPPH sequestration and β-carotene/linoleic acid methods. The authors linked these results to the presence of the following compounds: prenylated benzophenones, epinemorosone, xanthochymol, gambogenone, and aristophenone A. Wiwekowati et al. [73] also attributed the high antioxidant potential of *A. mellifera* to the structure of its flavonoids and phenolic acids, which was evaluated by inhibition of free radical DPPH.

4.5 Antihyperglycemic and antidiabetic activities

Cunha et al. [74] evaluated, *in vivo*, the control of postprandial hyperglycemia by performing a test of oral glucose tolerance in normoglycemic mice. After glucose overload, the mice treated with *A. mellifera* showed, after 30 min, reduced hyperglycemia peak and blood glucose values, as well as normalization of water intake. These results are similar to that showed by metformin, a first-line medication for the treatment of type 2 diabetes. Control of postprandial hyperglycemia has been linked with reduced vascular damage in diabetic patients.

5. Conclusions

Propolis essential oil presents various biological properties, being active against microorganisms such as bacteria, fungi, and viruses. It is evident that climatic factors are able to influence the chemical composition of the *Apis mellifera* propolis essential oil. In addition, the extraction technique chosen may also influence its yield and chemical composition.

Acknowledgements

Jorddy Neves Cruz appreciates the support of the Eliseu Alves Foundation (SAIC/Embrapa: 22500.18/0015-7).

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Author details

Jorddy Neves Cruz[1,2*], Adriane Gomes da Silva[2], Wanessa Almeida da Costa[3], Ely Simone Cajueiro Gurgel[4], Willison Eduardo Oliveira Campos[2], Renan Campos e Silva[3], Marcos Ene Chaves Oliveira[2], Antônio Pedro da Silva Souza Filho[2], Daniel Santiago Pereira[2], Sebastião Gomes Silva[1], Eloisa Helena de Aguiar Andrade[1] and Mozaniel Santana de Oliveira[1,4*]

1 Adolpho Ducke Laboratory, Emílio Goeldi Paraense Museum, Belém, PA, Brazil

2 Laboratory of Agro-Industry, Embrapa Eastern Amazon, Belém, Pará, Brazil

3 Program of Post-Graduation in Chemistry, Federal University of Para, Belém, PA, Brazil

4 Postgraduate Program in Biological Sciences - Tropical Botany, Federal Rural University of Amazonia and Emílio Goeldi Paraense Museum, Belém, PA, Brazil

*Address all correspondence to: jorddynevescruz@gmail.com and mozaniel.oliveira@yahoo.com.br

References

[1] Aygun A. Effects of Propolis on eggshell. In: Hester PY, editor. Egg Innov. Strateg. Improv. 1st ed. Cambridge, Massachusetts, EUA: Elsevier/Academic Press; 2017. pp. 145-156. DOI: 10.1016/B978-0-12-800879-9.00014-7

[2] Kocot J, Kiełczykowska M, Luchowska-Kocot D, Kurzepa J, Musik I. Antioxidant potential of propolis, bee pollen, and royal jelly: Possible medical application. Oxidative Medicine and Cellular Longevity. 2018;**2018**:1-29. DOI: 10.1155/2018/7074209

[3] Daffalla K, Mahmoud A. Propolis as a natural remedy. Journal of International Oral Health. 2016;**8**: 646-649. DOI: 10.2047/jioh-08-05-24

[4] Silva-Carvalho R, Baltazar F, Almeida-Aguiar C. Propolis: A complex natural product with a plethora of biological activities that can Be explored for drug development. Evidence-based Complementary and Alternative Medicine. 2015;**2015**:1-29. DOI: 10.1155/2015/206439

[5] Rufatto LC, dos Santos DA, Marinho F, Henriques JAP, Roesch Ely M, Moura S. Red propolis: Chemical composition and pharmacological activity. Asian Pacific Journal of Tropical Biomedicine. 2017;**7**:591-598. DOI: 10.1016/j.apjtb.2017.06.009

[6] Harvey AL, Edrada-Ebel R, Quinn RJ. The re-emergence of natural products for drug discovery in the genomics era. Nature Reviews. Drug Discovery. 2015;**14**:111-129. DOI: 10.1038/nrd4510

[7] Huang S, Zhang C-P, Wang K, Li G, Hu F-L. Recent advances in the chemical composition of propolis. Molecules. 2014;**19**:19610-19632. DOI: 10.3390/molecules191219610

[8] Miguel MG, Figueiredo AC. Propolis and geopropolis volatiles. In: Bee Products - Chemical and Biological Properties. Cham: Springer International Publishing; 2017. pp. 113-136. DOI: 10.1007/978-3-319-59689-1_6

[9] Bankova V, Popova M, Trusheva B. Propolis volatile compounds: Chemical diversity and biological activity: A review. Chemistry Central Journal. 2014;**8**:28. DOI: 10.1186/1752-153X-8-28

[10] Jihene A, Karoui IJ, Ameni A, Hammami M, Abderrabba M. Volatile compounds analysis of Tunisian propolis and its antifungal activity. Journal of Biosciences and Medicines. 2018;**06**: 115-131. DOI: 10.4236/jbm.2018.66009

[11] Pickett JA, Khan ZR. Plant volatile-mediated signalling and its application in agriculture: Successes and challenges. The New Phytologist. 2016;**212**:856-870. DOI: 10.1111/nph.14274

[12] Carvalho IT, Estevinho BN, Santos L. Application of microencapsulated essential oils in cosmetic and personal healthcare products - A review. International Journal of Cosmetic Science. 2016;**38**:109-119. DOI: 10.1111/ics.12232

[13] Gurgel ESC, de Oliveira MS, Souza MC, da Silva SG, de Mendonça MS, da Silva Souza Filhoe AP. Chemical compositions and herbicidal (phytotoxic) activity of essential oils of three Copaifera species (Leguminosae-Caesalpinoideae) from Amazon-Brazil. Industrial Crops and Products. 2019;**142**:111850. DOI: 10.1016/j.indcrop.2019.111850

[14] de Oliveira MS, da Cruz JN, Silva SG, da Costa WA, de Sousa SHB, Bezerra FWF, et al. Phytochemical profile, antioxidant activity, inhibition of acetylcholinesterase and interaction mechanism of the major components of the Piper divaricatum essential oil

obtained by supercritical CO_2. Journal of Supercritical Fluids. 2019;**145**:74-84. DOI: 10.1016/j.supflu.2018.12.003

[15] Silva SG, da Costa RA, de Oliveira MS, da Cruz JN, Figueiredo PLB, de Brasil DSB, et al. Chemical profile of Lippia thymoides, evaluation of the acetylcholinesterase inhibitory activity of its essential oil, and molecular docking and molecular dynamics simulations. PLoS One. 2019;**14**:e0213393. DOI: 10.1371/journal.pone.0213393

[16] Houdkova M, Rondevaldova J, Doskocil I, Kokoska L. Evaluation of antibacterial potential and toxicity of plant volatile compounds using new broth microdilution volatilization method and modified MTT assay. Fitoterapia. 2017;**118**:56-62. DOI: 10.1016/j.fitote.2017.02.008

[17] Rizzolo A, Bianchi G, Povolo M, Migliori CA, Contarini G, Pelizzola V, et al. Volatile compound composition and antioxidant activity of cooked ham slices packed in propolis-based active packaging. Food Packaging and Shelf Life. 2016;**8**:41-49. DOI: 10.1016/j.fpsl.2016.03.002

[18] Tongnuanchan P, Benjakul S. Essential oils: Extraction, bioactivities, and their uses for food preservation. Journal of Food Science. 2014;**79**:1231-1249. DOI: 10.1111/1750-3841.12492

[19] Aziz ZAA, Ahmad A, Setapar SHM, Karakucuk A, Azim MM, Lokhat D, et al. Essential oils: Extraction techniques, pharmaceutical and therapeutic potential - A review. Current Drug Metabolism. 2018;**19**:1100-1110. DOI: 10.2174/1389200219666180723144850

[20] El Asbahani A, Miladi K, Badri W, Sala M, Addi EHHA, Casabianca H, et al. Essential oils: From extraction to encapsulation. International Journal of Pharmaceutics. 2015;**483**:220-243. DOI: 10.1016/j.ijpharm.2014.12.069

[21] Azmir J, Zaidul ISM, Rahman MM, Sharif KM, Mohamed A, Sahena F, et al. Techniques for extraction of bioactive compounds from plant materials: A review. Journal of Food Engineering. 2013;**117**:426-436. DOI: 10.1016/j.jfoodeng.2013.01.014

[22] Jain SH, Ravikumar G. A brief review on essential oil extraction and equipment. Chemical Technology: An Indian Journal. 2010;**5**:19-24

[23] Moridi Farimani M, Mirzania F, Sonboli A, Moghaddam FM. Chemical composition and antibacterial activity of Dracocephalum kotschyi essential oil obtained by microwave extraction and hydrodistillation. International Journal of Food Properties. 2017;**20**:306-315. DOI: 10.1080/10942912.2017.1295987

[24] Rassem HHA, Nour AH, Yunus RM. Techniques for extraction of essential oils from plants: A review. Australian Journal of Basic and Applied Sciences. 2016;**10**:117-127

[25] Nora FMD, Borges CD. Ultrasound pretreatment as an alternative to improve essential oils extraction. Ciência Rural. 2017;**47**:1-9. DOI: 10.1590/0103-8478cr20170173

[26] Li Y, Fabiano-Tixier A-S, Chemat F. Essential Oils: From Conventional to Green Extraction. Cham: Springer International Publishing; 2014. pp. 9-20. DOI: 10.1007/978-3-319-08449-7_2

[27] Maia JGS, Andrade EHA. Database of the Amazon aromatic plants and their essential oils. Quimica Nova. 2009;**32**:595-622. DOI: 10.1590/S0100-40422009000300006

[28] Muhammad Z, Yusoff ZM, Nurhani K, Nordin MNN, Taib MN, Fazalul Rahiman MH, et al. Steam distillation with induction heating system: Analysis of kaffir lime oil compound and production yield at various temperatures. The Malaysian

Journal of Analytical Sciences. 2013;**17**:340-347

[29] Cassel E, Vargas RMF. Experiments and modeling of the Cymbopogon winterianus essential oil extraction by steam distillation article. Chemical Society. 2006;**50**:126-129

[30] Chemat F, Boutekedjiret C. Extraction // Steam Distillation. In: Reedijk J, editor. Reference Module in Chemistry, Molecular Sciences and Chemical Engineering. Amsterdam, Netherlands: Elsevier; 2015. pp. 1-12. DOI: 10.1016/b978-0-12-409547-2.11557-4

[31] Yadav AA, Chikate SS, Vilat RB, Suryawanshi MA, Student UG, Mumbai N, et al. Review on steam distillation: A promising Technology for Extraction of essential oil. International Journal of Advanced Research and Development. 2017;**4**:667-671. DOI: 10.21090/ijaerd.33095

[32] Bhusnure O, Gholve SB, Giram PS, Borsure VS, Jadhav PP, Satpute VV, et al. Importance of supercritical fluid extraction (SFE) in hair analysis. Indo American Journal of Pharmaceutical Research. 2015;**5**:3785-3801

[33] de Oliveira MS, da Cruz JN, Mitre GP, da Costa WA, da Silva Kataoka MS, Silva SG, et al. Antimicrobial, cytotoxic activity of the *Syzygium aromaticum* essential oil, molecular docking and dynamics molecular studies of its major chemical constituent. Journal of Computational and Theoretical Nanoscience. 2019;**16**:355-364. DOI: 10.1166/jctn.2019.8108

[34] Uquiche E, Cirano N, Millao S. Supercritical fluid extraction of essential oil from *Leptocarpha rivularis* using CO_2. Industrial Crops and Products. 2015;**77**:307-314. DOI: 10.1016/j.indcrop.2015.09.001

[35] Sodeifian G, Sajadian SA, Ardestani NS. Experimental optimization and mathematical modeling of the supercritical fluid extraction of essential oil from Eryngium billardieri: Application of simulated annealing (SA) algorithm. Journal of Supercritical Fluids. 2017;**127**:146-157. DOI: 10.1016/j.supflu.2017.04.007

[36] Sovilj MN, Nikolovski BG, Spasojević MD. Critical review of supercritical fluid extraction of selected spice plant materials. Macedonian Journal of Chemistry and Chemical Engineering. 2011;**30**:197-220

[37] Wrona O, Rafińska K, Możeński C, Buszewski B. Supercritical fluid extraction of bioactive compounds from plant materials. Journal of AOAC International. 2017;**100**:1624-1635. DOI: 10.5740/jaoacint.17-0232

[38] Reverchon E. Supercritical fluid extraction and fractionation related products. Drying Oils and Related Products. 1997;**10**:1-37

[39] Parhi R, Suresh P. Supercritical fluid technology: A review. Journal of Advanced Pharmaceutical Technology & Research. 2013;**1**:13-36. DOI: 10.14302/issn.2328-0182.japst-12-145

[40] de Oliveira MS, da Costa WA, Pereira DS, Botelho JRS, de Alencar Menezes TO, de Aguiar Andrade EH. Chemical composition and phytotoxic activity of clove (Syzygium aromaticum) essential oil obtained with supercritical CO_2. Journal of Supercritical Fluids. 2016;**118**:185-193. DOI: 10.1016/j.supflu.2016.08.010

[41] Sapkale GN, Patil SM, Surwase US, Bhatbhage PK. Supercritical fluid extraction. International Journal of Chemical Sciences. 2010;**8**:729-743. DOI: 10.1111/j.1467-8306.1965.tb00516.x

[42] Stashenko EE, Martínez JR, Ruíz CA, Arias G, Durán C, Salgar W, et al. Lippia origanoides chemotype

differentiation based on essential oil GC-MS and principal component analysis. Journal of Separation Science. 2010;**33**:93-103. DOI: 10.1002/jssc.200900452

[43] Maia JGS, Taveira FSN, Andrade EHA, da Silva MHL, Zoghbi M. Essential oils of Lippia grandis Schau. Flavour and Fragrance Journal. 2003;**18**:417-420. DOI: 10.1002/ffj.1241

[44] Maia JGS, da Silva MHL, Andrade EHA, Carreira LMM. Essential oil variation in Lippia glandulosa Schauer. Journal of Essential Oil Research. 2005;**17**:676-680. DOI: 10.1080/10412905.2005.9699030

[45] Raposo JDA, Figueiredo PLB, Santana RL, da Silva AQ Jr, Suemitsu C, da Silva R, et al. Seasonal and circadian study of the essential oil of Myrcia sylvatica (G. Mey) DC., a valuable aromatic species occurring in the lower Amazon River region. Biochemical Systematics and Ecology. 2018;**79**:21-29. DOI: 10.1016/j.bse.2018.04.017

[46] Abdel-Hameed ESS, Salman MS, Fadl MA, Elkhateeb A, Hassan MM. Chemical composition and biological activity of *Mentha longifolia* L. essential oil growing in Taif, KSA extracted by hydrodistillation, solvent free microwave and microwave hydrodistillation. Journal of Essential Oil-Bearing Plants. 2018;**21**:1-14. DOI: 10.1080/0972060X.2018.1454343

[47] Gobbo-Neto L, Lopes NP. Plantas medicinais: Fatores de influência no conteúdo de metabólitos secundários. Quimica Nova. 2007;**30**:374-381. DOI: 10.1590/S0100-40422007000200026

[48] Hussain AI, Anwar F, Hussain Sherazi ST, Przybylski R. Chemical composition, antioxidant and antimicrobial activities of basil (Ocimum basilicum) essential oils depends on seasonal variations. Food Chemistry. 2008;**108**:986-995. DOI: 10.1016/j.foodchem.2007.12.010

[49] Moghaddam M, Mehdizadeh L. Chemistry of essential oils and factors influencing their constituents. In: Soft Chemistry and Food Fermentation. Elsevier; 2017. pp. 379-419. DOI: 10.1016/B978-0-12-811412-4.00013-8

[50] Castelo AVM, Del Menezzi CHS, Resck IS. Seasonal variation in the yield and the chemical composition of essential oils from two Brazilian native Arbustive species. Journal of Applied Sciences. 2012;**12**:753-760. DOI: 10.3923/jas.2012.753.760

[51] Liber Z, Carović-Stanko K, Politeo O, Strikić F, Kolak I, Milos M, et al. Chemical characterization and genetic relationships among *Ocimum basilicum* L. cultivars. Chemistry & Biodiversity. 2011;**8**:1978-1989. DOI: 10.1002/cbdv.201100039

[52] Brant S, Eduardo J, Pereira B, Kelly S, Bertolucci V, Silva A, et al. Teores do óleo essencial de cidrão [Aloysia triphylla (L'Hérit) Britton (Verbenaceae)] em diferentes horários de colheita e processamentos pós-colheita. Ciência e Agrotecnologia. 2009:2065-2068

[53] Pino JA, Marbot R, Delgado A, Zumárraga C, Sauri E. Volatile constituents of propolis from honey bees and stingless bees from yucatán. Journal of Essential Oil Research. 2006;**18**:53-56. DOI: 10.1080/10412905.2006.9699384

[54] Oliveira AP, França HS, Kuster RM, Teixeira LA, Rocha LM. Chemical composition and antibacterial activity of Brazilian propolis essential oil. Journal of Venomous Animals and Toxins Including Tropical Diseases. 2010;**16**:121-130. DOI: 10.1590/S1678-91992010005000007

[55] Anjum SI, Ullah A, Khan KA, Attaullah M, Khan H, Ali H, et al.

Composition and functional properties of propolis (bee glue): A review. Saudi Journal of Biological Sciences. 2019;**26**:1695-1703. DOI: 10.1016/j.sjbs.2018.08.013

[56] Lavinas FC, Macedo EHBC, Sá GBL, Amaral ACF, Silva JRA, Azevedo MMB, et al. Brazilian stingless bee propolis and geopropolis: Promising sources of biologically active compounds. The Brazilian Journal of Pharmacognosy. 2019;**29**:389-399. DOI: 10.1016/j.bjp.2018.11.007

[57] de Albuquerque IL, Alves LA, Lemos TLG, Dorneles CA, de Morais MO. Constituents of the essential oil of brazilian green propolis from Brazil. Journal of Essential Oil Research. 2008;**20**:414-415. DOI: 10.1080/10412905.2008.9700044

[58] Kusumoto T, Miyamoto T, Higuchi R, Doi S, Sugimoto H, Yamada H. Isolation and structures of two new compounds from the essential oil of Brazilian propolis. Chemical & Pharmaceutical Bulletin. 2001;**49**:1207-1209. DOI: 10.1248/cpb.49.1207

[59] Janes K, Bumba V. Composition of bee glue (propolis). Die Pharmazie. 1974;**29**:544-545

[60] Bankova VS, de Castro SL, Marcucci MC. Propolis: Recent advances in chemistry and plant origin. Apidologie. 2000;**31**:3-15. DOI: 10.1051/apido:2000102

[61] Pellati F, Prencipe FP, Benvenuti S. Headspace solid-phase microextraction-gas chromatography-mass spectrometry characterization of propolis volatile compounds. Journal of Pharmaceutical and Biomedical Analysis. 2013;**84**:103-111. DOI: 10.1016/j.jpba.2013.05.045

[62] Kujumgiev A, Tsvetkova I, Serkedjieva Y, Bankova V, Christov R, Popov S. Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. Journal of Ethnopharmacology. 1999;**64**:235-240. DOI: 10.1016/S0378-8741(98)00131-7

[63] Betul D, Atac U, Fatih D. Volatile composition of Anatolian propolis by headspace-solid-phase microextraction (HS-SPME), antimicrobial activity against food contaminants and antioxidant activity. Journal of Medicinal Plants Research. 2013;**7**:2140-2149. DOI: 10.5897/jmpr2013.4470

[64] Sena-Lopes Â, Bezerra FSB, das Neves RN, de Pinho RB, de Silva MTO, Savegnago L, et al. Chemical composition, immunostimulatory, cytotoxic and antiparasitic activities of the essential oil from Brazilian red propolis. PLoS One. 2018;**13**:e0191797. DOI: 10.1371/journal.pone.0191797

[65] Naik DG, Vaidya HS, Namjoshi TP. Essential oil of Indian propolis: Chemical composition and repellency against the honeybee *Apis florea*. Chemistry & Biodiversity. 2013;**10**:649-657. DOI: 10.1002/cbdv.201200165

[66] Li YJ, Xuan HZ, Shou QY, Zhan ZG, Lu X, Hu FL. Therapeutic effects of propolis essential oil on anxiety of restraint-stressed mice. Human & Experimental Toxicology. 2012;**31**:157-165. DOI: 10.1177/0960327111412805

[67] Rios N, Yánez C, Rojas L, Mora F, Usubillaga A, Vit P. Chemical composition of essential oil of *Apis mellifera* propolis from Falcón state, Venezuela. Emirates Journal of Food and Agriculture. 2014;**26**:639-642. DOI: 10.9755/ejfa.v26i7.18198

[68] Awale S, Shrestha SP, Tezuka Y, Ueda JY, Matsushige K, Kadota S. Neoflavonoids and related constituents from nepalese propolis and their nitric oxide production inhibitory activity. Journal of Natural Products. 2005;**68**:858-864. DOI: 10.1021/np050009k

[69] Amaral TY, Padilha IG, Presídio GA, da Silveira EAAS, Duarte AWF, Barbosa APF, et al. Antimicrobial and anti-inflammatory activities of *Apis mellifera* honey on the helicobacter pylori infection of wistar rats gastric mucosa. Food Science and Technology. 2017;**37**:34-41. DOI: 10.1590/1678-457X.31016

[70] Alves RRN, Neta ROS, Trovão DMBM, Barbosa JEL, Barros AT, Dias TLP. Traditional uses of medicinal animals in the semi-arid region of northeastern Brazil. Journal of Ethnobiology and Ethnomedicine. 2012;**8**:34-36. DOI: 10.1186/1746-4269-8-41

[71] Han SM, Lee KG, Yeo JH, Baek HJ, Park K. Antibacterial and anti-inflammatory effects of honeybee (*Apis mellifera*) venom against acne-inducing bacteria. Journal of Medicinal Plants Research. 2010;**4**:459-464. DOI: 10.5897/JMPR09.427

[72] de Souza ECA, da Silva EJG, Cordeiro HKC, Lage Filho NM, da Silva FMA, dos Reis DLS, et al. Chemical compositions and antioxidant and antimicrobial activities of propolis produced by frieseomelitta longipes and *Apis mellifera* BEES. Quimica Nova. 2018;**41**:485-491. DOI: 10.21577/0100-4042.20170208

[73] Wiwekowati, Astawa P, Jawi IM, Sabir A. Antioxidant activity of *Apis mellifera* sp. Propolis extract from Java (Indonesia). International Research Journal of Engineering, IT and Scientific Research. 2017;**3**:18. DOI: 10.21744/irjeis.v3i5.530

[74] da Cunha JSM, Alfredo TM, dos Santos JM, Junior VVA, Rabelo LA, Lima ES, et al. Antioxidant, antihyperglycemic, and antidiabetic activity of *Apis mellifera* bee tea. PLoS One. 2018;**13**:1-17. DOI: 10.1371/journal.pone.0197071

Section 3

Antimicrobial Activity of Essential Oils

Chapter 8

Chemical Composition and Antibacterial Activity of the Essential Oil of *Mesosphaerum suaveolens* (Lamiaceae)

José Weverton Almeida Bezerra, Felicidade Caroline Rodrigues, Ma Aparecida Barbosa Ferreira Gonçalo, Marcos Aurélio Figuereido dos Santos, Gledson Ferreira Macedo, Janete de Souza Bezerra, Priscilla Augusta de Sousa Fernandes, Emanoel Messias Pereira Fernando, Carlos Henrique Silva de Oliveira, Viviane Bezerra da Silva, Isabella Hevily Silva Torquato, Niwiarakelly da Silva Monte, Luciano Temoteo dos Santos and Henrique Douglas Melo Coutinho

Abstract

Mesosphaerum suaveolens (Lamiaceae) is a medicinal plant commonly used in Brazil for the treatment of diseases related to the digestive tract and respiratory diseases, so we hypothesized that the essential oil of this species may have antibacterial activity. Thus, we aimed to evaluate the *in vitro* antibacterial and modulatory activity of the essential oil of *M. suaveolens* as well as to characterize its chemical composition. The identification of the constituents was performed by gas chromatography-flame ionization detector (GC-FID) and the antibacterial and modulating activity by the plate microdilution method. We found the oil had sesquiterpene β-caryophyllene as the major component. This compound may account for the antibacterial activity against *Staphylococcus aureus* strains, since the essential oil had a MIC of 64 μg/mL for the standard strain and 256 μg/mL for the multiresistant strain, demonstrated that the oil does not exhibit drug modulating activity. Thus, *M. suaveolens* oil has bioactive compounds which can be used in the preparation of drugs.

Keywords: bacteria, *Hyptis suaveolens*, bamburral, β-caryophyllene, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*

1. Introduction

Bacterial infections are major problems in medicine due to the indiscriminate use of antibiotics that eventually select resistant microorganisms, which in turn proliferate [1]. Among the bacteria that cause infections stand out *Pseudomonas aeruginosa* (Pseudomonadaceae), *Escherichia coli* (Enterobacteriaceae) and *Staphylococcus aureus* (Staphylococcaceae) [2].

The bacterium, *P. aeruginosa*, is a gram-negative bacterium that is responsible for causing hospital infections, especially in patients who have compromised immune systems, and in rarer cases, it can lead to pneumonia, resulting in the death of 60% of infected [3, 4]. Although strains of *E. coli* colonize the human digestive tract, in large quantities they are capable of causing intestinal problems such as diarrhea. While *S. aureus* causes several acute infections such as pneumonia, osteomyelitis, endocarditis, myocarditis, pericarditis, and meningitis [1].

It has been reported that the mechanisms of bacterial resistance include the efflux pumps, which expel the antibiotic, in addition, the bacteria are capable of altering the target of the antibiotic for mutation or enzymatic inactivation and alteration of the permeability of the bacterium to the drug [5]. Thus, antibiotics alone cannot inhibit bacterial growth so that substances that modulate their effect are necessary in order to potentiate the action of the drug [6, 7].

These substances capable of modulating standard drugs can be found in plants, since these have constituents with antibacterial actions derived from their secondary metabolism, mainly the aromatic herbs, because their essential oils have diverse biological and pharmacological activities [8, 9]. Among the botanical families most

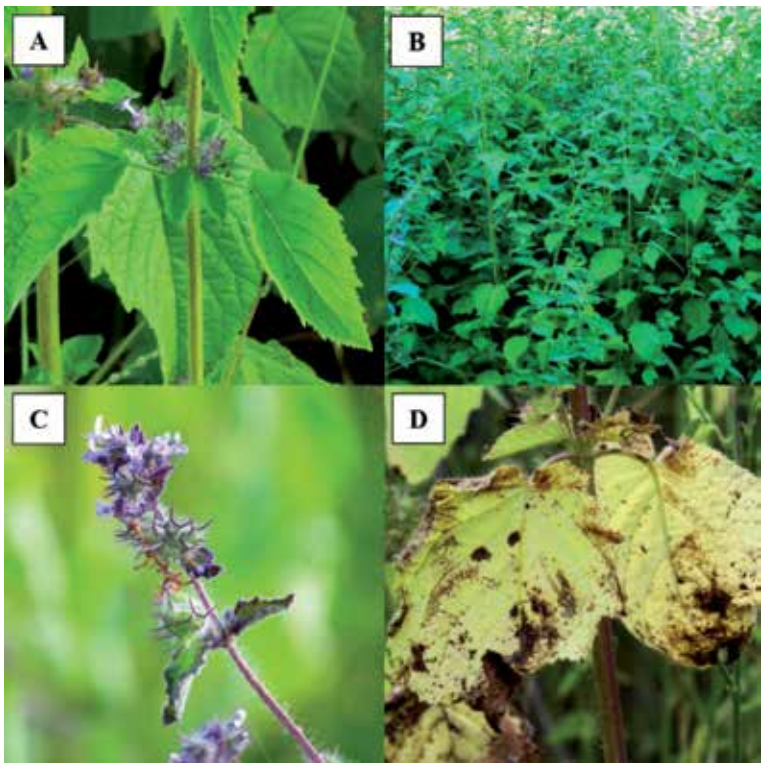

Figure 1.
Mesosphaerum suaveolens. *(a) Leaves and stem. (b) Population of* M. suaveolens *in Quixelô—CE, Brazil. (c) Highlight of flowers. (d) Leaves in senescence.*

rich in aromatic plants is Lamiaceae, which is well known for its representatives as sources of essential oils used in cooking, aromatherapy and medicine [10, 11]. Among the species of this family, the species *Mesosphaerum suaveolens* (L.) Kuntze (**Figure 1**), known in Brazil as "bamburral" and "alfazema-brava," is popularly used in the treatment of diseases related to gastrointestinal and respiratory tract [12], so that we hypothesize that the species is abundant in phytochemical constituents, which present biological activity against strains of pathogenic microorganisms, such as bacteria. This hypothesis is supported by the pharmacological and biological activities of these species already evidenced in the literature, such as antioxidant activity [13], neuroprotective [14], gastro-protective [15], antitumor [16], antinociceptive [17], anti-inflammatory [18], antifungal [19], anti-bacterial [20], insecticide [21], larvicide [8], and allelopathic action [22].

Thus, due to increasing bacterial resistance to drugs and the search for new bioactive sources, this research aims to evaluate the *in vitro* antibacterial and modulatory activity of *M. suaveolens* essential oil as well as to characterize its chemical compounds.

2. Results

2.1 Chemical composition of essential oil

The essential oil of *M. suaveolens* presented a total of 44 phyto-constituents, with β-caryophyllene (20.37%) being the major constituent (**Figure 2**). Following this, the oil presented as secondary compounds were sabinene (15.94%) and espatulenol (11.09%). As constituents traces (<1%), 26 constituents were found (**Table 1**).

2.2 Minimal inhibitory concentration (MIC)

According to **Table 2**, the essential oil of *M. suaveolens* showed no activity against gram-negative bacteria (*P. aeruginosa* and *E. coli*), both standard strains and multiresistant strains, since they have MIC ≥1024 μg/mL. However, the oil presented antibacterial action against *S. aureus* with MIC of 64 μg/mL for the standard strain (ATCC) and 256 μg/mL for the multiresistant strain.

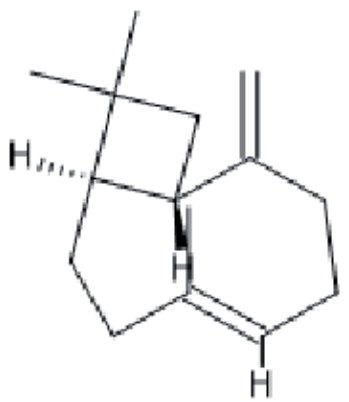

Figure 2.
Chemical structure of sesquiterpene β-caryophyllene.

Compounds	Molecular formula	RI[a]	RI[b]	Oil
				%
α-Thujene	$C_{10}H_{16}$	989	931	1.09
Sabinene	$C_{10}H_{16}$	976	976	15.94
β-Pinene	$C_{10}H_{16}$	980	980	2.01
α-Phellandrene	$C_{10}H_{16}$	1006	1005	1.38
α-Terpinene	$C_{10}H_{16}$	1019	1018	1.05
Limonene	$C_{10}H_{16}$	1031	1031	5.19
1-8-Cineole	$C_{10}H_{18}O$	1037	1033	3.04
γ-Terpinene	$C_{10}H_{16}$	1060	1061	2.47
Terpinen-4-ol	$C_{10}H_{18}O$	1178	1177	6.62
δ-Elemene	$C_{15}H_{24}$	1335	1338	1.17
β-Caryophyllene	$C_{15}H_{24}$	1421	1418	20.37
γ-elemene	$C_{15}H_{24}$	1435	1433	1.04
α-humulene	$C_{15}H_{24}$	1453	1454	1.17
Germacrene D	$C_{15}H_{24}$	1481	1480	5.21
Bicyclogermacrene	$C_{15}H_{24}$	1501	1488	7.02
Spathulenol	$C_{15}H_{24}O$	1576	1576	11.09
Caryophyllene oxide	$C_{15}H_{24}O$	1580	1581	3.18
Cubenol	$C_{15}H_{26}O$	1641	1642	1.07
Monoterpene hydrocarbons	**$C_{10}H_n$**			**29.13**
Sesquiterpene hydrocarbons	**$C_{15}H_n$**			**35.98**
Phenylpropanoids	**$C_nH_nO_n$**			**25.00**
Total identified (%)				**90.11**

Relative proportions of the essential oil constituents were expressed as percentages.
[a]Retention indices experimental (based on homologous series of n-alkane C_7-C_{30}).
[b]Retention indices from literature.

Table 1.
Main constituents (>1%) of Mesosphaerum suaveolens *essential oil.*

Strains	*Pseudomonas aeruginosa*	*Escherichia coli*	*Staphylococcus aureus*
Strains standards (ATCC)	≥1024	≥1024	64
Multi-resistant Strains	≥1024	≥1024	256

Table 2.
Minimal inhibitory concentration (μg/mL) of essential oil of Mesosphaerum suaveolens *against conventional bacterial (ATCC) and multiresistant strains.*

2.3 Modulation of drugs

According to **Figure 3**, it was demonstrated that the essential oil of *M. suaveolens* does not have the capacity to modulate the antibiotics, gentamicin, imipinem, and norfloxacin, since there was no significant difference between the control group and the treatments.

3. Discussion

Although the leaves of *M. suaveolens* are used in folk medicine for the treatment of diseases related to the gastrointestinal and respiratory tract [12], it has been demonstrated that the volatile terpenes of the species are not related to this action, since in the in the present study, this product did not present antibacterial action at concentrations of clinical relevance for two of the three strains used [23].

However, it is possible to observe that there is antibacterial action against the standard strains of *S. aureus* multiresistants. This can be explained by the mechanisms of action that some natural products have, such as the ability to disintegrate their cytoplasmic membranes, as well as destabilization of the proton motive force, electron flow, active transport and cellular content coagulation [24]. In addition, activity against *S. aureus* can be linked to the major compound of the study oil, β-caryophyllene, since this sesquiterpene exhibits antibacterial activity, especially against Gram-positive bacteria [25].

Thus, the oil has a source of β-caryophyllene, such sesquiterpene is found to be the majority compound; however, the oil of this species shows heterogeneity according to internal (genetic) and external factors (origin, mode of collection, collection period, etc.) [26]. To avoid large variations in the chemical composition of the oil, the collections should be standardized, such as collection times, period of the year, as well as to identify if the individual is under herbivorous attack [8].

This variation in the essential oil explains why some works show the antibacterial action of the essential oil, as Tesch et al. [27], where the oil showed activity against *E. coli* ATCC 25922 (MIC 350 μL/mL), *Klebsiella pneumoniae* ATCC 23357 (MIC 300 μL/mL), *Salmonella* Typhi CDC57 (MIC 400 μL/mL). In this study, the natural product presented eucalyptol ($C_{10}H_{18}O$) and fenchone ($C_{10}H_{16}O$) as the main compounds.

In addition to antimicrobial activities, the products of plant origin can have a drug modulating action, and although *M. suaveolens* does not present such action, it is seen that in members of the Lamiaceae family, some species present such action. Among the species is *Origanum vulgare* L., where its essential oil has a modulating action of the tetracycline drug against bacterial strains of *S. aureus* IS-58, which had the TetK tetracycline efflux protein [28].

4. Materials and methods

4.1 Collection of botanical material

Fresh leaves of *M. suaveolens* were collected in the municipality of Quixelô located in the state of Ceará (Brazil) under coordinates −6°14′22.40″S, −39°16′14.29″W in March 2015 (**Figure 4**). The collection area is characterized as being part of the Caatinga, a seasonally dry tropical forest. The leaves were dried in an oven at 30°C. The plant material was identified and a voucher specimen was deposited in the Herbarium Caririense Dárdano of Andrade-Lima – HCDAL under #12.104.

4.2 Extraction of essential oil

After drying, the leaves were packed in a volumetric flask containing 4 L of distilled water and subjected to constant boiling for 2 hours. Then the essential oil was collected and stored in an amber bottle under constant refrigeration until the conduction of the chemical analyzes and microbiological tests [8].

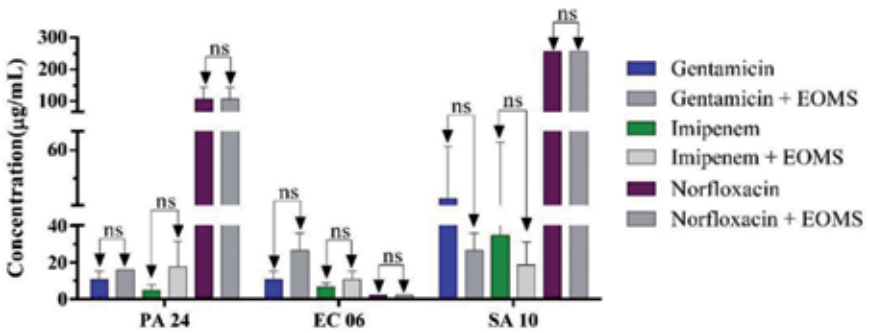

Figure 3.
Minimum inhibitory concentration of antibiotic modulation in combination with essential oil of Mesosphaerum suaveolens. *PA 24,* Pseudomonas aeruginosa *24; EC 06,* Escherichia coli *06; SA 10,* Staphylococcus aureus *10; EOMS, essential oil of* Mesosphaerum suaveolens.

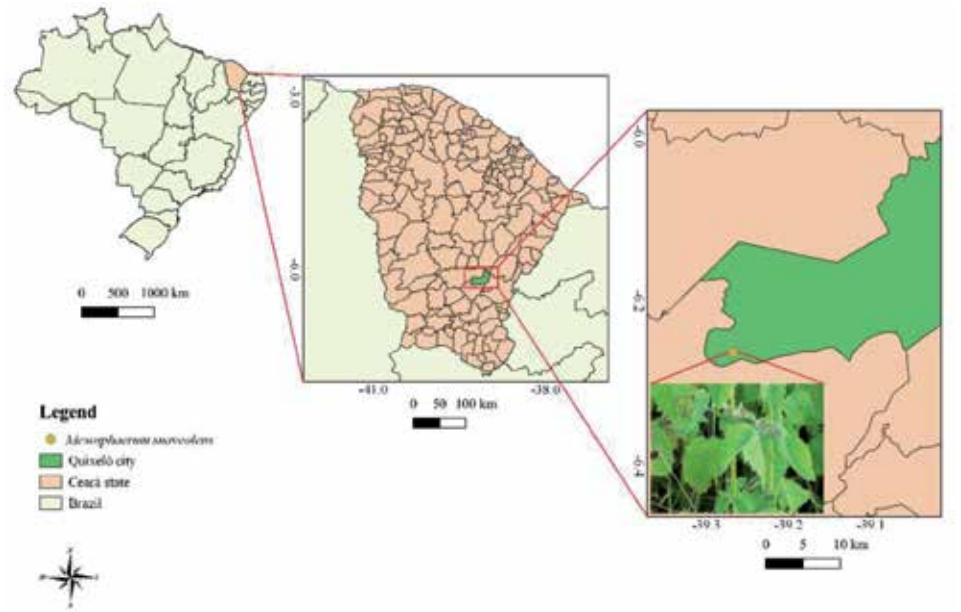

Figure 4.
Map of the collection of the species Mesosphaerum suaveolens *in the municipality of Quixelô—CE, Brazil.*

4.3 Phytochemical analysis of essential oil by gas chromatography (GC-FID)

For gas chromatography (GC), the Agilent Technologies 6890 N GC-FID system, equipped with DB-5 capillary column with the following specifications: 30 m of length, 0.32 mm and 0.50 µm of film thickness was used, which was connected to an FID detector. The temperature ramp consisted of: Initial temperature of 60°C for 1 min and was raised to 3° C/min until reaching 180°C [8].

4.4 Identification of the components

As for the identification, the terpenes were identified as to the of retention index (RI). In addition, they were compared with two spectral libraries, Nist and Wiley, and data in the literature [23].

Bacteria	Origin	Resistance profile
Escherichia coli 06	Urine culture	Cephalothin, cephalexin, cefadroxil, ceftriaxone, cefepime, ampicillin-sulbactam
Pseudomonas aeruginosa 03	Uroculture	Amikacin, imipenem, ciprofloxacin, levofloxacin, piperacillin-tazobactam, ceftazidime, meropenem, cefepime
Staphylococcus aureus 10	Rectal swab culture	Cefadroxil, cephalexin, cephalothin, oxacillin, penicillin, ampicillin, amoxicillin, moxifloxacin, ciprofloxacin, levofloxacin, ampicillin-sulbactam, amoxilin/ac. Clavulanic, erythromycin, clarithromycin, azithromycin, clindamycin

Source: Laboratory of Microbiology and Molecular Biology—LMBM—regional University of Cariri—URCA.

Table 3.
Isolated clinical bacterial strains used for MIC and modulation tests with their antibiotic resistance and origin profile.

4.5 Antibacterial activity

4.5.1 Bacterial strains, culture media and drugs

For the antibacterial tests, standard strains were used to determine minimum inhibitory concentration (MIC), being *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 25853 and *Staphylococcus aureus* ATCC 25923. While for the modulation and also MIC tests, strains resistant cells (**Table 3**), being *Escherichia coli* 06, *Pseudomonas aeruginosa* 03 and *Staphylococcus aureus* 10.

As for the culture medium for the antibacterial assays, Brain Heart Infusion (BHI) was prepared according to the measures recommended by the manufacturer. While for *in vitro* modulation assays, the drugs used were Gentamicin from class aminoglycoside, Norfloxacin, belonging to the classes of fluoroquinolones and Imipenem of the carbapenem class.

4.5.2 Minimal inhibitory concentration (MIC)

It was followed the methodology employed in the work Bezerra et al. [3] for the determination of the Minimum Inhibitory Concentration (MIC). In this study, concentrations ranging from 1 to 1024 μg/mL of the essential oil of *M. suaveolens* against pathogenic bacteria were evaluated. For that, the inoculants of the strains were mixed with BHI (10%), being distributed in microdilution plates with the natural product. After 24 hours of microbial growth at a temperature of 37°C, the MIC was evaluated with the addition of resazurin.

4.5.3 Effect modulator of antibiotics

To assess the modulating effect of essential oil, sub-inhibitory concentrations (MIC/8) of the product against multidrug-resistant bacteria were used. For that, concentrations of standard antibiotics (1–1024 μg/mL) were added to microdilution plates containing BHI (10%) and bacteria inoculum, as well as volatile *M. suaveolens* terpenes in sub-inhibitory concentrations. After 24 hours in a bacteriological oven (37°C), a resazurin solution was added to determine the MIC [7].

4.6 Statistical analysis

The results were analyzed in the GraphPad Prism program, version 6, in which the data were analyzed by Anova One-way and followed by post hoc Tukey test and were considered significant when $p < 0.05$.

5. Conclusion

The essential oil of *Mesosphaerum suaveolens* exhibits antibacterial activity against strains of *Staphylococcus aureus* so that its phytochemicals can be used in the formulation of new drugs. Further studies on toxicity should be performed in order to ascertain the tolerable concentrations that can be used of this oil.

Acknowledgements

The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and the Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP).

Conflict of interest

The authors declare no conflict of interest.

Funding

This research received no external funding.

Author details

José Weverton Almeida Bezerra[1*], Felicidade Caroline Rodrigues[1], Ma Aparecida Barbosa Ferreira Gonçalo[2], Marcos Aurélio Figuereido dos Santos[2], Gledson Ferreira Macedo[2], Janete de Souza Bezerra[2], Priscilla Augusta de Sousa Fernandes[2], Emanoel Messias Pereira Fernando[3], Carlos Henrique Silva de Oliveira[3], Viviane Bezerra da Silva[2], Isabella Hevily Silva Torquato[2], Niwiarakelly da Silva Monte[2], Luciano Temoteo dos Santos[2] and Henrique Douglas Melo Coutinho[2]

1 Federal University of Pernambuco (UFPE), Recife, PE, Brazil

2 Regional University of Cariri (URCA), Crato, CE, Brazil

3 Federal University of Campina Grande (UFCG), Patos, PB, Brazil

*Address all correspondence to: weverton.almeida@urca.br; rodriguescaroline26@gmail.com

References

[1] Rodrigues FC, Santos ATL, Machado AJT, Bezerra CF, Freitas TS, Coutinho HDM, et al. Chemical composition and anti-Candida potencial of the extracts of *Tarenaya spinosa* (Jacq.) Raf.(Cleomaceae). Comparative Immunology, Microbiology & Infectious Diseases. 2019;**64**:14-19. DOI: 10.1016/j.cimid.2019.02.005

[2] Santos FSM, Bezerra JWA, Kamdem JP, Boligon AA, Anraku MM, Silva ARP, et al. Polyphenolic composition, antibacterial, modulator and neuroprotective activity of *Tarenaya spinosa* (Jacq.) Raf. (Cleomaceae). Asian. Pac. Journal of Tropical Medicine. 2019;**9**:12-17. DOI: 10.4103/22211691.250264

[3] Bezerra JWA, Costa AR, Freitas MA, Rodrigues FC, Souza MA, Silva ARP, et al. Chemical composition, antimicrobial, modulator and antioxidant activity of essential oil of *Dysphania ambrosioides* (L.) Mosyakin & Clemants. Comparative Immunology, Microbiology & Infectious Diseases. 2019;**65**:58-64. DOI: 10.1016/j.cimid.2019.04.010

[4] Ferreira H, RPL E. *Pseudomonas aeruginosa*: An alert to the professionals of health. Revista Panamericana de Infectologia. 2010;**12**:44-50

[5] Veras HN, Rodrigues FF, Botelho MA, Menezes IRA, Coutinho HDM, Costa JGM. Enhancement of aminoglycosides and β-lactams antibiotic activity by essential oil of *Lippia sidoides* Cham. and the Thymol. Arabian Journal of Chemistry. 2017;**10**:2790-2795. DOI: 10.1016/j.arabjc.2013.10.030

[6] Costa AR, Silva JL, Lima KRR, Rocha MI, Barros LM, Costa JGM, et al. *Rhaphiodon echinus* (Nees & Mart.) Schauer: Chemical, toxicological activity and increased antibiotic activity of antifungal drug activity and antibacterial. Microbial Pathogenesis. 2017;**107**:280-286. DOI: 10.1016/j.micpath.2017.04.001

[7] Coutinho HDM, Costa JGM, Lima EO, Falcao-Silva VS, Siqueira-Junior JP. *In vitro* interference of *Momordica charantia* and chlorpromazine in the resistance to aminoglycosides. Pharmaceutical Biology. 2008;**47**:1056-1059. DOI: 10.3109/13880200902991540

[8] Bezerra JWA, Costa AR, Silva MAP, Rocha MI, Boligon AA, Rocha JBT, et al. Chemical composition and toxicological evaluation of *Hyptis suaveolens* (L.) Poiteau (LAMIACEAE) in *Drosophila melanogaster* and *Artemia salina*. South African Journal of Botany. 2017;**113**:437-442. DOI: 10.1016/j.sajb.2017.10.003

[9] Duarte AE, Menezes IRA, Morais-Braga MFB, Leite NF, Barros LM, Waczuk EP, et al. Antimicrobial activity and modulatory effect of essential oil from the leaf of *Rhaphiodon echinus* (Nees & Mart) Schauer on some antimicrobial drugs. Molecules. 2016;**21**:743-756. DOI: 10.3390/moléculas21060743

[10] Raja RR. Medicinally potential plants of Labiatae (Lamiaceae) family: An overview. Research Journal of Medicinal Plant. 2012;**6**:203-213. DOI: 10.3923/rjmp.2012.203.213

[11] Uritu CM, Mihai CT, Stanciu GD, Dodi G, Alexa-Stratulat T, Luca A, et al. Medicinal plants of the family Lamiaceae in pain therapy: A review. Pain Research & Management. 2018;**2018**:1-44. DOI: 10.1155/2018/7801543

[12] Albuquerque UP, Medeiros PM, Almeida ALS, Monteiro JM, Neto EMDFL, Melo JG, et al. Medicinal plants of the caatinga

(semi-arid) vegetation of NE Brazil: A quantitative approach. Journal of Ethnopharmacology. 2007;**114**:325-354. DOI: 10.1016/j.jep.2007.08.017

[13] Campos DVB, Rios R, Bessa C, Costa MD, Teixeira-Castro A, Maciel P, et al. Antioxidant and neuroprotective effects of *Hyptis suaveolens*, *Hyptis pectinata* and *Hyptis marrubioides* in *Caenorhabditis elegans*. Chinese Medicine-Uk. 2018;**13**:55-55

[14] Ghaffari H, Ghassam BJ, Nayaka SC, Kini KR, Prakash HS. Antioxidant and neuroprotective activities of *Hyptis suaveolens* (L.) Poit. against oxidative stress-induced neurotoxicity. Cellular and Molecular Neurobiology. 2014;**34**:323-331. DOI: 10.1007/s10571-013-0016-7

[15] Jesus NZT, Falcão HS, Lima GRM, Caldas Filho MRD, Sales IRP, Gomes IF, et al. *Hyptis suaveolens* (L.) Poit (Lamiaceae), a medicinal plant protects the stomach against several gastric ulcer models. J. Ethnopharmacology. 2013;**150**:982-988. DOI: 10.1016/j.jep.2013.10.010

[16] Brindha P, Sridharan G, Pradeep V, Sasikumar S. Antitumor activity and in vivo antioxidant status of *Hyptis suaveolens* against Ehrich ascites carcinoma in swiss albino mice. Indian Drugs. 2008;**45**:801-808

[17] Santos TC, Marques MS, Menezes IRA, Dias KS, Silva AB, Mello IC, et al. Antinociceptive effect and acute toxicity of the *Hyptis suaveolens* leaves aqueous extract on mice. Fitoterapia. 2007;**78**:333-336. DOI: 10.1016/j.fitote.2007.01.006

[18] Grassi P, Reyes TSU, Sosa S, Tubaro A, Hofer O, Zitterl-Eglseer K. Anti-inflammatory activity of two diterpenes of *Hyptis suaveolens* from El Salvador. Journal of Biosciences. 2006;**61**:165-170. DOI: 10.1515/znc-2006-3-402

[19] Mbatchou VC, Abdullatif S, Glover R. Phytochemical screening of solvent extracts from *Hyptis suaveolens* LAM for fungal growth inhibition. Pakistan Journal of Nutrition. 2010;**9**:358-361. DOI: 10.3923/pjn.2010.358.361

[20] Hossan MS, Jindal H, Maisha S, Samudi-Raju C, Devi-Sekaran S, Nissapatorn V, et al. Antibacterial effects of 18 medicinal plants used by the Khyang tribe in Bangladesh. Pharmaceutical Biology. 2018;**56**:201-208. DOI: 10.1080/13880209.2018.1446030

[21] Benelli G, Flamini G, Canale A, Cioni PL, Conti B. Toxicity of some essential oil formulations against the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (*Diptera Tephritidae*). Crop Protection. 2012;**42**:223-229. DOI: 10.1016/j.cropro.2012.05.024

[22] Bezerra JWA, Santos MAF, Meiado MV, Linhares KV, Boligon AA, Leandro CS, et al. Allelopathy of aromatic species on the germination of *Cereus jamacaru* DC. Subsp. *jamacaru* (Cactaceae). The Journal of Agricultural Science. 2018;**10**:337-348. DOI: 10.5539/jas.v9n11p99

[23] Houghton PJ, Howes MJ, Lee CC, Steventon G. Uses and abuses of *in vitro* tests in ethnopharmacology: Visualizing an elephant. Journal of Ethnopharmacology. 2007;**110**:391-400

[24] Sikkema J, Bont JA, Poolman B. Interactions of cyclic hydrocarbons with biological membranes. The Journal of Biological Chemistry. 1994;**269**:8022-8028

[25] Dahham S, Tabana Y, Iqbal M, Ahamed M, Ezzat M, Majid A, et al. The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-caryophyllene from the essential oil of *Aquilaria crassna*.

Molecules. 2015;**20**:11808-11829. DOI: 10.3390/moléculas200711808

[26] Martins FT, Santos MH, Polo M, Barbosa LC. Variação química do óleo essencial de *Hyptis suaveolens* (L.) Poit., sob condições de cultivo. Química Nova. 2006;**29**:1203-1209. DOI: 10.1590/S0100-40422006000600011

[27] Tesch NR, Márquez-Yánez R, Mendoza-Rojas X, Rojas-Fermín L, Velasco Carrillo J, Díaz T, et al. Composición química y actividad antibacteriana del aceite esencial de *Hyptis suaveolens* (L.) Poit. (Lamiaceae) de los Llanos venezolanos. Revista Peruana de Biología. 2015;**22**:103-107. DOI: 10.15381/rpb.v22i1.11127

[28] Cirino ICS, Menezes-Silva SMP, Silva HTD, Souza EL, Siqueira-Júnior JP. The essential oil from *Origanum vulgare* L. and its individual constituents carvacrol and thymol enhance the effect of tetracycline against *Staphylococcus aureus*. Chemotherapy. 2014;**60**:290-293. DOI: 10.1159/000381175

Chapter 9

Essential Oil as Antimicrobial Agents: Efficacy, Stability, and Safety Issues for Food Application

Hamdy A. Shaaban

Abstract

The use of natural antimicrobial compounds in food has gained much attention by the consumers and the food industry. This is primarily due to two major factors. First, the misuse and mishandling of antibiotics has resulted in the dramatic rise of a group of microorganisms including foodborne pathogens that are not only antibiotic resistant but also more tolerant to several food processing and preservation methods. In addition, increasing consumers' awareness of the potential negative impact of synthetic preservatives on health versus the benefits of natural additives has generated interest among researchers in the development and use of natural products in foods. Essential oils are volatile, natural, complex compounds characterized by a strong odor and are formed by aromatic plants as secondary metabolites. The bioactivity properties of essential oils are generally determined by the major compounds present in them. They have been widely used for bactericidal, virucidal, fungicidal, antiparasitical, insecticidal, medicinal, and antioxidant applications. The biological activity of the oils can be compared with the activity of synthetically produced pharmacological preparations. Thus, essential oils are promising natural extracts that need further evaluation for possible application as supplement, preservatives, or antioxidants in food or pharmaceutical industries.

Keywords: essential oil, antimicrobial activities, natural preservatives, pathogenic bacteria, microorganisms

1. Introduction

Aromatic plants have been used since ancient times for their preservative and medicinal properties, and to impart aroma and flavor to food. Hippocrates, sometimes referred to as the "father of medicine," prescribed perfume fumigations. The pharmaceutical properties of aromatic plants are partially attributed to essential oils. The term "essential oil" was used for the first time in the sixteenth century by Paracelsus von Hohenheim, who named the effective component of a drug, "Quinta essential" [1]. By the middle of the twentieth century, the role of essential oils had been reduced almost entirely to use in perfumes, cosmetics and food flavorings, while their use in pharmaceutical preparations had declined.

The natural mixtures of volatile and aromatic compounds (Essential oils) are secondary aromatic plant metabolites. Essential oils are complex, multi-component systems composed mainly of terpenes in addition to some other non-terpene components. Several techniques can be used to extract essential oils from different parts of the aromatic plant, including hydrodistillation (HD), solvent extraction and supercritical fluid extraction (SFE) [2]. Essential oils are derived from various parts of the plant, including leaves, flowers, fruits, seeds, rhizomes, roots, and bark. In the plant, these constituents serve several physiological purposes for the plant protection from pests and microorganisms, attraction of pollinating insects or birds, providing photoprotection to the plant, and allelopathy.

Essential oils are usually obtained by steam or hydro-distillation first developed in the Middle Ages by Arabs. Known for their antiseptic, i.e., bactericidal, virucidal and fungicidal, and medicinal properties and their fragrance, they are used in embalmment, preservation of foods and as antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic and locally anesthesic remedies. Up to the present day, these characteristics have not changed much except that more is now known about some of their mechanisms of action, particularly at the antimicrobial level. In nature, essential oils play an important role in the protection of the plants as antibacterials, antivirals, antifungals, insecticides, and also against herbivores by reducing their appetite for such plants. They also may attract some insects to favor the dispersion of pollens and seeds, or repel undesirable others. Essential oils are extracted from various aromatic plants generally localized in temperate to warm countries like Mediterranean and tropical countries where they represent an important part of the traditional pharmacopeia. They are liquid, volatile, limpid and rarely colored, lipid soluble and soluble in organic solvents. Essential oils can be synthesized by all plant organs, i.e., buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood or bark, and are stored in storage cells like cavities, canals, epidermic cells or glandular trichomes [3, 4]. Most of the commercialized essential oils are chemotyped by gas chromatography and mass spectrometry analysis. Analytical monographs have been published (European Pharmacopoeia, ISO, WHO, Council of Europe; [5]) to ensure good quality of essential oils.

Essential oils have been largely employed for their properties already observed in nature, i.e., for their antibacterial, antifungal and insecticidal activities. At present, approximately 3000 essential oils are known, 300 of which are commercially important especially for the pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries. Essential oils or some of their components are used in perfumes and make-up products, in sanitary products, in dentistry, in agriculture, as food preservers and additives, and as natural remedies. For example, d-limonene, geranyl acetate or d-carvone are employed in perfumes, creams, soaps, as fragrant components and in food, as natural flavoring agents fragrances for household cleaning products and as industrial solvents. Moreover, essential oils are used in massages as mixtures with vegetal oil or in baths but most frequently in aromatherapy. Some essential oils appear to exhibit particular medicinal properties that have been claimed to cure one or another organ dysfunction or systemic disorder [6–8].

Essential oils have traditionally been used to impart flavoring or preservative effects to foods, or to instill fragrances in cosmetics and aromatherapy. Since ancient times, numerous civilizations have also valued essential oils for their therapeutic qualities in disease prevention and treatment. Later, the Greeks and Romans absorbed Egyptian practices of using essential oils in aromatherapy and expanded it to their baths for promotion of well-being. For instance, baths infused

with the oils of jasmine, lavender, or ylang-ylang stimulated mental relaxation. Similarly, current interest in essential oils arises from the various bioactive effects they display, including antioxidant [9, 10], anti-inflammatory [11, 12], antimicrobial [13, 14], antiviral [15, 16], and anticarcinogenic [17]. In developed countries, the benefits derived from using essential oils appear optimistic. Demand for plant essential oils has risen as a consequence of consumers searching for cheaper, more 'natural' alternatives to disease-fighting medications. In food and cosmetic applications, essential oils are considered to be biodegradable, readily available, and 'less toxic' than synthetic preservative agents. As such, this optimism has raised concerns and stimulated studies to evaluate the safety and efficacy of essential oils in various systems in order to better understand their pharmacological properties and roles in health.

Today there is significant consumer demand for foods that are minimally processed and free from synthetic chemical preservatives with the perception of being "natural" [18, 19]. As a result the food industry is facing great challenges to produce naturally occurring food antimicrobials and antioxidants to reduce the use of synthetic chemical preservatives and still produce safe foods that are also regarded as healthy. Spices and herbs are well known for their antimicrobial and antioxidant properties and have the ability to produce multidimensional flavors in food [20]. The clove, cinnamon, oregano and rosemary are considered as the most common spices and herbs with strong antimicrobial activity. Their essential oils containing chemical compounds such as carvacrol, cinnamaldhyde, eugenol and camphor are identified as the major chemical components responsible for exerting antimicrobial activity [21–24]. Some studies reported that there is a highly positive linear relationship between antioxidant activity, antibacterial activity and total phenolic content in some spices and herbs [25, 26].

Antimicrobials are used in food for two main reasons: (1) to control natural spoilage processes (food preservation) and (2) to prevent/control growth of micro-organisms, including pathogenic micro-organisms (food safety). Natural antimicrobials are derived from animal, plant and microbial sources. There is considerable potential for utilization of natural antimicrobials in food, especially in fresh fruits and vegetables. However, methods and mechanisms of action, as well as the toxicological and sensory effects of natural antimicrobials, are not completely understood [18, 27–30].

There are more than 1340 plants with defined antimicrobial compounds, and over 30,000 components have been isolated from phenol group-containing plant-oil compounds and used in the food industry. However, commercially useful characterizations of preservative properties are available for only a few EOs. There is a need for more evaluation of EOs in field and food systems. Food-preservative utilization of spices and their EOs as natural agents has recently been focused on extending the shelf life of foods, reducing or eliminating pathogenic bacteria, and increasing overall quality of food products [27, 31–35].

2. Composition of essential oils

Essential oils are very complex natural mixtures which can contain about 20–60 components at quite different concentrations. They are characterized by two or three major components at fairly high concentrations (20–70%) compared to others components present in trace amounts. For example, carvacrol (30%) and thymol (27%) are the major components of the *Origanum compactum* essential oil, linalool (68%) of the *Coriandrum sativum* essential oil, a- and b-thujone (57%)

and camphor (24%) of the *Artemisia herba-alba* essential oil, 1,8-cineole (50%) of the *Cinnamomum camphora* essential oil, α-phellandrene (36%) and limonene (31%) of leaf (of what)? and carvone (58%) and limonene (37%) of seed *Anethum graveolens* essential oil, menthol (59%) and menthone (19%) of *Mentha piperita* essential oil. Generally, these major components determine the biological properties of the essential oils. The components include two groups of distinct biosynthetical origin [36–39]. The main group is composed of terpenes and terpenoids and the other of aromatic and aliphatic constituents, all characterized by low molecular (see **Figure 1**).

The essential oil of juniper berry (*Juniperus drupacea* L.) was analyzed by chromatographic analysis and it was found that α-pinene (44.2%), thymol methyl ether (22.2%) and camphor (10.2%) present in higher concentration [40]. The major volatile compounds found in caper (*Capparis ovata* desf. Var. *caescens*) bud oil were benzyl alcohol (20.4%), furfural (7.4%), ethanol methyl pentyl acetal (5.9%), thymol (5.1%) as well as the major volatile compound found in capers leaves were methyl isocyanate (20.0%), thymol (15.5%) [41].

The following paragraphs will descript some features that characterize each chemical group constituent of essential oils.

2.1 Terpenes

Terpenes form structurally and functionally different classes. They are made from combinations of several 5-carbon-base (C5) units called isoprene. The biosynthesis of the terpenes consists of synthesis of the isopentenyl diphosphate (IPP) precursor, repetitive addition of IPPs to form the prenyldiphosphate precursor of the various classes of terpenes, modification of the allylic prenyldiphosphate by terpene specific synthetases to form the terpene skeleton and finally, secondary enzymatic modification (redox reaction) of the skeleton to attribute functional properties to the different terpenes. The main terpenes are the monoterpenes (C10) and sesquiterpenes (C15), but hemiterpenes (C5), diterpenes (C20), triterpenes

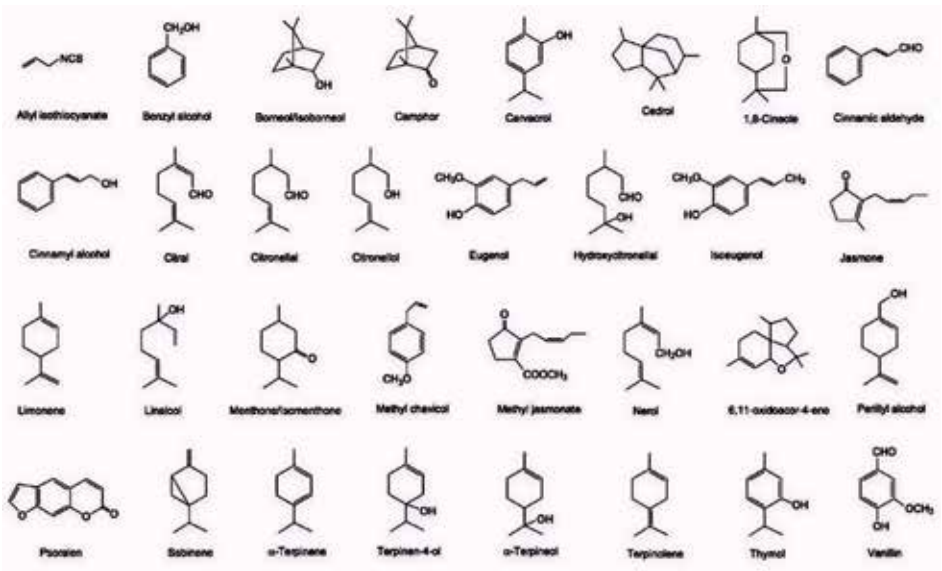

Figure 1.
The structure of the chemicals discussed in this chapter with respect to their biological activity in alphabetical order.

(C30) and tetraterpenes (C40) also exist. A terpene containing oxygen is called a terpenoid.

The monoterpenes are formed from the coupling of two isoprene units (C10). They are the most representative molecules constituting 90% of the essential oils and allow a great variety of structures. They consist of several functions:

2.1.1 Carbures

- Acyclic: e.g., myrcene and ocimene, etc.
- Monocyclic: e.g., terpinenes, p-cimene and phellandrenes, etc.
- Bicyclic: e.g., pinenes, −3-carene, camphene, sabinene, etc.

2.1.2 Alcohols

- Acyclic: e.g., geraniol, linalol, citronellol, lavandulol, and nerol, etc.
- Monocyclic: e.g., menthol, α-terpineol and carveol.
- Bicyclic: e.g., borneol, fenchol, chrysanthenol, thuyan-3-ol, etc.

2.1.3 Aldehydes

- Acyclic: e.g., geranial, neral, citronellal, etc.

2.1.4 Ketone

- Acyclic: e.g., tegetone, etc.
- Monocyclic: e.g., menthones, carvone, pulegone and piperitone, etc.
- Bicyclic: e.g., camphor, fenchone, thujone, ombellulone, pinocamphone and pinocarvone, etc.

2.1.5 Esters

- Acyclic: e.g., linalyl acetate or propionate, citronellyl acetate, etc.
- Monocyclic: e.g., menthyl or α-terpinyl acetate, etc.
- Bicyclic: e.g., isobornyl acetate, etc.

2.1.6 Ethers

- 1,8-cineole, menthofurane, etc.
- Peroxydes: e.g., ascaridole, etc.
- Phenols (aromatic ethers): e.g., thymol, carvacrol, etc.

When the molecule is optically active, the two enantiomers are very often present in different plants. For example, (+)α-pinene from *Pinus palustris*, (−)

β-pinene from *Pinus caribaea* and from *Pinus pinaster*. Another example is linalool from coriander is (+); however, linalool from lavender oil is (−). In some cases, it is the racemic form which is the most frequently encountered. (±)citronellol is widespread, the form (+) is characteristic of *Eucalyptus citriodor*, the form (−) is common to the rose and geranium essential oils.

The sesquiterpenes are formed from the assembly of three isoprene units (C15). The extension of the chain increases the number of cyclisations which allows a great variety of structures. The structure and function of the sesquiterpenes are similar to those of the monoterpenes. Examples of plants containing these compounds are angelica, bergamot, caraway, celery, citronella, coriander, eucalyptus, geranium, juniper, lavandin, lavander, lemon, lemongrass, mandarin, mint, orange, peppermint, petitgrain, pine, rosemary, sage, thyme.

2.2 Aromatic compounds

Derived from phenylpropane, the aromatic compounds occur less frequently than the terpenes. The biosynthetic pathways concerning terpenes and phenylpropanic derivatives generally are separated in plants but may coexist in some, with one major pathway taking over (e.g., cinnamom oil with cinnamaldehyde as major and eugenol as minor constituents, also clove oil, fennel, etc.).

Aromatic compounds comprise:

1. **Aldehyde**: e.g., cinnamaldehyde

2. **Alcohol**: e.g., cinnamic alcohol

3. **Phenols**: e.g., chavicol and eugenol

4. **Methoxyderivatives**: e.g., anethole, elemicine, estragole and methyleugenols

5. **Methylene dioxy compounds**: e.g., apiole, myristicine and safrole

The principal plant sources for these compounds are anise, cinnamon, clove, fennel, nutmeg, parsley, sassafras, star anise, tarragon, and some botanical families (Apiaceae, Lamiaceae, Myrtaceae, Rutaceae).

Nitrogenous or sulfured components such as glucosinolates or isothiocyanate derivatives (garlic and mustard oils) are also characteristic as secondary metabolites of diverse plants or of torrefied, grilled or roasted products.

3. Effects of essential oils as antibacterial agents

Various studies showed that essential oils also have antibacterial properties against a wide range of bacterial strain such as *Listeria monocytogenes*, *L. innocua*, *Salmonella typhimurium*, *Escherichia coli*, *Shigella dysenteria*, *Bacillus cereus*, *Staphylococcus aureus*, and *Salmonella typhimurium* [42]. Direct inhibition correlation due to presence of thymol and carvacrol in the essential oils of thyme and oregano can inhibit some pathogenic bacterial strains such as *E. coli*, *Salmonella enteritidis*, *Salmonella choleraesuis*, and *Salmonella typhimurium* [43]. The same correlation was also confirmed for oils rich in carvacrol alone. Eugenol and carvacrol showed an inhibitory effect against the growth of four strains of *Escherichia coli* O157:H7 and *Listeria monocytogenes* [44]. The carvacrol showed strong antibacterial activity due to presence of phenolic hydroxyl group. Some essential

oils demonstrated antibacterial activity against zoonotic enteropathogens including *Salmonella spp*., *Escherichia coli* O157, *Campylobacter jejuni*, and *Clostridium perfringens*. Thus, these oils could possibly be used as an alternative to antibiotics in animal feed [45]. Essential oils with high concentrations of thymol and carvacrol e.g., oregano, savory and thyme, usually inhibit Gram-positive more than Gram-negative pathogenic bacteria. However the essential oil of *Achillea clavennae* exhibited strong antibacterial activity against the Gram-negative *Haemophilus influenzae* and *Pseudomonas aeruginosa* respiratory pathogens, while Gram-positive *Streptococcus pyogenes* was the most resistant to the oil. Most antiseptic agents can damage the skin, leading to a change in microbial flora, and an increased shedding of the original protective bacterial flora of the hand leads to an increased risk of transmission of pathogenic microorganisms [46]. Reports suggest that repeated use of formulations containing tea tree essential oil (TTO) does not lead to dermatological problems, nor affect the original protective bacterial flora of the skin [47], so the antibacterial activity of some skin-wash formulas containing TTO as well as pure TTO was evaluated against *Staphylococcus aureus*, *Acinetobacter baumannii*, *Escherichia coli*, and *Pseudomonas aeruginosa.* The antibacterial activity of tea tree essential oil has recently been reviewed. It was found that antibacterial property of TTO is mainly due to presence of terpinen-4-ol. The essential oil of oregano is found to effective against *Pseudomonas aeruginosa* and *Escherichia coli* [48]. *Ocimum gratissimum* essential oil can also inhibit extracellular protease and the expression of O-lipopolysaccharide rhamnose in virulence and multidrug-resistant strains of 22 Shigellae. Thus, the oil may find a use as a therapeutic measure against shigellosis. Methicillin-resistant *Staphylococcus aureus* can also be inhibited by the application of peppermint and spearmint essential oils. Essential oils could be used as antibacterial agents against some respiratory tract pathogens. The oil of *Achillea clavennae* showed its maximum activity against *Klebsiella pneumoniae* and penicillin-susceptible and penicillin resistant *Streptococcus pneumoniae*. The oil also exhibited strong activity against *Haemophilus influenzae* and *Pseudomonas aeruginosa*. An increased density of Helicobacter pylori in the gastric mucosa is associated with severe gastritis and an increased incidence of peptic ulcers [49]. The activities of 60 essential oils against *H. pylori* P1 were evaluated: 30 oils were able to affect the growth in vitro, and 15 showed strong activity. Among the individual constituents of these oils, carvacrol, isoeugenol, nerol, citral and sabinene exhibited the strongest anti-*H. pylori* effects. Further investigations are underway regarding the ability of essential oils to control *H. pylori* infections [50]. Croton cajucara Benth essential oil was found to be toxic for some pathogenic bacteria and fungi associated with oral cavity disease and may be useful for controlling the microbial population in patients with fixed orthodontic appliances. A 6-month controlled clinical study demonstrated that a mouth rinse containing essential oils showed a comparable antiplaque and anti-gingivitis activity to that containing the synthetic antibacterial agent, chlorhexidine [51]. Mouth rinses containing essential oils (specially phenolic rich types) with chlorhexidine gluconate are commonly used as preprocedural preparations to prevent possible disease transmission, decrease chances of postoperative infection, decrease oral bacterial load and decrease aerosolization of bacteria Mouth washes containing essential oils could also be used as a part of plaque control routine since they can penetrate the plaque biofilm, kill pathogenicplaque—forming microorganisms by disrupting their cell walls and inhibiting their enzymatic activity. In addition, essential oils in mouth washes prevent bacterial aggregation, slow the multiplication and extract bacterial endotoxins. The mechanisms by which essential oils can inhibit microorganisms involve different modes of action, and in part may be due to their hydrophobicity. As a result, they get partitioned into the lipid bilayer of the cell membrane, rendering it more permeable, leading to leakage

of vital cell contents [52]. Impairment of bacterial enzyme systems may also be a potential mechanism of action. Essential oils show bactericidal activity against oral and dental pathogenic microorganisms and can be incorporated into rinses or mouth washes for pre-procedural infection control, general improvement of oral health, inter-dental hygiene and to control oral malodor [53].

4. Effects of essential oils as antifungal agents

Some EOs demonstrate a broad range of natural fungicidal effects against post-harvest pathogens, especially because of their bioactivity in the vapor phase for storage applications [54]. However, more time is needed for vapor-phase bioactivity effect, possible absorption into the food material needed to be considered [55]. Antifungal activity might be affected by the targeted fungal physiological activity [56].

The reported effective compounds against food-borne fungi, including *Aspergillus niger*; *A. flavus;* and *A. parasiticus* is carvacrol and thymol [57]. Water-distilled EO from leaves and flowers of *Micromeria nubigena* H.B.K. (Lamiaceae) also showed anti-fungal properties [58]. Essential oils from thymol at 500 μg/ml concentration and at 1.0% and 100 μg/ml concentrations; cinnamic aldehyde and eugenol extracted from cinnamon and clove at 1.0% and 100 μg/ml concentrations also showed anti-fungal properties [59]. *Aspergillus parasiticus* growth and Aflatoxins production has been inhibited by *Thymus vulgaris* and *Citrus aurantifolia*. On the other hand *Mentha spicata* L., *Foeniculum miller*, *Azadirachta indica* A. Juss, *Conium maculatum*, and *Artemisia dracunculus* has only inhibited the fungal growth. While *Carum carvi* L. has controlled aflatoxin production without effect on the fungal growth [34]. Linalool and methyl chavicol and vanillin extracted from sweet basil and vanilla at 2000 μg/ml concentration has the same effect on aflatoxin production [59].

Hydro-distilled EOs of stems, leaves (at vegetative and flowering stages) and flowers of *Eugenia chlorophylla* O. Berg. (Myrtaceae) and various extracts of thyme, were active against molds and yeasts [59–61], respectively. Oleoresin extracted from cinnamon and clove inhibited mycotoxin-producing *Aspergillus* and *Penicillium* species at 2.0% w/v, 300 μg/ml concentrations [59].

Higher levels of phenolic compounds in thyme and clove showed antifungal effects. Phenolic compounds, such as allyl isothiocyanate and citralon in mustard and lemongrass, have been more effective as volatiles [62]; however, in EOs such as marjoram oil, they have been effectively antifungal in a matrix with mainly hydrocarbons [56].

5. Mechanism of action

It has been demonstrated that the antimicrobial effects of the Eos acts by causing structural and functional damages to the bacterial cell membrane. It is also indicated that the optimum range of hydrophobicity is involved in the toxicity of the EOs.

Application of antimicrobials by different exposure methods, such as vapor phase compared to direct contact method, of mustard and clove EOs showed noteworthy differences [63]. The stereochemistry, lipophilicity and other factors affected the biological activity of these compounds which might be altered positively or negatively by slight modifications [64]. It has been shown that plant substances affect microbial cells by various antimicrobial mechanisms, including

attacking the phospholipid bilayer of the cell membrane, disrupting enzyme systems, compromising the genetic material of bacteria, and forming fatty acid hydroperoxidase caused by oxygenation of unsaturated fatty acids [65–70]. Allyl isothiocyanate derived from mustard seems to have multi-targeted mechanisms of action in metabolic pathways, membrane integrity, cellular structure and statistically significant higher release of the cell compounds of *Escherichia coli* O157:H7 [71].

Carvacrol increases the heat shock protein 60 HSP 60 (GroEL) protein and inhibited the synthesis of flagellin highly significantly in *E. coli* O157:H7 [66]. There are concerns regarding the enhanced aroma and taste of oregano at the higher levels of application in food items, especially at 1% [72]. The apparent antimicrobial efficacy of plant origin antimicrobials depends on factors such as the method of extracting EOs from plant material, the volume of inoculum, growth phase, culture medium used, and intrinsic or extrinsic properties of the food such as pH, fat, protein, water content, antioxidants, preservatives, incubation time/temperature, packaging procedure, and physical structure of food [73, 74]. Another important parameter regarding effects of food preservatives is ability to reduce the pH level inside the bacterial cell (pHin). It has been shown that pHin of both *E. coli* and Salmonella has been reduced by the effect of mustard's EOs [71].

Generally, Gram-negative bacteria are less sensitive to the antimicrobials because of the lipopolysaccharide outer membrane of this group, which restricts diffusion of hydrophobic compounds. However, this does not mean that Gram-positive bacteria are always more susceptible [27]. Gram-negative bacteria are usually more resistant to the plant-origin antimicrobials and even show no effect, compared to Gram-positive bacteria [61, 75].

6. Synergistic and antagonistic effects of components

When the combined effect of substances is higher than the sum of the individual effects, this is synergy; antagonism happens when a combination shows less effect compared to the individual applications [27]. Synergistic effects of some compounds, in addition to the major components in the EOs, have been shown in some studies [76–78]. Application of a certain combination of carvacrol-thymol can improve the efficacy of Eos against pathogenic micro-organisms [79].

Synergism between carvacrol and p-cymene, a very weak antimicrobial, might facilitate carvacrol's transportation into the cell by better swelling the *B. cereus* cell wall [27]. Antimicrobial activity of combination of cinnamon and clove EOs in vapor phase showed better antimicrobial with less active concentration in the vapor phase compare to liquid phase [63]. Thymol and carvacrol showed synergistic and antagonistic effects, in different combinations of cilantro, coriander, dill and eucalyptus EOs (each containing several components) and mixtures of cinnamaldehyde and eugenol, against *Staphylococcus sp*., *Micrococcus sp*., Bacillus sp. and *Enterobacter sp*. [27]. An antagonistic effect on *Bacillus cereus* was seen in rice when carvacrol and p-cymene were used with salt; high-hydrostatic pressure showed a synergistic effect in combination with thymol and carvacrol against *L. monocytogenes*. Vacuum packing in combination with oregano EOs showed a synergistic effect against *L. monocytogenes* with 2–3 log10 reduction. Similar results have been recorded when clove and coriander EOs have been used against *Aeromonas hydrophila* on vacuum-packed pork. Application of oregano EO has a synergistic effect in modified-atmosphere packaging (MAP) including 40% CO_2, 30% N_2 and 30% O_2. The available oxygen is another factor antagonistic on EO activities; by decreasing the oxygen level, the sensitivity of micro-organisms to the EOs has been increased [27].

Residual hydrosols after distillation of EOs from plant materials can be used as economical sources of antimicrobial components [74].

Application of nisin with carvacrol or thymol has been positively effective against *Bacillus cereus* with temperatures increasing from 8 to 30°C [27]. Application of nisin with rosemary extract enhanced the bacteriostatic and bactericidal activity of the nisin [80]. Oregano EOs, in combination with modified-atmosphere packaging, have effectively increased the shelf life of fresh chicken [72]. Antimicrobial resistance did not develop in *Yersinia enterocolitica* and *Salmonella choleraesuis* after sub-inhibitory passes with cinnamon, by direct contact or vapor phase [63]. Combination of linalool and 1, 8-cineole (1:1) created more resistance in *E. coli*, compared to application of pure linalool. Either synergism or antagonism of 1, 8-cineole and linalool derived from *Cinnamosma fragrans* could happen against Gram-negative bacteria and *Fusarium oxysporum* [81]. The synergistic effect of different components could offer a way to prevent possible off flavor caused by clove and tea tree when used to protect against *Escherichia coli* O157:H7 and minimize off flavor effects in meat products [32]. Combinations of EOs of oregano and thyme, oregano with marjoram and thyme with sage had the most effects against *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli* O157:H7 and *L. monocytogenes* [82].

7. Effects of essential oils as antiviral agents

Synthetic antiviral drugs have been used for the curing of Herpes simplex virus (type I, II) that causes some of the most common viral infections in humans, and can be fatal but not all are efficacious in treating genital herpes infections. HSV-1 and HSV-2 have also developed resistance to one of these (acyclovir) mainly in immuno-compromised hosts. Essential oils are considered to cure these viral diseases because plant extracts have low toxicity as compared to synthetic antiviral drugs. Natural material is considered as potential alternative. The activity of multilamellar liposome showed better improvement by introduction of essential oils with it. Due to presence of citral and citronellal, the essential oil of *Melissa officinalis* L. can inhibit the replication of HSV-2 and the ability to replicate of HSV-1 can be suppressed by incubation with different essential oils in vitro [83]. Of these, lemongrass essential oil possessed the most potent anti-HSV-1 activity and completely inhibited viral replication after incubation for 24 h, even at a concentration of 0.1%. The virucidal activity was found at high levels in peppermint essential oil against HSV-1 and HSV-2. When the viruses were pretreated with essential oil before adsorption than its antiviral activity was confirmed. Junin virus was successfully inhibited by the essential oil of *Lippia junelliana* and *Lippia turbinate* [84]. The essential oils of eucalyptus, *Santolina insularis* and Australian tea tree showed the antiviral effects against HSV-1. These oils gave good result both before and after adsorption. The oil directly inactivated virus particles, thus preventing adsorption of virion to host cells. Isoborneol, a common monoterpene alcohol, showed dual virucidal activity against HSV-1 [85] and specifically inhibited glycosylation of viral polypeptides. The essential oils have shown good results as antiviral but unfortunately, according to our information till now there is no study about the antiviral properties against the major viruses of era such as HIV and hepatitis C viruses [86].

8. Effects of essential oils as preservatives

In-food studies depend on several additional factors, which have not been tested in similar in vitro studies [87]. Spices and herbs can be used as an alternative

preservative and pathogen-control method in food materials. Application of both extracts and EOs of plant-origin antimicrobials such as floral parts of *Nandina domestica* Thunb could be a potential alternative to synthetic preservatives [88]. Generally, effective EOs in decreasing order of antimicrobial activities are oregano > clove > coriander > cinnamon > thyme > mint > rosemary > mustard > cilantro/sage [27]. However, in another study, mint showed less antimicrobial effect compared to mustard [89]. There are differences between in vitro and in-food trials of plant-origin antimicrobials, mainly because only small percentages of EOs are tolerable in food materials. Finding the most inhibitory spices and herbs depends on a number of factors such as type, effects on organoleptic properties, composition and concentration and biological properties of the antimicrobial and the target micro-organism and processing and storage conditions of the targeted food product [82, 90, 91]. In a study on blanched spinach and minced cooked beef, using clove and tea tree EOs, three and four times the MIC in in vitro studies were needed to restrict *E. coli* O157:H7 populations in the food materials [32]. Despite some positive reports in regard to application of plant-origin natural antimicrobials, two major issues are faced regarding application of plant-origin antimicrobials in food: odors created mostly by the high concentrations, and the costs of these materials [68, 69].

9. Antimutagenic properties of essential oils

Many studies showed that the mutations can be prevented by the inhibition the penetration of mutagens into cells, by adding antioxidants which inactivate the free radicals produced by mutagens, also by activation of cell antioxidant enzymes and by detoxification of mutagens by activation of enzymes by using plant extracts [92]. Some antimutagenic compounds works in two ways those are by promotion of error-free DNA repair or by inhibition of error-prone DNA repair [93]. During recent years the role and reaction of reactive oxygen species (ROS) scavengers, such as glutathione, superoxide dismutase, catalase, N-acetylcystein, provitamins like retinoid, carotenoids and tocopherols, flavonoids and other polyphenols. Also the biochemistry of antimutagens has been published in various documents [94]. However, since the work of [93] on *Escherichia coli*, nobody has examined in more detail this type of antimutagenicity possibly involving interference with DNA repair via intracellular pro-oxidant reactions of the latter compounds or terpenic and phenolic compounds from aromatic plants. Natural compounds, tannic acid and apigenin, reduced the frequency induced by nitropyrenes in CHO cells [95]. *Matricaria chamomilla* essential oil inhibits SCEs (sister chromatid exchanges) induced by daunorubicin and methyl methane sulfonate in mouse bone marrow cells. The aromatic plant *Salvia officinalis* and major components thujone, 1,8-cineole, camphor and limonene inhibit UV-C-induced mutagenesis in *Salmonella typhimurium*, *Escherichia coli* and *Saccharomyces cerevisiae*. The chemical compounds extracted from plats such as α-terpinene, α-terpineol, 1,8-cineole, d-limonene, camphor, citronellal and citral modulate hepatic mono-oxygenase activity by interacting with promutagen or procarcinogen xenobiotic biotransformation [96]. In a more recent study, they showed in the same system that *Origanum compactum* essential oil and some of its sub-fractions and constituents are antimutagenic against the indirect-acting mutagen urethane and also against the direct-acting mutagen methyl methanesulfonate. It is now accepted that pro-oxidant activities can induce late apoptosis and necrosis. Pro-oxidant activities may damage cellular membranes, in particular those of mitochondria, and thus promote the release of Ca++, cytochrome C and ROS (reactive oxygen species). This leads to cell death, at least in mammalian cells, whereas yeast cells are able to

survive in spite of mitochondrial damage. It has been recently demonstrated in the yeast *Saccharomyces cerevisiae* that induction of mitochondrial damage transforming Rho+ cells into Rhoo cells and the induction of apoptosis/necrosis by a combined exposure to essential oils and nuclear mutagens caused a striking reduction of the frequency of nuclear genetic events [86]. Typical mutagenic agents were used such as ultraviolet C (UV-C) radiation which forms pyrimidine dimers and 6-4 photoproducts, 8-methoxypsoralen (8-MOP) activated by ultraviolet A (UVA) radiation which forms DNA mono- and biadducts, or methyl methanesulfonate (MMS) which methylates DNA bases. The reduction in mutant frequency in the presence of essential oils was accompanied by a strong synergistic induction of cytoplasmic "petite" mutants. The anti-mutagenic effect was independent of the type of mutations, i.e., reversion, intra- or intergenic recombination. The extent of this anti-mutagenic effect depended on the mutagen and oil concentrations. However, unexpectedly, the mechanism of the decrease of mutagenicity did not depend on the type of essential oil but on the type of mutagen, thus on the type of lesions and consequently on the DNA repair or lesion avoidance system involved. In fact, after combined treatment by UVC or 8-MOP/UVA plus essential oils, the transformation of Rho+ cells into rho0 cells resulted in a decrease of the frequency of mutants accompanied by a slight resistance of the survival [97]. After UVC or 8-MOP/UVA alone, less mutants were also found in a rho0 mutant, i.e., a complete BET-induced rho0 selected by the alcaloid lycorine, than in the wild type Rho+. In that case, the reduction in mutation frequency was the same as that after the combined treatments confirming the importance of mitochondrial dysfunction for these effects [98]. The same decrease of mutant frequencies was also found in a nucleotide excision repair (NER) defective rad3 mutant after UVC/essential oil combined treatment. Thus the error-free NER repair system does not play any role in this decrease and probably not the error-free homologous recombination in the case of 8-MOP/UVA. However, as a function of survival, this additional cytotoxicity caused a notable reduction of the mutant frequencies for a same survival level. The decrease of cell survival was also accompanied by a synergistic increase of cytoplasmic petite mutants. Thus, in this case, the essential oils contributed to the elimination of the cells already affected by MMS, leading potential mutants to death by late apoptosis and necrosis [97]. The reduction by essential oils of the frequency of mutations induced by the mutagens was always accompanied by a synergistic induction of complete petite mutants. Moreover, essential oils alone or in combined treatments mainly induced necrosis rather than apoptosis. This corroborates with the fact that petite mutants were true rho0 mutants unable to perform apoptosis but only able to passively undergo necrosis, since functional mitochondria are necessary to induce apoptosis [99].

10. Role of essential oils as antioxidants

Form the safety point of view, one of the important sources for the search of essential oils is herbs and spices. Since from prehistoric era, these had been used for flavoring and medicinal properties. However, recent reports showed that the radical scavenging activity of various essential oil is very high. The lag time in conjugated diene formation was dose-dependently prolonged by addition of the essential oils of some aromatic plants such as black cumin, cinnamon bark, ginger [100]. At a level of 200 μg/ml DPPH activity ranged from 39 to 90% in different plants. Phenolic compounds such as thymol, eugenol, linalool etc. are considered to be responsible for scavenging activity of essential oils. Biomolecules such as protein, amino acids, unsaturated lipids are mostly oxidized by free radicals and

reactive oxygen species. The human body is equipped with an inherent defense system which can destroy free radicals present in almost all cells [101]. Oxidative stress is the cause of imbalance between free radical production and their removal from the body. So to overcome oxidative stress antioxidants should be taken externally. Nowadays, there is more interest on natural antioxidants from plants sources to replace those of synthetic origin. Various studies have shown that essential oils are natural source of antioxidants. Aromatic plants are rich in natural antioxidants. The essential oils of coriander, Eucalyptus, juniper, cumin, basil, cinnamon, clove and thyme have proven radical-scavenging and antioxidant properties in the DPPH radical assay at room temperature [2, 40, 102]. The main constituents of the volatile extract of Egyptian corn silk were *cis*-R-terpineol (24.22%), 6,11-oxidoacor-4-ene (18.06%), citronellol (16.18%), *trans*-pinocamphone (5.86%), eugenol (4.37%), neo-iso-3-thujanol (2.59%), and *cis*-sabinene hydrate (2.28%).The water extract inhibited DPPH activity by 81.00 (6.00% at a level of 200 μg/ml). These results suggest that corn silk is a flavor ingredient source and a natural antioxidant supplement for various food products [41]. The antioxidant activity was attributed to the high content of the phenolics thymol and carvacrol (20.5 and 58.1%, respectively). *Thymus spathulifolius* essential oil also possessed an antioxidant activity due to the high thymol and carvacrol content 36.5 and 29.8%, respectively [103]. The activity is again attributed to the content of thymol and carvacrol (35.0 and 32.0%, respectively). Although dietary supplementation of oregano oil to rabbits delayed lipid oxidation, this effect was less than that of supplementation with the same concentration of tocopheryl acetate [104]. However, when tested on turkeys it showed an equivalent performance to the same concentration of α-tocopheryl acetate in delaying iron-induced, lipid oxidation [105]. The essential oils of *Salvia cryptantha* and *Salvia multicaulis* have the capacity to scavenge free radicals. The activity of these oils was higher than that of curcumin, ascorbic acid or BHT [106]. The essential oil of *Achillea millefolium subsp. millefolium* (Asteraceae) exhibited a hydroxyl radical scavenging effect in the Fe3 α-EDTA-H_2O_2 deoxyribose system and inhibited the non-enzymatic lipid peroxidation of rat liver homogenate [107]. In addition, *Curcuma zedoaria* essential oil was found to be an excellent scavenger for DPPH radical [108]. The antioxidant activity of essential oils cannot be attributed only to the presence of phenolic constituents; monoterpene alcohols, ketones, aldehydes, hydrocarbons and ethers also contribute to the free radical scavenging activity of some essential oils. For instance, the essential oil of *Thymus caespititius*, *Thymus camphoratus*, and *Thymus mastichina* showed antioxidant activity which in some cases was equal to that of alpha-tocopherol. Surprisingly, the three species are characterized by high contents of linalool and 1,8-cineole, while thymol or carvacrol are almost absent. The essential oil of lemon balm (*Melissa officinalis* L.) shows an antioxidant and free radical scavenging activity [109] with the most powerful scavenging constituents comprising neral/geranial, citronellal, isomenthone and menthone. Tea tree (*Melaleuca alternifolia*) oil has been suggested as a natural antioxidant alternative for BHT with the inherent antioxidant activity attributed mainly to the α-terpinene, β-terpinene and β-terpinolene content. Essential oils isolated from *Mentha aquatica* L., *Mentha longifolia* L. and *Mentha piperita* L., were able to reduce DPPH radicals into the neutral DPPHH form [110]. The most powerful scavenging constituents were found to be 1,8-cineole for the oil of *M. aquatica* while menthone and isomenthone were the active principles of *M. longifolia* and *M. piperita*. From the above studies, we may conclude that essential oils are rich sources of natural antioxidants. So we can use essential oils as natural sources of antioxidants instead of synthetic antioxidants to prevent from various degenerative diseases as well as to preserve food safely [111].

11. Photo toxicity of essential oils

The essential oils of some aromatic plants contain some photoactive compounds in their composition. Psoralens present in essential oil of citru bergamia were found to effective for binding of mono and biadducts produced under UV-light. These were found to mutagenic and cytotoxic [112]. However, in the dark, this oil is not cytotoxic or mutagenic by itself. It has been noted that *Fusanus spicatus* wood essential oil was not phototoxic but was very cytotoxic. In other words, cytotoxicity seems rather antagonistic to phototoxicity. In the case of cytotoxicity, essential oils damage the cellular and organelle membranes and can act as pro-oxidants on proteins and DNA with production of reactive oxygen species (ROS), and light exposures do not add much to the overall reaction. In the case of phototoxicity, essential oils penetrate the cell without damaging the membranes or proteins and DNA. Radical reactions by excitation of certain molecules and energy transfer with production of oxygen singlet occur when cells are exposed to activating light. This may cause damage to cellular macromolecules and in some cases the formation of covalent adducts to DNA, proteins and membrane lipids. Obviously, cytotoxicity or phototoxicity depends on the type of molecules present in the essential oils and their compartmentation in the cell, producing different types of radicals with or without light exposure. However, such an antagonism is not quite a strict rule [86]. It was also found that *Citrus aurantium* dulcis (*Citrus gracilis* subf. dulcis) and *Cymbopogon citratus* essential oils were phototoxic and cytotoxic. Therefore, the potential toxicity of essential oil should be considered before use as antibacterial for human as well as for animals [113].

12. Other activities

EOs and their monoterpenes affected bone metabolism when added to the food of rats. It was demonstrated that these lipophilic compounds inhibited bone resorption [114]. It was reported that (2*E*,6*R*)-8-hydroxy-2,6-dimethyl-2-octenoic acid, a novel monoterpene, from *Cistanche salsa* had antiosteoporotic properties [115].

Pine EOs prevented bone loss in an osteoporosis model (ovariectomized rats). The monoterpenes borneol, thymol and camphor directly inhibited osteoclast resorption [114]. It was observed that inactive monoterpenes can be metabolized to their active forms *in vivo*; thus, *cis*-verbenol, a metabolite of α-pinene, inhibited osteoclastic resorption activity, in contrast to the parent compound α-pinene.

Potential activities for the treatment of Alzheimer's disease were demonstrated in a pilot open-label study involving oral administration of the EO of *Salvia lavandulaefolia* Vahl. known as Spanish sage [7].

Chinese angelica (*Angelica sinensis*) is the most important female tonic remedy in Chinese medicine. The effects of angelica EO in three assays in mice (elevated plus maze, light/dark and stress-induced hyperthermia test) suggested that angelica EO exhibited an anxiolytic-like effect [116]. A link to emotion and cognitive performance with the olfactory system was reported [117]. Moreover, the EOs could affect mood, concentration and sleep [118], while other studies had shown that EOs were potentially important to boost the immune system [119, 120].

EOs from different *Lippia alba* chemotypes showed behavioral effects. Greater effects were presented by chemotype 2 (with citral and limonene), while chemotype 1, containing citral, myrcene and limonene, decreased only the number of rearings in the open-field test [121]. The EO of lemon was found to modulate the behavioral and neuronal responses related to nociception, pain and

anxiety [122, 123]. Thus, there is widespread and increasing interest in complementary and alternative medicines using EOs [124].

Aloe vera gel enhanced the antiacne properties of *Ocimum gratissimum* L.oil; the oil or its combination with *Aloe vera* gel was more effective than 1% clindamycin in the treatment of *Acne vulgaris* [124]. Linalool-rich EO was potent against promastigotes and amastigotes of *Leishmania amazonensis* [125].

13. Plant extracts

Plant extracts have shown a considerable promise in a range of applications in the food industry and several plant extracts enjoy GRAS status. The antimicrobial activities of plant extracts may reside in a variety of different components and several extracts owing to their phytochemical constituents have been shown to have antimicrobial activity. The antibacterial activity is most likely due to the combined effects of adsorption of polyphenols to bacterial membranes with membrane disruption and subsequent leakage of cellular contents [126, 127], and the generation of hydroperoxides from polyphenols [128]. Plant extracts also showed antifungal activity against a wide range of fungi, antioxidant and antimutagenic activities [129] and inhibited lipid oxidation in foods [130]. Although numerous studies have been done *in vitro* to evaluate the antimicrobial activity of plant extracts, very few studies are available for food products, probably because plant extracts did not produce as marked inhibition as many of the pure compounds in foods. The reduced effectiveness may be attributed to the use of crude extracts in most studies. As the crude extracts generally contain flavonoids in glycosidic form, where the sugar present in them decreases effectiveness against some bacteria [131, 132].

Dietary herbs and spices have been traditionally used as food additives throughout the world not only to improve the sensory characteristics of foods but also to extend their shelf life by reducing or eliminating survival of pathogenic bacteria. Herbs and spices are rich in phenolic compounds and besides exerting antimicrobial effect they may preserve the foods by reducing lipid oxidation as they are reported to have significant antioxidant activity [133]. A wide variety of phenolic substances derived from herbs and spices possess potent bio- logical activities, which contribute to their preservative potential [134]. Careaga et al. [135] reported that 1.5 ml/100 g of capsicum ex- tract was sufficient to prevent the growth of *Salmonella typhimurium* in raw beef but that 3 ml/100 g was required for a bactericidal effect against *P. aeruginosa*. Treatment with hydrosol of thyme, black cumin, sage, rosemary and bay leaf was reported to reduce *S. typhimurium* and *E. coli* O157:H7 in apple and carrots [136]. Black cumin ethanolic extract applied in a marinade base for raw trout was found to reduce aerobic plate count, yeast, and coliforms [137]. Lee et al. [138] observed that the addition of green tea or rosemary (1 or 3%) to rice cakes significantly reduced the levels of *B. cereus* and *S. aureus* during 3 days storage at room temperature (22°C). Ahn et al. [139] reported that a range of plant extracts are useful for reduction of pathogens associated with cooked beef, however, Uhart et al. [140] reported that spices inactivate *S. typhimurium* DT104 in *in vitro* condition, but the activity decreased considerably when added to a complex food system such as ground beef. Kim et al. [141] observed that ground beef samples did not show significant difference in *L. monocytogenes*, *S. aureus* and total bacterial counts after treatment with green and jasmine tea as compare to untreated samples, however, a slight reduction in viable count of *Salmonella enterica Serotype Enteritidis* and *Listeria monocytogenes* in ground beef by water-soluble arrowroot tea extract (upto 6% w/w) was reported [142]. Combination of

different plant extracts showed better preservative effects on meat as rosemary extracts and dry powders of orange and lemon applied to beef meatballs were found to be effective in controlling bacterial spoilage during 12 days storage period at 8°C [143]. Mixtures of Scutellaria, honeysuckle, Forsythia and cinnamon or cinnamon, rosemary and clove oil showed 1.81–2.32-log reductions in microbial counts as compare to control in vacuum-packaged fresh pork during 28 days storage [144]. Yin and Cheng [145] reported that the antimicrobial properties of garlic are due to organosulfur compounds. Freshly ground garlic, when added to mayonnaise at a concentration of 1% reduced *Salmonella* count [146]. Garlic also has been shown to reduce the levels of *E. coli* in ground meat [147]. Sallam et al. [148] observed that addition of fresh garlic and garlic powder controlled microbial contamination and preserved chicken sausages. Species of the genus *Mentha* (family *Lamiaceae*) are a rich source of polyphenolic compounds, flavonoids, terpenoids, and other volatile compounds, which imparts it a strong antimicrobial property [149]. Nguyen and Mittal [150] reported more than 8 log reductions in the artificially inoculated pasteurized tomato juice when mint was used as a preservative.

Turmeric, a tropical herb of *Zingiberaceae* family is used in Indian cuisine mainly for its coloring and flavoring characteristics, and curcumin is the active constituent of turmeric responsible for its preservative action [151]. Even, the byproducts of curcumin manufacture were found to have high biological activity [152, 153]. Turmeric extract (1.5%, v/v) alone or in combination with shallot extract (1.5% each, v/v) were found to retain quality characteristics of vacuum-packaged rainbow trout (*Oncorhynchus mykiss*) during a refrigerated storage of over a period of 20 days [154].

Cinnamon is the source of cinnamon bark, fruit, leaf and their essential oils and many *Cinnamomum* species yield a volatile oil on distillation with different aroma characteristics and composition [155, 156]. Extracts of the cinnamon bark and fruit and cinnamon oil have been reported to possess antimicrobial, antioxidant and antimutagenic activities [157]. Cinnamon was found to reduce the levels of *E. coli* in apple juice [158]. Yuste and Fung [159] reported up to 6 log cfu/ml reductions of artificially inoculated *L. monocytogenes* in pasteurized apple juice with 0.1–0.3% (w/v) of ground cinnamon after 1 h of incubation, and no further growth of the microorganism occurred during 7 days of storage. Ceylan et al. [158] reported that the addition of 0.3% (w/v) cinnamon powder gradually decreased the counts of *E. coli* O157:H7 in pasteurized apple juice, whereas only 2 log cfu/ml reduction of *E. coli* O157:H7 in unpasteurized apple cider was reported even by addition of 2% (w/v) cinnamon powder [160].

Punica granatum L. has a rich history of traditional use of its bark, leaves, flowers, fruits and seeds to ameliorate diseases. The presence of phytocompounds in the pomegranate extracts such as phenols, tannins and flavonoids as major active constituents may be responsible for these medicinal values [161, 162]. Several studies have reported the efficacy of various extracts from the different parts of pomegranate plant against the growth of Gram positive and Gram negative bacteria [163]. Aqueous and ethanolic fruit shell extracts of *P. granatum* were found to have antibacterial activity against different strains of *E. coli* [164], *Salmonella* Typhi [165], and it inhibited Staphylococcal enterotoxin A production [166]. Various other solvent extracts from the rind of *P. granatum* also showed antibacterial activity against enterohaemorrhagic *E. coli* and food spoilage bacteria [162]. Pomegranate peel extracts were used to enhance the shelf life of chicken meat products by controlling oxidative rancidity and bacterial growth [167].

Various species of *Garcinia* contains several secondary metabolites which exhibit a wide range of biological and pharmacological activities such as antimicrobial, antioxidant, antitumour-promoting and cytotoxic activities [168].

Likhitwitayawuid et al. [169, 170] reported antimalarial activity of xanthones isolated from the bark of *G. dulcis* and *G. cowa*. Crude extracts as well as partially purified compounds from different parts of some species of Garcinia have shown antibacterial potential [171]. A polyisoprenylated benzophenone (garcinol) isolated from stem bark of *G. huillensis* has been shown to be active against Gram positive and Gram negative cocci, mycobacteria and fungi but inactive against Gram negative enteric bacilli, yeast and viruses [172]. Alpha-mangostin, rubraxanthone, and xanthochymol isolated from *G. mangostana*, *G. diocia* and *G. subelliptica*, respectively, showed strong antibacterial activity against both methicillin-resistant and methicillin-sensitive *S. aureus* [173, 174]. Crude extracts of leaves, fruits, root, stem and trunk bark of *G. atroviridis* exhibited antibacterial activity with the root extract showing the strongest inhibition, while the fruit and leaf extracts exhibited significant antifungal activity against *Cladosporium herbrum* [168]. Crude extracts of *G. indica* also showed antiaflatoxigenic properties [175].

Seabuckthorn has been widely used in traditional medicines, mainly of Tibetian, Mongolian, Chinese and Middle Asian cultures [176, 177] for the treatment of asthma, skin diseases, gastric ulcers, lung disorders, cough, diarrhea, and menstrual disorder [178]. The health benefits of *Hippophae rhamnoides* oils, juice, leaves and bark are also well known and they have been used to treat several diseases [179]. All parts of the seabuckthorn plant are considered to be rich source of a large number of bioactive substances and are reported to have antimicrobial [180], antioxidant [181], and antimutagenic activities [182]; and antitumoral, hepato-protective and immunumodulatory [183], anti-platelet aggregating [184], anti-inflammatory [185], and radio-protective properties [186]. The leaf extract was reported to have better immunomodulatory effect than fruit extracts [183]. Jelly prepared by using seabuckthorn berries showed microbiological stability at ambient temperature and 37°C for a period of 6 months [187]. Various other plant extracts were found to be effective against *L. monocytogenes* in refrigerated meat products [188, 189]. The effect of a mixture of oregano and cranberry (0.1 mg of phenolic/ml) on beef slices and cod fish filet was studied by Lin et al. [190] and they observed that at pH 7, phytochemicals have no significant effect on cell numbers after 18 h of incubation, but at pH 6.0, differences in viable cell counts were observed in beef and fish slices. The oregano-cranberry extract mixture showed higher log reduction in viable counts than the slices treated with either oregano or cranberry extract [190]. Ruiz et al. [191] also reported that although rosemary extract was not able to completely eliminate *L. monocytogenes* in ready-to-eat vacuum-packaged diced turkey and ham, it significantly decreased the counts when used along with nisin. Cranberry powder alone at 1, 2, and 3% levels resulted in 2–4 log cfu/g reduction in growth of *L. monocytogenes* compared to the control (treated with nitrite, $p \leq 0.05$), and similar effect on growth was seen when it was combined with cherry powder, lime powder and grape seed extracts in a cured cooked meat model system [192]. Grape seed extract and pine bark extract were used to control the growth of artificially inoculated bacteria on the surface of raw ground beef during refrigerated storage [193]. The combination of grape seed extract and nisin gave the greatest inhibitory activity with reductions of *L. monocytogenes* populations to undetectable levels after 21 days indicating potential of natural antimicrobials to control the growth and recontamination of *L. monocytogenes* on meat products [194].

Dried plum puree was found to reduce *E. coli* and *Salmonella* in ground meat [195]. Karapinar and Sengun [196] recommended use of unripe grape juice for enhancing the safety of salad vegetables. Grape pomace extract and olive extracts showed antimicrobial activity in apple juice [197]. Grape seed extract (1%) and rosemary oleoresin (1%) reduced the populations of *E. coli* O157:H7, *S. typhimurium* and *L. monocytogenes* after 9 days in raw ground beef [193]. Owen and Palombo

[198] investigated the ability of *Eremophila duttonii* and *E. alternifolia* to control the growth of *L. monocytogenes* in full cream milk, skim milk, diluted homogenates of salami, pate and brie cheese, and reported that both the extracts inhibited the growth of *L. monocytogenes* in salami at 37°C, only *E. duttonii* extract was effective in pate at 4°C storage, and growth of *L. monocytogenes* was not affected by both the extracts in other products. Reduction in microbial load by water-soluble extract from pine needles of *Cedrus deodara* in fresh-squeezed tomato juice [199] and by the extracts from cinnamon stick, oregano, clove, pomegranate peel and grape seeds in raw pork over 9 days storage at ambient temperature was reported by Shan et al. [130].

14. Future potential

The identification and evaluation of natural products for the control of pathogens and to assure consumers a safe, wholesome and nutritious food supply is a challenge. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Even though pharmacological industries have produced a number of new antibiotics in the last few decades, resistance to these drugs by microorganisms has increased. Plants contain thousands of constituents and are valuable sources of new and biologically active molecules possessing antimicrobial properties. The current focus in natural preservatives is on a small number of antimicrobial agents, which have been used for many years, and there is a need to expand this list for their food application to ensure safety and quality of the food products. There is no shortage of candidates to become the food preservatives of the future, but still many obstacles exist on the road to all-natural preservation. There are very few natural antimicrobials that can be used as direct replacements for existing preservatives owing to their lower effectiveness, higher cost and product organoleptic quality deterioration. Further, if a natural antimicrobial with potential as a food preservative can be shown to be sufficiently effective in foods, it will need regulatory approval before it can be used as a food additive. Once declared additive on the label, consumers will have different perspectives about these antimicrobials, but it is possible to classify them as processing aids, thus consumer perception of them being an additive can be avoided. Therefore, for the successful exploitation of the natural antimicrobials as food preservatives, probably it will not only require changes in legislation but also require better consumer education.

The information available to date demonstrates that different antimicrobials of plant origin can effectively reduce or inhibit pathogenic and spoilage microorganism, and thus have a potential to become a good alternative to synthetic antimicrobials. The development of cost effective isolation and purification procedures that avoid loss of functional properties of active compounds will aid in wider use and acceptance of plant extracts as natural preservatives. However, too much focus on the use of single compounds over mixtures may not be compatible with complex plant extracts in which valuable bioactive molecules are often present in mixed form and the biological activity of plant extracts mostly results from additive or synergistic effects of these components. Further, the use of natural antimicrobials in combination with another or with other technologies in a multi-hurdle preservation system can enhance the performance of natural antimicrobials. Studies have demonstrated that natural antimicrobial agents may offer unique advantages for food processing, and in addition to improving the shelf life and safety of foods; they may allow novel food products with enhanced quality and nutritional properties. The applications of natural antimicrobial agents are likely to grow steadily in the future

because of consumer demand for minimal processing and food containing naturally derived preservatives is on rise. Further, it is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens. The impact of product formulation and storage parameters on the efficacy of natural antimicrobials as well as safety and toxicology evaluation of these natural antimicrobials require an in-depth study.

Antimicrobial and antioxidative packaging systems containing natural biological active substances may have high potential for commercial food packaging applications. Consumers would prefer to obtain better food safety of fresh produce and minimally processed foods using this type of packaging system. However, it is necessary that new active packaging systems must satisfy food safety regulations, which are different in each country. Greater food safety and quality assurance may be improved by the use of both antimicrobial and antioxidative packaging systems incorporating natural active agents. Some commercial products, such as films coated with wasabi extract, are already on the market and satisfy the regulations and consumer needs of particular countries. Most materials containing natural active agents are more effective when there is direct contact of the packaging materials with the food product. For new packaging systems to be introduced into the market effectively, careful design is required. Food contact layers with the appropriate concentrations of the active agents may be laminated or coated onto the barrier layer of the package structure. There are some suggestions that before long many countries would adopt the new active packaging concepts into their packaging regulations. Therefore, new applications of antimicrobial and antioxidative packaging are likely to be available in the market sooner or later.

Also, the products which produced by nanotechnology as (Aquanova product) the product description include "Aquanova's recent nanotech antioxidant system for essential oils and flavours is a clear signpost of where food ingredient technology in the twenty-first century is headed." It is designed to help manufacturers introduce antioxidants into food and beverage products easily and effectively. The product micelle allows to create crystal clear solutions ("solubilisates") of lipophilic or waterinsoluble substances not just from a visual standpoint. The product micelle is stable with respect to pH and temperature and has a diameter of approximately 30 nm. Here, where microemulsions and liposomes prove to be problematical and unsuitable, the product micelle represents the optimum solution in the fields of foodstuffs (functional food), cosmetics, pharmaceuticals and biotechnology (nutritional solutions for cell and bacterial cultures). The 100% water-soluble micelle can be integrated directly and independently of recipe characteristics into final products in the quoted fields. The product micelle is proving to be an optimum carrier system of hydrophobic substances for a higher and faster intestinal and dermal resorption and penetration of active ingredients.

On the other hand, it is important we must be realize that further investigations into the deleterious or adverse biological effects of essential oils in *in vivo* models need to be performed. By doing so, we can better understand their mechanisms of action in combating disease, and better evaluate the quantities at which they best exert their beneficial actions to improving human health.

15. Conclusion

Natural materials should be considered as potential alternative. Owing to the new attraction for natural products like essential oils and plant extracts, despite their wide use, it is important to develop a better understanding of their mode of biological action for new applications in human health, agriculture and the

environment. To preserve food safely and to prevent human beings from various degenerative diseases we can use essential oils as natural antioxidants and anti-mutagenic preferably over synthetic. Essential oils can be incorporated into rinses or mouth washes as antibacterial to protect from infection and for general improvement of oral health. The essential oils have also shown good antiviral activity but antiviral activity against the major viruses of twenty-first century such as HIV and hepatitis C viruses should also be studied. As the essential oils and plant extracts have useful biological properties, so their uses in food and pharmaceutical industry should be more as natural ingredients instead of synthetic chemicals, to save and protect the ecological equilibrium.

Author details

Hamdy A. Shaaban
Chemistry of Flavor and Aroma Department, National Research Center, Dokki, Cairo, Egypt

*Address all correspondence to: hamdy.shaaban64@gmail.com

References

[1] Guenther E. The Essential Oil. Vol. IV. New York: D.Van Nostrand; 1950

[2] El-Ghorab AH, Fadel HM, El-massry KF. The Egyptian *Eucalyptus camaldulensis* var. brevirostris: Chemical composition of the fruit volatile oil and antioxidant activity. Flavour and Fragrance Journal. 2002;**17**:306-312

[3] Angioni A, Barra A, Coroneo V, Dessi S, Cabras P. Chemical composition, seasonal variability, and antifungal activity of *Lavandula stoechas* L. ssp. stoechas essential oils from stem/leaves and flowers. Journal of Agricultural and Food Chemistry. 2006;**54**:4364-4370

[4] Masotti V, Juteau F, Bessiere JM, Viano J. Seasonal and phenological variations of the essential oil from the narrow endemic species *Artemisia molinieri* and its biological activities. Journal of Agricultural and Food Chemistry. 2003;**51**:7115-7121

[5] Smith RL, Cohen SM, Doull J, Feron VJ, Goodman JI, Marnett LJ, et al. A procedure for the safety evaluation of natural flavor complexes used as ingredients in food: Essential oils. Food and Chemical Toxicology. 2005;**43**:345-363

[6] Hajhashemi V, Ghannadi A, Sharif B. Anti-inflammatory and analgesic properties of the leaf extracts and essential oil of *Lavandula angustifolia* mill. Journal of Ethnopharmacology. 2003;**89**:67-71

[7] Perry NS, Bollen C, Perry EK, Ballard C. Salvia for dementia therapy: Review of pharmacological activity and pilot tolerability clinical trial. Pharmacology, Biochemistry, and Behavior. 2003;**75**:651-659

[8] Silva J, Abebe W, Sousa SM, Duarte VG, MIL M, FJA M. Analgesic and anti-inflammatory effects of essential oils of *Eucalyptus*. Journal of Ethnopharmacology. 2003;**89**:277-283

[9] Dorman HJD, Surai P, Deans SG. In vitro antioxidant activity of a number of plant essential oils and phytoconstituents. Journal of Essential Oil Research. 2000;**12**:241-248

[10] Lee KG, Shibamoto T. Determination of antioxidant potential of volatile extracts isolated from various herbs and spices. Journal of Agricultural and Food Chemistry. 2002;**50**:4947-4952

[11] Park KK, Chun KS, Lee JM, Lee SS, Surh YJ. Inhibitory effects of [6]-gingerol, a major pungent principle of ginger, on phorbol ester-induced inflammation, epidermal ornithine decarboxylase activity and skin tumor promotion in ICR mice. Cancer Letters. 1998;**129**:139-144

[12] Vazquez B, Avila G, Segura D, Escalante B. Antiinflammatory activity of extracts from aloe vera gel. Journal of Ethnopharmacology. 1996;**55**:69-75

[13] Elgayyar M, Draughon FA, Golden DA, Mount JR. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. Journal of Food Protection. 2001;**64**:1019-1024

[14] Lima ED, Gompertz OF, Paulo MD, Giesbrecht AM. In vitro antifungal activity of essential oils against clinical isolates of dermatophytes. Revista de Microbiologia. 1992;**23**:235-238

[15] Schnitzler P, Koch C, Reichling J. Susceptibility of drug-resistant clinical herpes simplex virus type 1 strains to essential oils of ginger, thyme, hyssop, and sandalwood. Antimicrobial Agents and Chemotherapy. 2007;**51**: 1859-1862

[16] Schnitzler P, Schon K, Reichling J. Antiviral activity of Australian tea tree oil and eucalyptus oil against herpes simplex virus in cell culture. Pharmazie. 2001;**56**:343-347

[17] Aruna K, Sivaramakrishnan VM. Anticarcinogenic effects of the essential oils from cumin, poppy and basil. Phytotherapy Research. 1996;**10**:577-580

[18] Gaysinsky S, Weiss J. Aromatic and spice plants: Uses in food safety. Stewart Postharvest Review. 2007;**4**(5):1-9

[19] Zink DL. The impact of consumer demands and trends on food processing. Emerging Infectious Diseases. 1997;**3**(4):467-469

[20] Uhl SR. Handbook of Spices, Seasonings, and Flavorings. Lancaster, PA: Technomic Publishing; 2000

[21] El-Massry KF, Farouk A, Abou-Zeid M. Free radical scavenging activity and lipoxygenase inhibition of rosemary (*Rosmarinus officinalis* L) volatile oil. Journal of Essential Oil-Bearing Plants. 2008;**11**(5):536-543

[22] Kordali S, Cakir A, Ozer H, Cakmakci R, Kesdek M, Mete E. Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish *Origanum acutidens* and its three components, carvacrol, thymol and p-cymene. Bioresource Technology. 2008;**99**(18):8788-8795

[23] Singh G, Maurya S, de Lampasona MP, Catalan CAN. A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. Food and Chemical Toxicology. 2007;**45**(9):1650-1661

[24] Zawirska-Wojtasiak R, Wasowicz E. GC analysis of rosemary aroma isolated traditionally by distillation and by SPME. Journal of Essential Oil Research. 2009;**21**(1):8-15

[25] Shan B, Cai YZ, Brooks JD, Corke H. The in vitro antibacterial activity of dietary spice and medicinal herb extracts. International Journal of Food Microbiology. 2007;**117**(1):112-119

[26] Shan B, Cai YZ, Sun M, Corke H. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. Journal of Agricultural and Food Chemistry. 2005;**53**(20):7749-7759

[27] Burt S. Essential oils: Their antibacterial properties and potential applications in foods—A review. International Journal of Food Microbiology. 2004;**94**:223-253

[28] Davidson PM. Food antimicrobials: Back to nature. Acta Horticulturae. 2006;**709**(ISHS):29-33

[29] Patrignani F, Iucci L, Belletti N, Gardini F, Guerzoni ME, Lanciotti R. Effects of sub-lethal concentrations of hexanal and 2-(E)-hexenal on membrane fatty acid composition and volatile compounds of *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella enteritidis* and *Escherichia coli*. International Journal of Food Microbiology. 2008;**123**(1-3):1-8

[30] Ponce AG, Roura SI, Del Valle CE, Moreira MR. Antimicrobial and antioxidant activities of edible coatings enriched with natural plant extracts: In vitro and in vivo studies. Postharvest Biology and Technology. 2008;**49**(2):294-300

[31] Lopez-Malo Vigil A, Palou E, Alzamora SM. Naturally occurring compounds plant sources. In: Davidson PM, Sofos JN, Branen AL, editors. Antimicrobials in Food. 3rd ed. Boca Raton, Florida: CRC Press; 2005. pp. 429-446

[32] Moriera MR, Ponce AG, Del Valle CE, Roura S. Effects of clove and tea tree oils on *Escherichai coli* O157:H7

in blanching spinach and minced cooked beef. Journal of Food Processing and Preservation. 2007;**31**:379-391

[33] Periago PM, Conesa R, Delgado B, Fernȗndez PS, Palop A. *Bacillus megaterium* spore germination and growth inhibition by a treatment combining heat with natural antimicrobials. Food Technology and Biotechnology. 2006;**44**(1):17-23

[34] Razzaghi-Abyaneh M, Shams-Ghahfarokhi M, Rezaee MB, Jaimand K, Alinezhad S, Saberi R, et al. Chemical composition and anti aflatoxigenic activity of *Carum carvi* L., *Thymus vulgaris* and *Citrus aurantifolia* essential oils. Food Control. 2009;**20**:1018-1024

[35] Zaika LL. Spices and herbs: Their antimicrobial activity and its determination. Journal of Food Safety. 1988;**9**(2):97-118

[36] Betts TJ. Chemical characterisation of the different types of volatile oil constituents by various solute retention ratios with the use of conventional and novel commercial gas chromatographic stationary phases. Journal of Chromatography. A. 2001;**936**:33-46

[37] Bowles EJ. Chemistry of Aromatherapeutic Oils. NSW, Australia: Allen & Unwin; 2003. Available from: www.allenandunwin.com

[38] Croteau R, Kutchan TM, Lewis NG. Natural products (secondary metabolites). In: Buchanan B, Gruissem W, Jones R, editors. Biochemistry and Molecular Biology of Plants. Rockville, USA: American Society of Plant Physiologists; 2000

[39] Pichersky E, Noel JP, Dudareva N. Biosynthesis of plant volatiles: Nature's diversity and ingenuity. Science. 2006;**311**:808-811

[40] El-Ghorab AH, Shaaban HA, El-massry KF, Shibamoto T. Chemical composition of volatile extract and biological activities of volatile and less-volatile extracts if juniper berry (*Juniperus drupacea* L.) fruit. Journal of Agricultural and Food Chemistry. 2008;**56**:5021-5025

[41] El-Ghorab AH, Shibamoto T, Ozcan MM. Chemical composition and antioxidant activities of buds and leaves of capers (*Capparis ovata* Desf. Var. canescens) cultivated in Turkey. Journal of Essential Oil Research. 2007;**19**:72-77

[42] Hulin V, Mathot A, Mafart P. Les propriétés antimicrobiennes des huiles essentielles et composés d'arômes. Sciences des Aliments. 1998;**18**:563-582

[43] Penalver P, Huerta B, Borge C, Astorga R, Romero R, Perea A. Antimicrobial activity of five essential oils against origin strains of the Enterobacteriaceae family. APMIS. 2005;**113**:1-6

[44] Santoyo S, Cavero S, Jaime L, Ibanez E, Senorans J, Reglero G. Supercritical carbon dioxide extraction of compounds with antimicrobial activity from *Origanum vulgare* L.: Determination of optimal extraction parameters. Journal of Food Protection. 2006;**69**:369-375

[45] Wannissorn B, Jarikasem S, Siriwangchai T, Thubthimthed S. Antibacterial properties of essential oils from Thai medicinal plants. Fitoterapia. 2005;**76**:233-236

[46] Larson E. Hygiene of the skin: When is clean too clean? Emerging Infectious Diseases. 2001;**7**:225-230

[47] Carson C, Riley T. Toxicity of the essential oil of *Melaleuca alternifolia* or tea tree oil. Journal of Toxicology. Clinical Toxicology. 1995;**33**:193-195

[48] Bozin B, Mimica-Dukic N, Simin N, Anackov G. Characterization of the volatile composition of essential oils

of some *Lamiaceae* spices and the antimicrobial and antioxidant activities of the entire oils. Journal of Agricultural and Food Chemistry. 2006;**54**:1822-1828

[49] Kelly D. The physiology and metabolism of the human gastric pathogen (*Helicobacter pylori*). Advances in Microbial Physiology. 1998;**40**:137-189

[50] O'Gara E, Hill D, Maslin D. Activities of garlic oil, garlic powder, and their diallyl constituents against *Helicobacter pylori*. Applied and Environmental Microbiology. 2000;**66**:2269-2273

[51] Charles C, Mostler K, Bartels L, Mankodi S. Comparative antiplaque and antigingivitis effectiveness of a chlorhexidine and an essential oil mouthrinse: 6-month clinical trial. Journal of Clinical Periodontology. 2004;**31**:878-884

[52] Juven J, Kanner J, Schved F, Weisslowicz H. Factors that interact with antimicrobial action of thyme essential oil and its active constituents. The Journal of Applied Bacteriology. 1994;**76**:626-631

[53] Yengopal V. Preventative dentistry: Essential oils andoral malodour. SADJ. 2004;**59**:204-206

[54] Tripathi P, Dubey NK, Shukla AK. Use of some essential oils as postharvest botanical fungicides in the management of grey mould of grapes caused by *Botrytis cinerea*. World Journal of Microbiology and Biotechnology. 2008;**24**:39-46

[55] Fisher K, Phillips C. Potential antimicrobial uses of essential oils in food: Is citrus the answer? Trends in Food Science and Technology. 2008;**19**(2):156-164

[56] Daferera DJ, Ziogas BN, Polissiou MG. GC-MS analysis of essential oils from some Greek aromatic plants and their fungi toxicity on *Penicillium digitatum*. Journal of Agricultural and Food Chemistry. 2000;**48**(6):2576-2581

[57] Razzaghi-Abyaneh M, Shams-Ghahfarokhi M, Yoshinari T, Rezaee MB, Jaimand K, Nagasawa H, et al. Inhibitory effects of *Satureja hortensis* L. essential oil on growth and aflatoxin production by *Aspergillus parasiticus*. International Journal of Food Microbiology. 2008;**123**(3):228-233

[58] El-Seedi HR, Khattab A, Gaara AHM, Mohamed TK, Hassan NA, Elkattan AE. Essential oil analysis of *Micromeria nubigena* H.B.K. and its antimicrobial activity. Journal of Essential Oil Research. 2008;**20**:452-456

[59] Davidson PM, Naidu AS. Phytophenols. In: Naidu AS, editor. Natural Food Antimicrobial Systems. Boca Raton, Florida: CRC Press; 2000. pp. 265-295

[60] Nejad Ebrahimi S, Hadian J, Mirjalili MH, Sonboli A, Yousefzadi M. Essential oil composition and antibacterial activity of *Thymus caramanicus* at different phenological stages. Food Chemistry. 2008;**110**(4):927-931

[61] Stefanello MEA, Cervi AC, Ito IY, Salvador MJ, Wisniewski A Jr, Simionatto EL. Chemical composition and antimicrobial activity of essential oils of *Eugenia chlorophylla* (Myrtaceae). Journal of Essential Oil Research. 2008;**20**(1):75-78

[62] Suhr KI, Nielsen PV. Antifungal activity of essential oils evaluated by two different application techniques against rye bread spoilage fungi. Journal of Applied Microbiology. 2003;**94**(4):665-674

[63] Goni P, Lopez P, Sanchez C, Gomez-Lus R, Becerril R,

Nerin C. Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. Food Chemistry. 2009;**116**:982-989

[64] Veluri R, Weir TL, Bais HP, Stermitz FR, Vivanco JM. Phytotoxic and antimicrobial activities of catechin derivatives. Journal of Agricultural and Food Chemistry. 2004;**52**:1077-1082

[65] Arques JL, Rodriguez E, Nunez M, Medina M. Inactivation of gramnegative pathogens in refrigerated milk by reuterin in combination with nisin or the lactoperoxidase system. European Food Research and Technology. 2008;**227**(1):77-82

[66] Burt SA, Der Zee RV, Koets AP, De Graaff AM, Van Knapen F, Gaastra W, et al. Carvacrol induces heat shock protein 60 and inhibits synthesis of flagellin in *Escherichia coli* O157:H7. Applied and Environmental Microbiology. 2007;**73**:4484-4490

[67] Lanciotti R, Gianotti A, Patrignani F, Belletti N, Guerzoni ME, Gardin F. Use of natural aroma compounds to improve shelf life and safety of minimally processed fruits. Trends in Food Science and Technology. 2004;**15**(3-4):201-208

[68] Proestos C, Boziaris I, Kapsokefalou SM, Komaitis M. Natural antioxidant constituents from selected aromatic plants and their antimicrobial activity against selected pathogenic microorganisms. Food Technology and Biotechnology. 2008;**46**:151-156

[69] Silva FG, Oliveira CBA, Pinto JEBP, Nascimento VE, Santos SC, Seraphin JC, et al. Seasonal variability in the essential oils of wild and cultivated *Baccharis trimera*. Journal of the Brazilian Chemical Society. 2007;**18**:990-997

[70] Skocibusic M, Bezic N, Dunkic V. Phytochemical composition and antimicrobial activities of the essential oils from *Satureja subspicata* Vis. growing in Croatia. Food Chemistry. 2006;**96**(1):20-28

[71] Turgis M, Han J, Caillet S, Lacroix M. Antimicrobial activity of mustard essential oil against *Escherichia coli* O157:H7 and *Salmonella typhi*. Food Control. 2009;**20**:1073-1079

[72] Chouliara E, Karatapanis A, Savvaidis IN, Kontominas MG. Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4 _C. Food Microbiology. 2007;**24**(6):607-617

[73] Brandi G, Amagliani G, Schiavano GF, De Santi M, Sisti M. Activity of *Brassica oleracea* leaf juice on food borne pathogenic bacteria. Journal of Food Protection. 2006;**69**(9):2274-2279

[74] Lis-Balchin M, Steyrl H, Krenn E. The comparative effect of novel Pelargonium essential oils and their corresponding hydrosols as antimicrobial agents in a model food system. Phytotherapy Research. 2003;**17**:60-65

[75] Rameshkumar KB, George V, Shiburaj S. Chemical constituents and antibacterial activity of the leaf oil of *Cinnamomum chemungianum* Mohan et Henry. Journal of Essential Oil Research. 2007;**19**:98-100

[76] Abdalla AEM, Darwish SM, Ayad EHE, El-Hamahmy RM. Egyptian mango by-product 2: Antioxidant and antimicrobial activities of extract and oil from mango seed kernel. Food Chemistry. 2007;**103**(4):1141-1152

[77] Becerril R, Gomez-Lus R, Goni P, Lopez P, Nerin C. Combination of analytical and microbiological techniques to study the antimicrobial activity of a new active food packaging containing cinnamon or oregano

against *E. coli* and *S. aureus*. Analytical and Bioanalytical Chemistry. 2007;**388**(5-6):1003-1011

[78] Rota MC, Herrera A, Martinez RM, Sotomayor JA, Jordan MJ. Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. Food Control. 2008;**19**:681-687

[79] Lambert RJW, Skandamis PN, Coote PJ, Nychas GJE. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. Journal of Applied Microbiology. 2001;**91**(3):453-462

[80] Thomas LV, Isak T. Nisin synergy with natural antioxidant extracts of the herb rosemary. Acta Horticulturae. 2006;**709**:109-113

[81] Randrianarivelo R, Sarter S, Odoux E, Brat P, Lebrun M, Romestand B, et al. Composition and antimicrobial activity of essential oils of *Cinnamosma fragrans*. Food Chemistry. 2009;**114**:680-684

[82] Gutierrez J, Barry-Ryan C, Bourke P. The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. International Journal of Food Microbiology. 2008;**124**(1):91-97

[83] Allahverdiyev A, Duran N, Ozguven M, Koltas S. Antiviral activity of the volatile oils of *Melissa officinalis* L. against herpes simplex virus type-2. Phytomedicine. 2004;**11**:657-661

[84] Garcia C, Talarico L, Almeida N, Colombres S, Duschatzky C, Damonte B. Virucidal activity of essential oils from aromatic plants of San Luis, Argentina. Phytotherapy Research. 2003;**17**:1073-1075

[85] Armaka M, Papanikolaou E, Sivropoulou A, Arsenakis M. Antiviral properties of isoborneol, a potent inhibitor of herpes simplex virus type-1. Antiviral Research. 1999;**43**:79-92

[86] Edris AE. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents. Phytotherapy Research. 2007;**21**:308-323

[87] Stoicov C, Saffari R, Houghton JM. Green tea inhibits *Helicobacter* growth in vivo and in vitro. International Journal of Antimicrobial Agents. 2009;**33**:473-478

[88] Bajpai VK, Rahman A, Kang SC. Chemical composition and inhibitory parameters of essential oil and extracts of Nandina domestica Thunb. To control food-borne pathogenic and spoilage bacteria. International Journal of Food Microbiology. 2008;**125**(2):117-122

[89] Sofia PK, Prasad R, Vijay VK, Srivastava AK. Evaluation of antibacterial activity of Indian spices against common food borne pathogens. International Journal of Food Science and Technology. 2007;**42**:910-915

[90] Naidu AS. Chapter 1: Overview. In: Naidu AS, editor. Natural Food Antimicrobial Systems. Boca Raton, Florida: CRC Press; 2000. pp. 1-16 E

[91] Romeo FV, De Luca S, Piscopo A, Poiana M. Antimicrobial effect of some essential oils. Journal of Essential Oil Research. 2008;**20**(4):373-379

[92] Ramel C, Alekperov UK, Ames BN, Kada T, Wattenberg LW. International commission for protection against environmental mutagens and carcinogens. ICPEMC publication No. 12. Inhibitors of mutagenesis and their relevance to carcinogenesis. Report by ICPEMC expert group on antimutagens and desmutagens. Mutation Research. 1986;**168**:47-65

[93] Kada T, Shimoi K. Desmutagens and bio-antimutagens—Their modes of action. BioEssays. 1987;**7**:113-116

[94] Odin AP. Vitamins as antimutagens: Advantages and some possible mechanisms of antimutagenic action. Mutation Research. 1997;**386**:39-67

[95] Kuo ML, Lee KC, Lin JK. Genotoxicities of nitropyrenes and their modulation by apigenin, tannic acid, ellagic acid and indole-3-carbinol in the *Salmonella* and CHO systems. Mutation Research. 1992;**270**:87-95

[96] De-Oliveira ACAX, Ribeiro-Pinto LF, Paumgartten FJR. In vitro inhibition of CYP2B1 monooxygenase by b myrcene and other monoterpenoid compounds. Toxicology Letters. 1997;**92**:39-46

[97] Bakkali F, Averbeck S, Averbeck D, Idaomar D. Biological effects of essential oils. A review. Food and Chemical Toxicology. 2008;**46**:446-475

[98] Bakkali F, Averbeck S, Averbeck D, Zhiri A, Baudoux D, Idaomar M. Antigenotoxic effects of three essential oils in diploid yeast (*Saccharomyces cerevisiae*) after treatments with UVC radiation, 8-MOP plus UVA and MMS. Mutation Research. 2006;**606**:27-38

[99] Van Houten B, Woshner V, Santos JH. Role of mitochondrial DNA in toxic responses to oxidative stress. DNA Repair. 2006;**5**:145-152

[100] El-massry KF, El-Ghorab AH. Effect of essential oils and non-volatile extracts of some aromatic plants on Cu++_induced oxidative modification of human low-density lipoprotein(LDL). JEOBP. 2006;**3**:292-299

[101] Halliwell B, Gutteridge J. The antioxidants of human extracellular fluids. Archives of Biochemistry and Biophysics. 1990;**280**:1-8

[102] Tomaino A, Cimino F, Zimbalatti V. Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. Food Chemistry. 2005;**89**:549-554

[103] Sokmen A, Gulluce M, Akpulat A. The in vitro antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic *Thymus spathulifolius*. Food Control. 2004;**15**:627-634

[104] Botsoglou N, Florou-Paneri P, Christaki E, Giannenas I, Spais A. Performance of rabbits and oxidative stability of muscle tissues as affected by dietary supplementation with oregano essential oil. Archives of Animal Nutrition. 2004;**58**:209-218

[105] Papageorgiou G, Botsoglou N, Govaris A, Giannenas I, Iliadis S, Botsoglou E. Effect of dietary oregano oil and alpha-tocopheryl acetate supplementation on iron-induced lipid oxidation of Turkey breast, thigh, liver and heart tissues. Journal of Animal Physiology and Animal Nutrition. 2003;**87**:324-335

[106] Tepe B, Donmez E, Unlub M. Antimicrobial and antioxidative activities of the essential oils and methanol extracts of *Salvia cryptantha* (Montbret et Aucher ex Benth.) and *Salvia multicaulis* (Vahl). Food Chemistry. 2004;**84**:519-525

[107] Candan F, Unlu M, Tepe B. Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. millefolium Afan. (Asteraceae). Journal of Ethnopharmacology. 2003;**87**:215-220

[108] Mau J, Laib E, Wang N, Chen C, Chang C, Chyau C. Composition and antioxidant activity of the essential oil from *Curcuma zedoaria*. Food Chemistry. 2003;**82**:583-591

[109] Mimica-Dukic N, Bozin B, Sokovic M, Simin N. Antimicrobial and antioxidant activities of *Melissa officinalis* L. (Lamiaceae) essential oil. Journal of Agricultural and Food Chemistry. 2004;**52**:2485-2489

[110] Mimica-Dukic N, Bozin B, Sokovic M, Mihajlovic B, Matavulj M. Antimicrobial and antioxidant activities of three Mentha species essential oils. Planta Medica. 2003;**69**:413-419

[111] Pokorny J. Chapter no. 10: Sources of natural antioxidants: Vegetables, fruits, herbs, spices and teas. In: Yanishlieva-Maslarova NV, editor. Antioxidant in Food. UK: Woodhead Publishing Ltd; 2001

[112] Averbeck D, Averbeck S, Dubertret L, Young AR, Morliere P. Genotoxicity of bergapten and bergamot oil in *Saccharomyces cerevisiae*. Journal of Photochemistry and Photobiology. B. 1990;**7**:209-229

[113] Dijoux N, Guingand Y, Bourgeois C, Durand S, Fromageot C, Combe C, et al. Assessment of the phototoxic hazard of some essential oils using modified 3T3 neutral red uptake assay. Toxicology In Vitro. 2006;**20**:480-489

[114] Muhlbauer RC, Lozano A, Palacio S, Reinli A, Felix R. Common herbs, essential oils, and monoterpenes potently modulate bone metabolism. Bone. 2003;**32**:372-380

[115] Yamaguchi K, Shinohara C, Kojima S, Sodeoka M, Tsuji T. (2E,6R)-8-hydroxy-2,6-dimethyl-2-octenoic acid, a novel antiosteoporotic monoterpene, isolated from *Cistanche salsa*. Bioscience, Biotechnology, and Biochemistry. 1999;**63**:731-735

[116] Wei Chen S, Min L, Li WJ, Kong WX, Li JF, Zhang YJ. The effects of angelica essential oil in three murine tests of anxiety. Pharmacology Biochemistry and Behavior. 2004;**79**:377-382

[117] Broughan C. Odours, emotions, and cognition—How odours may affect cognitive performance. International Journal of Aromatherapy. 2002;**12**(2):92-98

[118] Svoboda KP, Karavia AN, McFarlane V. Bioactivity of essential oils and their components. International Journal of Aromatherapy. 2002;**12**:67

[119] Alexander M. Part IV: Modulating immunity with aromatherapy: Conditioning, suppression and stimulation of the immune system. International Journal of Aromatherapy. 2002;**12**(1):49-56

[120] Standen MD, Myers SP. The roles of essential oils in the modulation of immune function and inflammation: survey of aromatherapy educators. International Journal of Aromatherapy. 2004;**14**:150-161

[121] Vale TG, Matos FJA, de Lima TCM, Viana GSB. Behavioral effects of essential oils from *Lippia alba* (Mill.) N.E. Brown chemotypes. Journal of Ethnopharmacology. 1999;**167**:127-133

[122] Aloisi AM, Ceccarelli I, Masi F, Scaramuzzino A. Effects of the essential oil from citrus lemon in male and female rats exposed to a persistent painful stimulation. Behavioural Brain Research. 2002;**36**:127-135

[123] Ceccarelli I, Lariviere WR, Fiorenzani P, Sacerdote P, Aloisi AM. Effects of long-term exposure of lemon essential oil odor on behavioral, hormonal and neuronal parameters in male and female rats. Brain Research. 19 March 2004;**1001**(1-2):78-86

[124] Orafidiya LO, Agbani EO, Adelusola KA, et al. A study on the effect of the essential oil of *Ocimun gratissimum* Linn. on cyclophosphamide induced inhibited hair growth in pulp rats. International Journal of Aromatherapy. 2004;**14**:119-128

[125] Rosa MSS, Mendonça-Filho RR, Bizzo HR, Rodrigues IA, Soares RM, Souto-Padrón T, et al. Antileishmanial activity of linalool-rich essential oil from *Croton cajucara* Benth.

Antimicrobial Agents and Chemotherapy. 2003;**47**:1895-1901

[126] Ikigai H, Nakae T, Hara Y, Shimamura T. Bactericidal catechins damage the lipid bilayer. Biochemistry et Biophysics Acta. 1993;**1147**:132-136

[127] Otake S, Makimura M, Kuroki T, Nishihara Y, Hirasawa M. Anticaries effects of polyphenolic compounds from Japanese green tea. Caries Research. 1991;**25**:438-443

[128] Akagawa M, Shigemitsu T, Suyama K. Production of hydrogen peroxide by polyphenols and polyphenol-rich beverages under quasi-physiological conditions. Bioscience, Biotechnology, and Biochemistry. 2003;**67**:2632-2640

[129] Boubaker J, Mansour HB, Ghedira K, Chekir-Ghedira L. Antimutagenic and free radical scavenger effects of leaf extracts from *Accacia salicina*. Annals of Clinical Microbiology and Antimicrobials. 2011;**10**:37-46

[130] Shan B, Yi-Zhong C, Brooks JD, Corke H. Antibacterial and antioxidant effects of five spice and herb extracts as natural preservatives of raw pork. Journal of the Science of Food and Agriculture. 2009a;**89**:1879-1885

[131] Kapoor N, Narain U, Misra K. Bioactive conjugates of curcumin having ester, peptide, thiol and disulphide links. Journal of Scientific and Industrial Research. 2007;**66**:647-650

[132] Parvathy KS, Negi PS, Srinivas P. Antioxidant, antimutagenic and antibacterial activities of curcumin-β-diglucoside. Food Chemistry. 2009;**115**:265-271

[133] Yanishlieva NV, Marinova E, Pokorny J. Natural antioxidants from herbs and spices. European Journal of Lipid Science and Technology. 2006;**108**:776-793

[134] Surh YJ. Molecular mechanisms of chemopreventive effects of selected dietary and medicinal phenolic substances. Mutation Research. 1999;**428**:305-327

[135] Careaga M, Fernandez E, Dorantes L, Mota L, Jaramillo ME, Hernandez-Sanchez H. Antibacterial activity of *Capsicum* extract against *Salmonella typhimurium* and *Pseudomonas aeruginosa* inoculated in raw beef meat. International Journal of Food Microbiology. 2003;**83**:331-335

[136] Tornuk F, Cankurt H, Ozturk I, Sagdic O, Bayram O, Yetim H. Efficacy of various plant hydrosols as natural food sanitizers in reducing *Escherichia coli* O157:H7 and *Salmonella typhimurium* on fresh cut carrots and apples. International Journal of Food Microbiology. 2011;**148**:30-35

[137] Elgayyar M, Draughton FA. Use of extracts of Nigella sativa to inhibit spoilage and pathogenic microorganisms in rainbow trout. In: Annual Meeting of International Association of Milk, Food and Environmental Sanitarians, Detroit. 1-4 August 1999

[138] Lee SY, Gwon SY, Kim SJ, Moon BK. Inhibitory effect of commercial green tea and rosemary leaf powders on the growth of foodborne pathogens in laboratory media and oriental-style rice cakes. Journal of Food Protection. 2009;**71**:1107-1111

[139] Ahn J, Grun IU, Mustapha A. Effects of plant extracts on microbial growth, color change, and lipid oxidation in cooked beef. Food Microbiology. 2007;**24**:7-14

[140] Uhart M, Maks N, Ravishankar S. Effect of spices on growth and survival of *Salmonella typhimurium* DT 104 in ground beef stored at 4 and 8 °C. Journal of Food Safety. 2006;**26**:115-125

[141] Kim S, Ruengwilysup C, Fung DY. Antibacterial effect of water-soluble tea extracts on foodborne pathogens in laboratory medium and in a food model. Journal of Food Protection. 2004;**67**:2608-2612

[142] Kim S, Fung DY. Antibacterial effect of water-soluble arrowroot (*Puerariae radix*) tea extracts on foodborne pathogens in ground beef and mushroom soup. Journal of Food Protection. 2004;**67**:1953-1956

[143] Fernandez-lopez J, Zhi N, Aleson-Carbonell L, Perez-Alvarez JA, Kuri V. Antioxidant and antibacterial activities of natural extracts: Application in beef meatballs. Meat Science. 2005;**69**:371-380

[144] Kong B, Wang J, Xiong YL. Antimicrobial activity of several herb and spice extracts in culture medium and in vacuum-packaged pork. Journal of Food Protection. 2007;**70**:641-647

[145] Yin MC, Cheng WS. Antioxidant and antimicrobial effects of four garlicderived organosulfur compounds in ground beef. Meat Science. 2003;**63**:23-28

[146] Leuschner RGK, Zamparini J. Effect of spices on growth and survival of *E. coli* O157 and *Salmonella enterica* serovar Enteritidis in broth model systems and mayonnaise. Food Control. 2002;**13**:399-404

[147] Ceylan E, Kang DE, Fung DYC. Reduction of *E. coli* O 157:H7 in ground meat by selected spices. In: Annual Meeting of Institute of Food Technologist, Atlanta. 20-24 June 1998

[148] Sallam KI, Ishioroshi M, Samejima K. Antioxidant and antimicrobial effects of garlic in chicken sausage. LWT—Food Science and Technology. 2004;**37**:849-855

[149] Gulluce M, Sahin F, Sokmen M, Ozer H, Daferera D, Sokmen A, et al. Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp. Longifolia. Food Chemistry. 2007;**103**:1449-1456

[150] Nguyen P, Mittal GS. Inactivation of naturally occurring microorganisms in tomato juice using pulsed electric field (PEF) with and without antimicrobials. Chemical Engineering and Processing. 2007;**46**:360-365

[151] Majeed M, Badmaev V, Shivakumar U, Rajendran R. Curcuminoids—Antioxidant Phytonutrients. Piscataway: Nutriscience Publishers Inc.; 1995

[152] Jayaprakasha GK, Negi PS, Anandharamakrishnan C, Sakariah KK. Chemical composition of turmeric oil—A byproduct from turmeric oleoresin industry and its inhibitory activity against different fungi. Zeitschrift für Naturforschung. 2001;**56C**:40-44

[153] Jayaprakasha GK, Negi PS, Jena BS, Sakariah KK. Evaluation of antioxidant activities and antimutagenicity of turmeric oil: A byproduct from curcumin production. Zeitschrift für Naturforschung. 2002;**57C**:828-835

[154] Pezeshk S, Rezaei M, Hosseini H. Effects of turmeric, shallot extracts, and their combination on quality characteristics of vacuum-packaged rainbow trout stored at 4±1 °C. Journal of Food Science. 2011;**76**:M387-M391

[155] Jayaprakasha GK, Rao JML, Sakariah KK. Chemical composition of the volatile oils from the fruits of *Cinnomomum zeylanicum* blume. Flavour and Fragrance Journal. 1997;**12**:331-333

[156] Kaul PN, Bhattacharya AK, Rao BRR, Syamasundar KV, Ramesh S. Volatile constituents of essential oils isolated from different parts of cinnamon (*Cinnamomum zeylanicum* Blume).

Journal of the Science of Food and Agriculture. 2003;**83**:53-55

[157] Kamath JV, Rana AC, Choudhury AR. Prohealing effect of *Cinnamomum zeylanicum* bark. Phytotherapy Research. 2003;**17**: 970-972

[158] Ceylan E, Sabah JR, Fung DYC. Effect of cinnamon on *E. coli* O 157:H7 in apple cider. In: Annual Meeting of Institute of Food Technologist, Chicago. 25-28 July 1999

[159] Yuste J, Fung DYC. Inactivation of *Listeria monocytogenes* Scott a 49594 in apple juice supplemented with cinnamon. Journal of Food Protection. 2002;**65**:1663-1666

[160] Lu J, Mittal GS, Griffiths MW. Reduction in levels of *Escherichia coli* O157:H7 in apple cider by pulsed electric fields. Journal of Food Protection. 2001;**64**:964-969

[161] Jurenka JMT. Therapeutic applications of pomegranate (*Punica granatum* L.): A review. Alternative Medicine Review. 2008;**13**:128-144

[162] Seeram N, Risa Schulmann PN, Heber D. Pomegranates: Ancient Roots to Modern Medicine. Boca Raton: CRC Press; 2006

[163] Rani P, Khullar N. Antimicrobial evaluation of some medicinal plants for their potential against multidrug resistant *Salmonella typhi*. Phytotherapy Research. 2004;**18**:670-673

[164] Voravuthikunchai S, Lortheeranuwat A, Jeeju W, Sririrak T, Phongpaichit S, Supawita T. Effective medicinal plants against enterohaemorrhagic *Escherichia coli* O157:H7. Journal of Ethnopharmacology. 2004;**94**:49-54

[165] Perez C, Anesini C. In vitro antibacterial activity of Argentinian folk medicinal plants against *Salmonella typhi*. Journal of Ethnopharmacology. 1994;**44**:41-46

[166] Braga LC, Shupp JW, Cummings C, Jett M, Takahasti JA, Carmo LS, et al. Pomegranate extract inhibits *Staphylococcus aureus* growth and subsequent enterotoxin production. Journal of Ethnopharmacology. 2005;**96**:335-339

[167] Kanatt SR, Chander R, Sharma A. Antioxidant and antimicrobial activity of pomegranate peel extract improves the shelf life of chicken products. International Journal of Food Science and Technology. 2010;**45**:216-222

[168] Mackeen NM, Ali AM, Lajis NH, Kawazu K, Hassan Z, Amran M, et al. Antimicrobial, antioxidant, antitumourpromoting and cytotoxic activities of different plant part extracts of *Garcinia atroviridis* griff. ex T. anders. Journal of Ethnopharmacology. 2000;**72**:395-402

[169] Likhitwitayawuid K, Padungcharoen T, Krungkrai J. Antimalarial xanthones from *Garcinia cowa*. Planta Medica. 1998a;**64**:70-72

[170] Likhitwitayawuid K, Chanmahasathien W, Ruangrungsi N, Krungkrai J. Xanthones with antimalarial activity from *Garcinia dulcis*. Planta Medica. 1998b;**64**:281-282

[171] Negi PS, Jayaprakasha GK, Jena BS. Evaluation of antioxidant and antimutagenic activities of the extracts from the fruit rinds of *Garcinia cowa*. International Journal of Food Properties. 2010;**13**:1256-1265

[172] Bakana P, Claeys M, Totte J, Pieters LA, Van Hoof C, Tamba-Vemba L, et al. Structure and chemotherapeutical activity of a polyisoprenylated benzophenone from the stem bark of *Garcinia huillensis*. Journal of Ethnopharmacology. 1987;**21**:75-84

[173] Iinuma M, Tosa H, Tanaka T, Asai F, Kobayashi Y, Shimano R, et al. Antibacterial activity of xanthones from guttiferaeous plants against methicillinresistant *Staphylococcus aureus*. Journal of Pharmacy and Pharmacology. 1996a;**48**:861-865

[174] Iinuma M, Tosa H, Tanaka T, Kanamaru S, Asai F, Kobayashi Y, et al. Antibacterial activity of some Garcinia benzophenone derivatives against methicillin-resistant Staphylococcus aureus. Biological and Pharmaceutical Bulletin. 1996;**19**:311-314

[175] Tamil Selvi A, Goseph GS, Jayaprakasha JP. Inhibition of growth and aflatoxin production in *Aspergillus flavus* by *Garcinia indica* extracts and its antioxidant activity. Food Microbiology. 2003;**20**:455-460

[176] Xu MY, Sun XX, Tong WX. Medical research and development of seabuckthorn. Hippophae. 1994;**7**;32-40

[177] Yang B, Kallio H, Tahvonen R, Kalimo K, Mattila L, Kallio S, et al. Effects of dietary supplementation of Seabuckthorn (*Hippophae rhamnoides*) oils on fatty acids in patients with atopic dermatitis. Journal of Nutrition and Biochemistry. 2000;**1**:338-340

[178] Ranjith A, Kumar SK, Venugopalan VV, Arumughan C, Sawhney RC, et al. Fatty acids, tocols and carotenoides in pulp oil of three seabuckthorn species (*H. rhamnoides*, *H. salicifolia* and *H. tibetana*) grown in the Indian Himalayas. Journal of the American Oil Chemists' Society. 2006;**83**:359-384

[179] Li TSC, Wang LCH. Physiological components and health effects of ginseng, echinacea and seabuckthorn. In: Mazza G, editor. Functional Foods: Biochemical and Processing Aspects. Lancaster: Technomic Publishing; 1998. pp. 329-356

[180] Gupta SM, Gupta AK, Ahmed Z, Kumar A. Antibacterial and antifungal activity in leaf, seed extract and seed oil of Seabuckthorn (*Hippophae salicifolia* D. Don) plant. Journal of Plant Pathology and Microbiology. 2011;**2**:105

[181] Negi PS, Chauhan AS, Sadia GA, Rohinishree YS, Ramteke RS. Antioxidant and antibacterial activities of various Seabuckthorn (*Hippophae rhamnoides* L.) seed extracts. Food Chemistry. 2005;**92**:119-124

[182] Edenharder R, Speth C, Decker M, Kolodzig H, Kayser O, Platt KL. Inhibition of mutagenesis of 2-amino-3-methyl imidazo [4,5-f] quinoline by coumarins and furanocoumarins, chromanones and furanochromanones. Mutation Research. 1995;**345**:57-71

[183] Geetha S, SaiRam M, Singh V, Ilavazhagan G, Sawhney RC. Antioxidant and immunomodulatory properties of seabuckthorn (*Hippophae rhamnoides* L.)—An in vitro study. Journal of Ethnopharmacology. 2002;**79**:373-378

[184] Chen IS, Lin YC, Tsai IL, Teng CM, Ko FN, Ishikawa T, et al. Coumarins and anti-platelet aggregation constituents from *Zanthoxylum schinifolium*. Phytochemistry. 1995;**39**:1091-1097

[185] Ganju L, Padwad Y, Singh R, Karan D, Chanda S, et al. Anti-inflammatory activity of Seabuckthorn (*Hippophae rhamnoides*) leaves. International Immunopharmacology. 2005;**5**:1675-1684

[186] Chawla R, Arora R, Singh S, Sagar RK, Sharma RK, et al. Radioprotective and antioxidant activity of fractionated extracts of berries of *Hippophae rhamnoides*. Journal of Medicinal Food. 2007;**10**:101-109

[187] Selvamuthukumaran M, Khanum F, Bawa AS. Development of seabuckthorn mixed fruit jelly.

International Journal of Food Science and Technology. 2007;**42**:403-410

[188] Hao YY, Brackett RE, Doyle MP. Inhibition of listeria monocytogenes and *Aeromonas hydrophila* by plant extracts in refrigerated cooked beef. Journal of Food Protection. 1998;**61**:307-312

[189] Ward SM, Delaquis PJ, Holley RA, Mazza G. Inhibition of spoilage and pathogenic bacteria on agar and pre-cooked roast beef by volatile horseradish distillates. Food Research International. 1998;**31**:19-26

[190] Lin YT, Labbe RG, Shetty K. Inhibition of listeria monocytogenes in fish and meat systems by use of oregano and cranberry phytochemical synergies. Applied and Environmental Microbiology. 2004;**70**:5672-5678

[191] Ruiz A, Williams SK, Djeri N, Hinton A Jr, Rodrick GE. Nisin, rosemary, and ethylenediaminetetraacetic acid affect the growth of *Listeria monocytogenes* on ready-to-eat Turkey ham stored at four degrees celsius for sixty-three days. Poultry Science. 2009;**88**:1765-1772

[192] Xi Y, Sullivan GA, Jackson AL, Zhou GH, Sebranek JG. Use of natural antimicrobials to improve the control of *Listeria monocytogenes* in a cured cooked meat model system. Meat Science. 2011;**88**:503-511

[193] Ahn J, Grun IU, Mustapha A. Antimicrobial and antioxidant activities of natural extracts in vitro and in ground beef. Journal of Food Protection. 2004;**67**:148-155

[194] Sivarooban T, Hettiarachchy NS, Johnson MG. Inhibition of *Listeria monocytogenes* using nisin with grape seed extract on Turkey frankfurters stored at 4 and 10 degrees C. Journal of Food Protection. 2007;**70**:1017-1020

[195] Pszczola DE. Beefing up innovations for meat and poultry ingredients. Food Technology. 2002;**56**:54-67

[196] Karapinar M, Sengun IY. Antimicrobial effect of koruk juice against *Salmonella typhimurium* on salad vegetables. Food Control. 2007;**18**:702-706

[197] Serra AT, Matias AA, Nunes AVM, Leitao MC, Brito D, Bronze R, et al. In vitro evaluation of oliveand grape-based natural extracts as potential preservatives for food. Innovative Food Science and Emerging Technologies. 2008;**9**:311-319

[198] Palombo EA. Anti-listerial activity of ethanolic extracts of medicinal plants, *Eremophila alternifolia* and *Eremophila duttonii*, in food homogenates and milk. Food Control. 2007;**18**:387-390

[199] Zeng WC, He Q, Sun Q, Zhong K, Gao H. Antibacterial activity of watersoluble extract from pine needles of *Cedrus deodara*. International Journal of Food Microbiology. 2012;**153**:78-84

Section 4

Allelochemicals

Chapter 10

Roles of Terpenoids in Essential Oils and Its Potential as Natural Weed Killers: Recent Developments

Ahmed Abdulwahid Ali Almarie

Abstract

Weed control through the use of conventional chemical compounds presented by synthetic herbicides is a widely used and successful method to control weed by reducing the negative impact of weed and increase agricultural production gradually. However, although the losses in agricultural production arising from weed competition are decreased through the use of synthetic herbicides, the negative impacts of these compounds on the environment and human health have raised awareness and created grave concern of a number of parties to safeguard the environment and humans. The adverse effect of synthetic herbicides can still occur even if such herbicides are applied at the recommended rates. Control weed naturally presented by allelochemical compounds provides an attractive, alternative and safe way to control weed synthetic herbicides. Previous works indicated that terpenoids as the most important group of allelochemicals have shown to exhibit a good phytotoxic effect against a wide range of weed species by suppressing germination and reducing growth. This review was a highlight to detect the desirable phytotoxic effects of some terpenoid compounds as a major content in essential oils on various weed species and the possible uses as natural weed killers.

Keywords: essential oils, terpenoids, allelochemicals, weeds, natural killers

1. Introduction

Weed control defined as any method trying to stop weeds, especially noxious or injurious weeds from competing with desired plants. The evolution of weed control began many years ago when humans felt that it was necessary to remove and dispose of weeds in order to reduce their competition with the planted crops and thereby increase the yield qualitatively and quantitatively. Weed control involves processes where weed infestations are reduced, but not necessarily eliminated. In weed control, the degrees of control can be described by the state of weed reduction ranging from poor to excellent. The degrees of control depending on the types of weeds involved and the effectiveness of the control method adopted. It should be noted that in weed control, the weeds are generally not completely killed, however, their growth is somewhat contained while the crop continues a normal yield. Weed control is aimed at only reducing the weeds present by employing some form of

control measures. On the contrary, weed management is a systematic approach where the whole land use planning is carried out in advance to minimize the very invasion of weeds in aggressive forms and give crop plants a strong competitive advantage over the weeds. Weed control methods can be grouped into the culture, physical, biological and chemical [1]. Human efforts of controlling weeds began with the use of cultural practices such as tillage, planting, crop rotation, fertilizer application, irrigation, etc., that are adapted to create favorable conditions for the crop. If properly used, the practices can help in suppressing weeds. On the other hand, culture methods alone, cannot control weeds; it can only help to reduce the weed population. Culture methods therefore, can be effectively used in combination with other methods. Every method of weed control has its own advantages and disadvantages. No single method is successful under all weed situations. Most often, a combination of these methods gives effective and economic control than a single method. These methods of controlling weeds were later developed in the form of mechanical weed control such as hand pulling, hand hoeing, and planting in rows to facilitate machinery use, but again these methods did not attain the desired benefits [2].

Later, a new mechanism of weed control was developed through the use of chemical inputs. Chemical weed control began on a small scale. Since the 19th century, a combination of salt and ash powder was used to control weed plants which grow on either side of the railway. The use of synthetic herbicides, however, begun in the 1940s with the development of some organic herbicides, specifically the 2,4-D. This herbicide is considered as a growth regulator used in high doses to control broadleaf weeds [3]. Then, chemical weed control was widely used as a form of weed control and achieved a dominant role in the crop management, more efficient, economical and low costs as compared to other methods and contributed strongly to the increase in the agricultural yields and reduce losses due to weeds [4, 5]. As a result of using chemical weed control, the traditional method of weed control such as cultivation and hand weeding has been greatly been decreased [6]. A new method to control weeds was created by producing different types of synthetic herbicides according to the mode of action of these compounds against weed plants. By 1990s, the number of compounds that have been used in herbicides in many different formulas reached to more than 180 compounds [7]. According to a report of [8], the total value of the global's agrochemical market was between 31 and 35 billion US$ and of the products, herbicides accounted for 48% followed by fungicides at 22%. Nowadays, chemical weed control becomes as an integral part of the complex world of technical inputs required for modern agricultural production and have been accepted as a standard tool of the trade by farmers throughout the world despite the negative effects of synthetic herbicides on the ecosystem [1].

2. Negative impacts of the synthetic herbicides on human health and ecosystems

Although synthetic herbicides are considered the best and effective methods to be used as weed control, the risk is very high if it is used indiscriminately. The types, quantity and frequency of applications of the synthetic herbicides can bring about various harmful effects to the environment and its ecosystems and in fact a threat to human health. The uses of synthetic pesticides including herbicides have directly and indirectly brought about several adverse effects to the soil surface, groundwater and organisms as well as in the atmosphere. The above changes have negative ramifications for the community and disrupting the ecological balance, hence risking human life.

2.1 Human health

There is no segment of the population that is completely protectedc exposure and high risk groups are not only of the people of the developing countries but all the countries over the world. The hazards of synthetic pesticides are summarized by their impact through food commodities, surface water, groundwater and soil contamination and the effects on soil fertility (beneficial soil microorganisms) and non-target vegetation [9]. According to the report of Williams et al. [10] also highlighted the complaint of contacts diseases, acute ulcer, heart pain, skin rashes respiratory condition and eye problem of the people in the survey area. In another study conducted by Niemann et al. [11] on glyphosate; a common non-selective systemic herbicide promoted by the manufacturers as a safer herbicide, reported tracing of its residues in both humans and animal urine. It was then suggested that the use of glyphosate may have to be re-evaluated to reduce human exposure to the dangers of synthetic herbicides.

2.2 Ecosystems

Using synthetic herbicides even at recommended rates can lead to negative impacts on the ecosystems, especially the harmful effects that come from their residues. On the other hand, the efficiency of these compounds will be slowly decreased due to the increase in the resistance of the weed plants as a result of the continuous use of these compounds to control the same weed species. Hence, using these synthetic herbicides continuously becomes a double-edged sword. As it is well known, the insecticides are the most toxic to the environment, followed by fungicides and herbicides. But there are some herbicides that can be highly toxic and much more serious than the insecticides. Such problems that emanated from the utilization of synthetic herbicides cannot be overlooked regardless of the benefits accruing of its application [9, 12]. According to Williams et al. [10],the herbicide applied in agricultural lands can be leached and washed by rain or precipitation which leading to extending their risks to the wider areas which applied.

The herbicide effects on the soil and the environment were detected and found to contaminate the groundwater and the source of fresh drinking water even at recommended levels, causing an acute decrease in the biomass such as microorganisms, plant tissues and soil organic matter [13]. On the other hand, most of the synthetic herbicides not only affect the target plants, but it can also cause low growth rates, reproductive problems, changes in appearance or behavior or even death of non-target plant species, making the use of herbicides more harmful [14]. Furthermore, a wide range of non-targeted plant species are more sensitive to herbicides, especially during the reproductive stages of their life cycle. This is further compounded by the effect of external factors such as wind and rainfall, which can adversely affect this most important stage of the plant's life cycle. In this regard, Boutin et al. [15] mentioned the delays in flowering and reduction in seed production observed in a large number of non-target plant species, including climbing and hedgerow plants, through the spraying of herbicides within the vicinity of their growing locations.

3. Development of weed resistance to synthetic herbicides

Weed resistance to synthetic herbicides is considered to be the most difficult problem facing weed chemical control. The resistance comes when some of the weed species to be resistant to the herbicides naturally. Weed resistance can come in

different forms. One of the mechanisms is through the production of a new generation of weeds of different morphological and physiological characteristics with shorter life cycles that can survive from the application of the systemic herbicides. Thus, the number of these weeds surviving from the continuous application of herbicides which will gradually increase. While the other weed species in the same area will be controlled by applying herbicides [16]. Another way in the development of weed resistance comes from the application of contact herbicides, which are responsible for inhibiting the process of photosynthesis, such as attrition. The application of such herbicides, especially during unfavorable weather conditions can contribute to the absorption of a small amount of the active ingredient but ineffective to the targeted weeds. Over time, the continuous accumulation of contact herbicides developed a new generation of weed that is immune to the synthetic herbicides [17]. In September 2010, Dow AgroSciences' scientists stated that weeds which have become resistant to the glyphosate herbicide and the ensuing production of genetically modified plants are one of the solutions to resolve issues pertaining to weed competition [7]. Nowadays, there are more than 604 species of weed plants considered as resistance to synthetic herbicides, most of these weed species are resistant to herbicides which are responsible for inhibiting acetolactate synthase (ALS enzyme), photosystem II and acetyl-CoA carboxylase [18, 19]. **Figure 1**, showed the number of resistant plant species for several herbicides according to their modes of action [18].

In this regard, Sekutowski [20] and Weber and Golebiowska [21] reported that there are 10 species of weeds that pose the biggest threat to crops by causing yield losses. The weeds include the most important herbicide-resistant species which are characterized by multiple resistances such as rigid rye-grass (*Lolium rigidum* Gaud.), wild oat (*Avena fatua* L.) and redroot pigweed (*Amaranthus retroflexus*). As a result of weed resistance, many types of synthetic herbicide are re-evaluated as useless. Hence, a lot of conventional herbicides have been identified as resistance and ineffective against common weeds. For example, there are 116 useless conventional herbicides identified in Europe, where the highest number of herbicides is found in France (30 types) followed by Spain (26 types), United Kingdom (24 types), Belgium (18 types) and Germany (18 types) [22].

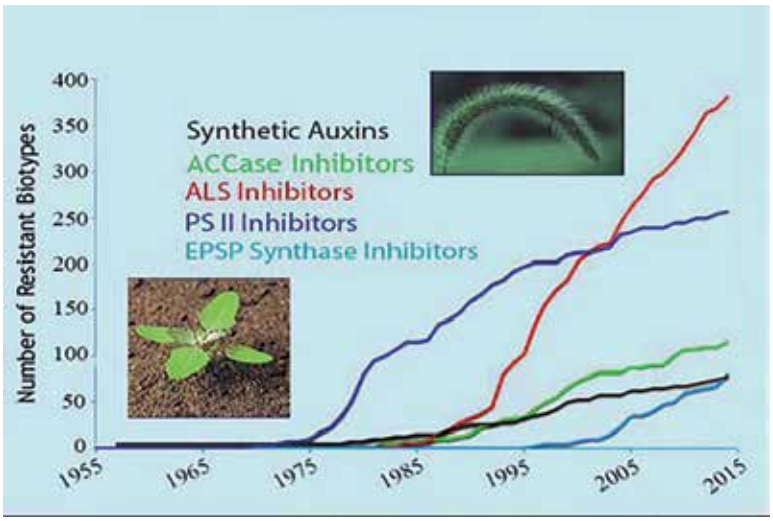

Figure 1.
Number of resistant plant species for several herbicides according to their modes of action [18].

Therefore, due to the similarity of the active ingredient in most of the herbicides that belong to the same group, there is no major new site-of-action herbicide has been introduced into the marketplace in the last two decades [23]. Conditions for the registration of synthetic herbicides also become strict and complicated. This is considered the greatest loss in the herbicides production sector. For example, the number of new herbicides active substances in Europe declined to 336 in 2009 from 945 in 1999 [23]. In fact, chemical herbicides development efforts worldwide whose market used to be monopolized by glyphosate-based herbicide have diminished since 1996 as a result of the glyphosate-resistant which is considered the most widely used pesticide in the world [24]. Therefore, the trend today is toward the use of natural alternative compounds, but still possess their herbicidal potential and safety versus the currently used synthetic and non-ecofriendly materials, wherein the plant defense tactic characterized by plant secondary metabolites, come to the forefront as promising solutions to replace the conventional herbicides.

4. Allelopathic compounds

The word allelopathy comes from the Greek words "Allelon" meaning "each other" and "Pathos" refers "to suffering". This allelopathic phenomenon whereby a plant response in defense of the neighboring plants, insects, microorganism and animals results in the production of natural chemicals called allelochemicals or phytochemicals [25, 26]. Allelopathy phenomenon is defined by Kato-Noguchi [27] as an important defense mechanism of the plant which results in the manufacturing of secondary metabolites. This term is also defined by the International Allelopathy Society in 1996th as a science that study any process involving secondary metabolites produced by plants, micro-organisms, viruses, and fungi that influence growth and development of agricultural and biological systems, excluding animals [28]. Usually, the allelochemical compounds released from donor plants caused negative effects on organisms found within the surrounding environment. However, some of the recent researchers reported the effects of the allelochemical produced by plants belonging to the plant family (Fabaceae) can be positive to the surrounding environment. As for an instance, the allelochemicals residues produced by these plant types such as legumes help to fix nitrogen from the air thus enriching the soil [29]. Most of the plants including weeds release allelochemical compounds as defense mechanisms. These compounds can be released from donor plant parts into the environment by leaching, volatilization, exudate from living plant tissue or by the decomposition of plant residues as shown in **Figure 2**. Hence, it was responsible for inhibiting the germination and growth of neighboring organisms [30]. One of the major advantages of allelopathy involves the release of plant biochemical compounds into the environment that inhibits germination or suppresses the growth of the surrounding vegetation. Another form of allelopathic potential can be tapped from trees that produce biochemical compounds for its survival and hence its reproduction. An example of such plants is eucalyptus; these types of plants can be used in agricultural production as cover crops to control weeds [31]. Lately, the allelopathic phenomenon has gained prominent attention and has been successfully employed in field crop production toward the improvement of crop productivity and for the protection of the environment through eco-friendly control of weeds, pests and crop diseases [32, 33].

The motivation for the use of the allelochemical compound in weed control is attributed to its phytotoxic effects similar to the phytotoxic effects of the synthetic herbicides in inhibiting seed germination and reducing seedling growth. The

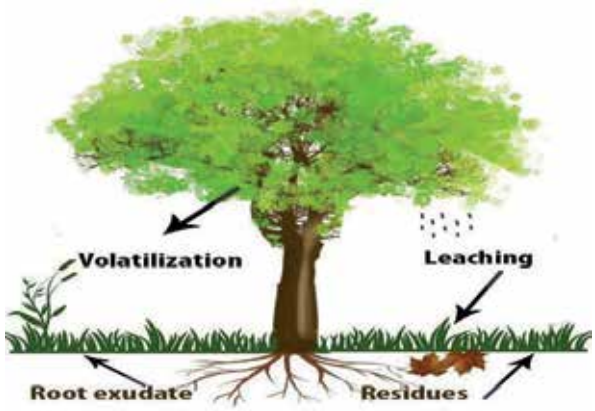

Figure 2.
Methods of allelochemical compounds released from the donor plant into the environment.

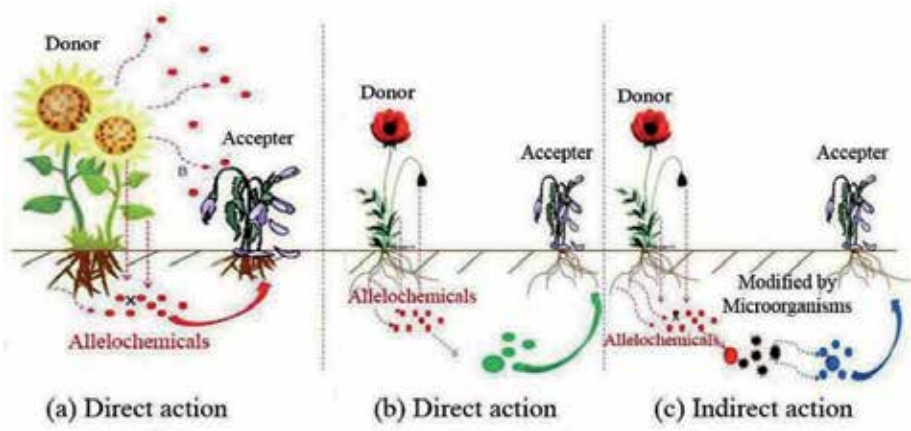

Figure 3.
Direct and indirect allelopathic mechanisms of donor plant to the targeted plants [19].

inhibition process includes several action sites such as cell division, nutrient uptake, photosynthesis and specific enzymatic functions [34]. The benefit of using allelopathy will not be just from being an attractive alternative to conventional herbicides, but also in the possibility of applying at places where the use of synthetic herbicides is illegal such as in organic farming. Thus, the use of allelochemical compounds could be adopted to reduce damage resulting from weed competition of the areas where the use of synthetic herbicides is not allowed [35]. Plants produce secondary metabolites exhibited a few ecological advantages such as pollinator attractants, determinants of vegetation patterning, provide protection against predators and other enemies and more importantly in mediating plant-plant interactions known as allelopathy [36]. The responsible chemical compounds for demonstrating allelopathic influences are called allelochemicals or biochemicals compounds produced as offshoots from the primary metabolic pathways of plants [37].

Allelochemicals have been defined as compounds derived as metabolic by-products of that certain plant which, when introduced into the environment can cause growth inhibition as a result of different malfunctions inside targeted plants such as respiration, cell division, water and nutrient uptake. The symptoms of the "allelopathic effects" include leaf wilting and yellowing, or death of part or all of a

plant [3]. Allelochemical compounds, therefore, can act directly and indirectly when releasing from donor to the receiver plant as described in **Figure 3** which showed the direct and indirect allelopathic mechanisms of donor plant to the targeted plants [19]. The direct action of allelochemicals similar to the action of conventional herbicides hence may lead to being a new approach in herbicides technique to discover and select the most effective allelopathic plants which are the commonly used as natural herbicides [24]. The allelopathic potential on targeted or receiver plants are shown in different ways, such as the reduction in both the length and mass of radicle and roots, extension shoot and coleoptile, swelling or necrosis of root tips, destruction of cell wall, curling of the root axis, lack of root hairs, decrease in the number of seminal roots, reduced in plant dry weight accumulation, leaf discoloration and lower in reproductive capacity [25, 33, 38]. Allelopathic inhibition is complex and can involve the interaction of different classes of allelochemicals such as phenolics, flavonoids, terpenoids, alkaloids, steroids, carbohydrates and amino acids [37, 39].

The allelopathic mechanisms of allelochemical compounds on targeted plants that have been identified by Li et al. [40] can be summarized as below:

1. Changes in membrane permeability and inhibition of plant nutrient uptake.

2. Inhibition of cell division, elongation, and submicroscopic structure.

3. Effects on plant photosynthesis and respiration.

4. Effects on various enzymatic functions and activities.

5. Effects on the synthesis of plant endogenous hormones.

6. Effects on protein synthesis.

Allelochemical compounds have been classified into 10 categories depending on the structures and properties of these compounds according to [22, 40] namely the flavonoids, terpenoids, alkaloids, phenolics, tannins, coumarins, cinnamic acid and its derivatives, simple lactones, water-soluble organic acids and long-chain fatty acids. A wide range of these biochemicals are synthesized through the shikimate pathway or the isoprenoid pathway which are responsible for the essential oil production [41].

5. Essential oils

Essential oil is a concentrated volatile liquids consisting of different types of secondary plant metabolites but mainly composed of terpenoids and phenolics. Technically, essential oils are defined as odiferous bodies by oily nature obtained from plants by different ways, such as cold and hot pressing, distillation and extraction using organic solvents [42].

Essential oils produced from specific types of plants can be used for different purposes. Most of the essential oil usage is influenced by donor or producer plants and their surroundings such as scent to attract certain animals and insects, aiding in pollination, protection or as repellent agents, energy reserve, wound healing and prevent water evaporation. Essential oils can be obtained from different parts of plants such as the leaves, flowers, fruit, seeds, roots, rhizomes, bark and wood [43].

Biosynthetically, essential oil components composed of two groups. The first group is the terpenoids, which is considered the main group; mostly, of the monoterpenoids, sesquiterpenoids. The second group is non-terpenoids, which may contain aromatic compounds such as phenylpropanoids, short-chain aliphatic structures, nitrogenated and sulfuric substances [42]. Essential oils can be isolated from plants by several processes such as expressed oils, steam distillation, solvent extraction, fractional distillation and percolation and carbon dioxide extraction. The process of steam distillation is the most widely accepted method for the extracting of essential oils on a large scale.

The steam distillation process considered lower risk as compared with another process due to absence chemical compounds such as solvents and the thermal degradation as temperature generally not above 100°C [42]. Considering the multiple properties demonstrated with essential oils, such as pharmaceutical applications, antioxidant, food and cosmetic uses [44, 45]. Nowadays, essential oils are becoming increasingly important as a safer alternative to synthetic chemical products [46]. Essential oils also showed a broad spectrum of advantages against the pest, plant pathogenic and fungi ranging from bactericidal, fungicidal, insecticidal, antifeedant or repellent, oviposition deterrent, and growth regulatory and antivector activities [47, 48]. The application of essential oils and their constituents mainly; terpenoids for weed and pest management is currently being explored and is viewed as an important source of lead molecules in agriculture [49, 50].

Recently, the effectiveness of essential oils has been investigated on some weed species, demonstrating the ability to inhibit germination and the development of seedlings. The reasons that encouraged the use essential oils as alternative compounds to conventional herbicides are due to a less harmful effect on the environment and almost as effective as the synthetic herbicides. Furthermore, there are no contradictions and obstacles to be used as bioherbicides in all aspects of agriculture, specifically in organic farming as compared to the use of synthetic pesticides, which has attracted a lot of interest in the safety and health of the consumers [35].

6. Terpenoids

Terpenoids or terpenes are the most structurally varied classes and they are the largest family of compounds of plant products. Previous studies estimated the exiting of more than 23,000 of known terpenoid compounds, including carotenoids, tocopherol, phytol, steroids and hormones [51]. Terpenoids play an important and essential role in a broad range of biological functions like respiration, chain electron transport, cell wall and membrane biosynthesis. Also, terpenoids are involved extensively in the fields of pharmaceuticals, cosmetics, colorants, disinfectants, scents, flavorings and agrichemicals [52]. Biosynthetically, terpene compounds are classified as unsaturated hydrocarbons and basically synthesized from the isoprene unit which has the molecular formula ($CH_2{=}C(CH_3){-}CH{=}CH_2$). The molecular formulae of terpenoids are multiples of that, (C5H8) and where (n) is the number of linked isoprene units called the isoprene rule or the C5 rule; **Figure 4** shows the chemical structure of the isoprene unit.

The isoprene units may be joined together head-to-tail to form linear chains or they may be arranged to form rings. Chains of isoprene units are linked to building the terpene structure that synthesized sequentially to form hemiterpenes, monoterpenoids, sesquiterpenoids, diterpenes, sesterterpenes, triterpenes, and tetraterpenes depending on the number of isoprene units [53].

Terpenoids play a defensive role in trees. They are the major component of essential oils in many trees which are responsible for the aroma of the trees. They are released into the air by vaporization or leached in small amounts by water. These compounds caused poor growth of neighboring plants in addition to aggravating other problems site when released [54]. Among the terpenoids compounds, Monoterpenoids and sesquiterpenoids are the most available compounds in the secondary plant metabolites and widely used as antimicrobials and cosmetics. Monoterpenoids and sesquiterpenoids produce in plants as defensive chemicals against insects, fungi and surrounding plants [51].

Terpenoids can be classified according to the number of isoprene units in the molecule. A prefix in the name indicates the number of terpene units needed to assemble the molecule; **Table 1** lists of various classifications of isoprene units as well as the examples of the compounds associated with the classification. All the above different secondary plant metabolites are produced through the metabolism of primary metabolites depending on the pathway and the type of primary metabolites.

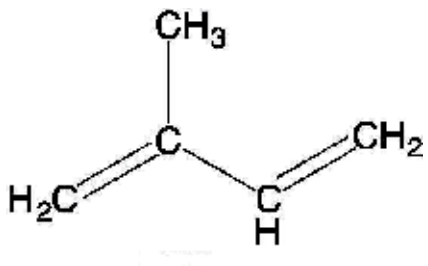

Figure 4.
Structure of one isoprene unit.

No.	Type of classification	Number of isoprene units	Structure formula	Example
1	Hemiterpenes	Single	C_5H_8	Prenol and isovaleric acid
2	Monoterpenoids	Two	$C_{10}H_{16}$	Geraniol, limonene, terpineol and myrcene
3	Sesquiterpenoids	Three	$C_{15}H_{24}$	Humulene, caryophyllene, and farnesol
4	Diterpenoids	Four	$C_{20}H_{32}$	Cafestol, kahweol, cembrene and taxadiene and phytol
5	Sesterterpenoids	Five	$C_{25}H_{40}$	Geranylfarnesol
6	Triterpenoids	Six	$C_{30}H_{48}$	Sterols
7	Sesquarterpenoids	Seven	$C_{35}H_{56}$	Ferrugicadiol and tetraprenylcurcumene
8	Tetraterpenoids	Eight	$C_{40}H_{64}$	Cyclic lycopene, carotenoids
9	Polyterpenes	More than eight	—	Natural rubber

Table 1.
Classification of terpenoids based on the number of isoprene units.

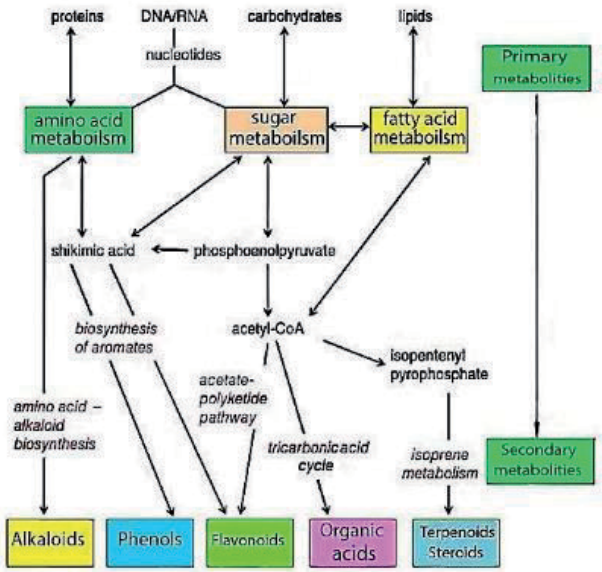

Figure 5.
General schematic biosynthetic pathways to produce major secondary metabolites including terpenoids [60].

6.1 Biosynthesis of terpenoids

Secondary metabolites including terpenoids are derived essentially from the modification of primary metabolites by different main pathways; the pathways responsible for synthesizing the primary metabolites. The secondary metabolite biosynthetic pathways are too numerous and cannot be easily determined. Nevertheless, there are a few typical pathways involved in the biosynthesis of major classes of these compounds. The shikimate pathway is considered the major pathway used by plants as well as different organisms like bacteria and fungi to synthesize primary metabolites which in turn form the basic building block for a wide range of phenolic and flavonoid compounds [55, 56]. The shikimate pathway involving multiple isoprene units (C_5H_8) linked together in a head-to-tail pattern can synthesize to terpenoids according to the number of isoprene units incorporated in the molecular skeleton [57]. Terpenoids can also be synthesized through an isopentenyl diphosphate pathway, which arose from the intermediate substrate, particularly, mevalonic acid (MVA) via the mevalonate pathway and a methylerythritol phosphate/deoxy-D-xylulose 5-phosphate pathway (MEP/DOXP) pathway. **Figure 5** showed the biosynthesis of terpenoids [58, 59].

7. Terpenoids as natural weed killers; mode of action

The term "mode of action" refers to the sequence of herbicides action beginning from absorption by plant tissues until death. Understanding herbicide mode of action is helpful in knowing what groups of weed killers. Generally, herbicides classified depending on their mode of action and the toxicity into two groups; contact and systemic herbicides [56]. Contact herbicides only kill the plant tissue which comes into contact with the spray solution. While systemic herbicides need to translocated in plant tissues until reaching the active site for causing the injury. Contact herbicides quite often the fastest acting by causing acute toxicity but the whole plant must be sprayed to be effective [60]. Herbicides also can further

classify according to their selectivity to selective or non-selective herbicides. Non-selective herbicides can kill most plants while selective herbicides designed to kill specific types of plants depends on the morphological and physiological differences between the two major plant groups, grassy or broadleaf. Moreover, herbicides can be divided into two groups as a result of its application timing; pre and postemergence. Preemergence herbicides normally targeted seeds by preventing the germination and suppress seedling development. While postemergence herbicides target weed biomass by reducing or inhibiting the biological processes in plant tissues.

Study of the injury symptoms of the targeted plant tissues resulting from the application of herbicides helps to determine how herbicides interact with the biological and physical systems of the targeted plant. Injury symptoms in targeted weed species depend on the type of herbicide, the rate of application, stage of growth, type of exposure, and the plant species receptor involved. All herbicides work by disrupting one or more than one of the natural mechanisms of the targeted plant tissues such as the stomatal system through the influence of the guard cells, photosynthesis by the distraction of chlorophyll pigment and targeting cell membrane and other cellular systems.

Herbicidal mechanisms of the secondary plant metabolites as post-contact formulations weed killers are strictly fast-acting. They generally disrupt the cuticular layer of the foliage which resulting in the rapid desiccations or burn-down of young tissues [35]. Membrane disruption can be considered as one of the underlying mechanisms of plants' phytotoxic effects, which result in cell death and growth inhibition. Secondary metabolites such as terpenoids are less specific and attack a multitude of proteins by building hydrogen, hydrophobic and ionic bonds and as a result of this, modulating their 3D structures and in consequence their bioactivities [61] (**Figure 6**).

Monoterpenes are considered lipophilic compounds; hence, there is, therefore, the possibility of plant cell membrane expansion as a result of accumulation monoterpenes, thereby destroying the membrane structure [62, 63]. **Figure 7** showed the interaction of terpenoids with the plant cell membrane [64]. Moreover, the monoterpenes compounds in essential oils uncoupled the oxidative photophosphorylation (transform ADP to form ATP using the energy of sunlight). As a result, monoterpenes cause a reduction in cellular respiration leading to a perturbation in the ATP production. Thus, disorders in physiological processes in plants are induced [33, 65] (**Table 2**).

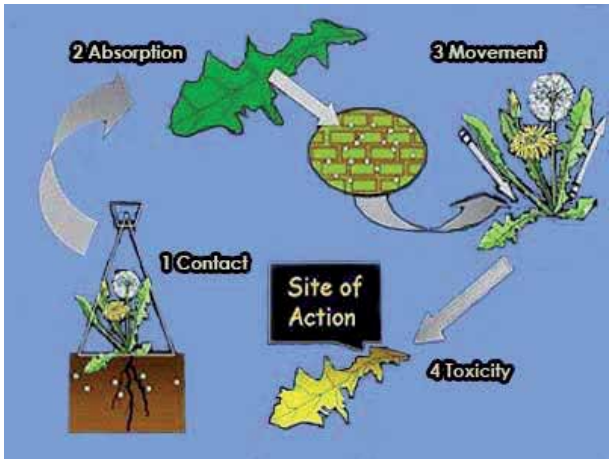

Figure 6.
Natural weed killers; mode of action [23].

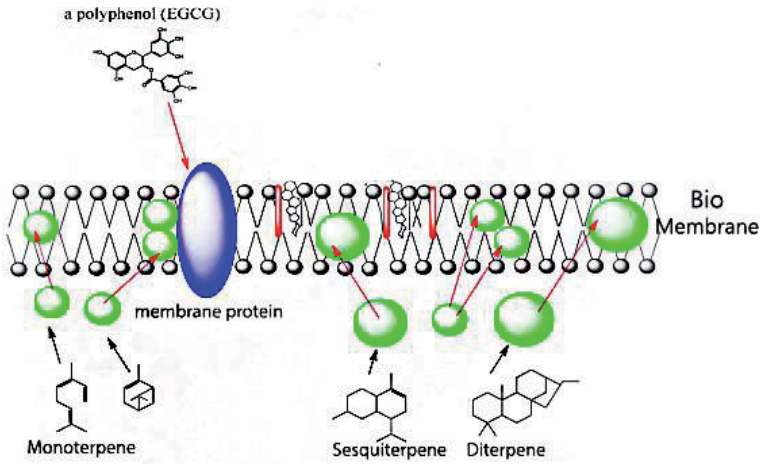

Figure 7.
Interaction of terpenoids with plant cell membrane [60].

Donor plant	Involved terpenoid compounds	Affected weed species	Ref.
Artemisia scoparia	p-Cymene Caryophyllene Germacrene D Limonene α-Pinene	*Achyranthes aspera,* *Cassia occidentalis* *Parthenium hysterophorus* *Echinochloa crus-galli,* *Ageratum conyzoides*	[26]
Nepeta meyeri	Nepetalactone	*Amaranthus retroflexus* *Portulaca oleracea* *Bromus danthoniae,* *Agropyron cristatum* *Lactuca serriola* *Bromus tectorum* *Bromus intermedius* *Chenopodium album* *Cynodon dactylon* *Convolvulus arvensis*	[66]
Leptospermum scoparium		*Digitaria sanguinalis*	[35]
—	Limonene	*Amaranthus viridis* L	[67]
Peumus boldus	Ascaridole p-Cymene 1,8-Cineole	*Amaranths hybrids* *P. oleracea*	[68]
Anisomeles indica	α-Bisabolol oxide	*Bidens pilosa* *C. occidentalis,* *A. viridis* *E. crus-galli*	[69]
Cistus ladanifer	Trans-pinocarveol Viridiflorol Bornyl acetate Ledol	*A. hybridus* *Conyza canadensis* *Parietaria judaica*	[50]
Eucalyptus salubris	1,8-Cineole α-Pinene ρ-Cymene Predominant	*Solanum elaeagnifolium*	[70]
Cupressus sempervirens	α-Pinene α-Cedrol δ-3-Carene Germacrene D	*L. rigidum* *Phalaris canariensis* *Trifolium campestre* *Sinapis arvensis*	[71]

Donor plant	Involved terpenoid compounds	Affected weed species	Ref.
Pinus pinea	Limonene α-pinene β-Pinene	*S. arvensis* *Trifolium campestre* *L. rigidum* *P. canariensis*	[72]
N. meyeri	Nepetalactone	*Bromus danthoniae* *Lactuca serriola*	[73]
Eucalyptus globulus	1,8-Cineole	*Amaranthus blitoide* *C. dactylon*	[74]
Cymbopogon citratus	Citral	*E. crus-galli*	[64]
Satureja khuzestanica *Satureja rechingeri*	Carvacrol Thymol	*Secale cereale*	[75]
Pinus brutia *Pinus pinea*	α-Pinene β-Pinene	*L. sativa* *Lepidium sativum* *P. oleracea*	[76]
Eupatorium adenophorum	γ-Cadinene γ-Muurolene	*Phalaris minor*	[77]
Pelargonium graveolens	Citronellol Geraniol	*Silybum marianum*	[78]
Artemisia judaica	Thujone Chrysanthenone	*S. marianum*	
Carum carvi	Carvone Limonene	*Phalaris canariensis*	[79]
Thymus daenensis	Thymol Carvacrol	*A. retroflexus* *Avena fatua* *Datura stramonium* *Lepidium sativum*	[80]
Eucalyptus citriodora	Citronellol	*A. viridis*	[67]
Plectranthus amboinicus	Carvacrol Thymol	*L. sativa* *Sorghum bicolor*	[81]
Tagetes minuta	Limonene piperitenone α-terpinolene Piperitone (E)-Tagetone (Z)-Ocimenone	*Chenopodium murale* *Ph. minor* *A. viridis*	[82]
Cupressus macrocarpa	Citronellal Thujene Thymol	*Digitaria australe* *A. hybridus*	[83]
Pelargonium radula	Cis-Geraniol Eudesmol	*Digitaria australe* *A. hybridus*	
Melaleuca bracteata	Methyl eugenol	*Panicum virgatum* *D. longiflora* *Stachytarpheta indica* *Aster subulatus*	[84]
C. citratus	Neral Geranial	*P. virgatum* *Chloris barbata,* *Euphorbia hirta* *Stachytarpheta indica*	[85]
Eucalyptus lehmannii	1,8-Cineole α-Thujene α-Pinene	*Sinapis arvensis* *Diplotaxis harra* *Trifolium campestre* *Desmazeria rigida* *Phalaris canariensis*	[86]

Donor plant	Involved terpenoid compounds	Affected weed species	Ref.
Eucalyptus cinerea	1,8-Cineole α-Pinene ρ-Cymene α-Terpineol	*S. arvensis* *Erica vesicaria* *Scorpiurus muricatus*	[87]
Nepeta cataria	Nepetalactone	*Hordeum spontaneum* *Taraxacum officinale* *Avena fatua*	[88]
Pinus nigra	Germacrene D δ-Cadinene Caryophyllene	*P. canariensis* *Trifolium campestre* *S. arvensis*	[89]
Zanthoxylum piperitum	Xanthoxyline	*Amaranthus tricolor*	[90]
Satureja montana	Carvacrol Thymol	*P. oleracea* *Lolium multiflorum* *E. crus-galli*	[91]
Eucalyptus citriodora	Citronellal Citronellol	*S. arvensis* *Sonchus oleraceus* *Xanthium strumarium* *A. fatua*	[92]
Copaifera duckei *Copaifera martii* *Copaifera reticulata*	Germacrene-D β-Caryophyllene, α-humulene, *δ*-elemene, and *δ*-cadinene	*Mimosa pudica L.* *Senna obtusifolia*	[93]

Table 2.
Allelopathic effects of terpenoids compounds in essential oils on seed germination and seedling development on different weed species 2010–current.

8. Conclusion

In this chapter, we presented a detail overview about roles of terpenoids in essential oils as a natural weed killer on a wide range of weed species according to the latest investigations conducted in the current decade. Terpenoids can be useful to control weeds which should be considered as a new approach agricultural sustainable to reduce weed losses and keeping the environment safe from the risks of synthetic herbicides. The current review also turns out that monoterpenoids showed the highest phytotoxicity in comparison to the sesquiterpenoids when these types considered the dominant compounds found in essential oils.

DOI: http://dx.doi.org/10.5772/intechopen.91322

Author details

Ahmed Abdulwahid Ali Almarie
College of Agriculture, University of Anbar, Anbar, Iraq

*Address all correspondence to: ahmedalmaree@yahoo.com;
ag.ahmed.abdalwahed@uoanbar.edu.iq

References

[1] Cobb AH, Reade JP. Herbicide uptake and movement. In: Herbicides Plant Physiology. 2nd ed. John Wiley & Sons. 2011. pp. 50-69. DOI: 10.1002/9781444327793

[2] Zimdahl RL. Fundamentals of Weed Science. Academic Press; 2018. Available from: https://www.elsevier.com/books/fundamentals-of-weed-science/zimdahl/978-0-12-811143-7

[3] Appleby AP. A history of weed control in the United States and Canada—A sequel. Weed Science. 2005;**53**(6):762-768. DOI: 10.1614/WS-04-210.1

[4] Cobb AH, Reade JP. Herbicide discovery and development. In: Herbicides Plant Physiology. 2nd ed. 2010. pp. 27-49. DOI: 10.1104/pp.114.241992

[5] Gianessi LP, Reigner NP. The value of herbicides in US crop production. Weed Technology. 2007;**21**(2):559-566. DOI: 10.1614/WT-06-130.1

[6] Gianessi LP. The increasing importance of herbicides in worldwide crop production. Pest Management Science. 2013;**69**(10):1099-1105. DOI: 10.1002/ps.3598

[7] Powles SB, Yu Q. Evolution in action: Plants resistant to herbicides. Annual Review of Plant Biology. 2010;**61**:317-347. DOI: 10.1146/annurev-arplant-042809-112119

[8] Zhang W. Global pesticide use: Profile, trend, cost/benefit and more. Proceedings of the International Academy of Ecological Environmental Science. 2018;**8**(1):1. Available from: http://www.iaees.org/publications/journals/piaees/articles/2018-8(1)/global-pesticide-use-profile-trend-cost-benefit.pdf

[9] Aktar W, Sengupta D, Chowdhury A. Impact of pesticides use in agriculture: Their benefits and hazards. Interdisciplinary Toxicology. 2009;**2**(1):1-12. DOI: 10.2478/v10102-009-0001-7

[10] Williams GM, Kroes R, Munro IC. Safety evaluation and risk assessment of the herbicide roundup and its active ingredient, glyphosate, for humans. Regulatory Toxicology and Pharmacology. 2000;**31**(2):117-165. DOI: 10.1006/rtph.1999.1371

[11] Niemann L, Sieke C, Pfeil R, Solecki R. A critical review of glyphosate findings in human urine samples and comparison with the exposure of operators and consumers. Journal für Verbraucherschutz und Lebensmittelsicherheit. 2015;**10**(1):3-12. DOI: 10.1007/s00003-014-0927-3

[12] Rashid B, Husnain T, Riazuddin S. Herbicides and pesticides as potential pollutants: A global problem. In: Plant Adaptation and Phytoremediation. Springer; 2010. pp. 427-447. DOI: 10.1007/978-90-481-9370-7_19

[13] Guzzella L, Pozzoni F, Giuliano G. Herbicide contamination of surficial groundwater in Northern Italy. Environmental Pollution. 2006;**142**(2):344-353. DOI: 10.1016/j.envpol.2005.10.037

[14] De Souza L. Long term toxicity of a roundup herbicide and a roundup-tolerant genetically modified maize. Food and Chemical Toxicology. 2013;**53**:440. DOI: 10.1016/j.fct.2012.08.005

[15] Boutin C, Strandberg B, Carpenter D, Mathiassen SK, Thomas PJ. Herbicide impact on non-target plant reproduction: What are the toxicological and ecological implications? Environmental Pollution. 2014;**185**:295-306. DOI: 10.1016/j.envpol.2013.10.009

[16] Yu Q, Han H, Vila-Aiub MM, Powles SB. AHAS herbicide resistance endowing mutations: Effect on AHAS functionality and plant growth. Journal of Experimental Botany. 2010;**61**(14):3925-3934. DOI: 10.1093/jxb/erq205

[17] Tranel PJ, Wright TR. Resistance of weeds to ALS-inhibiting herbicides: What have we learned? Weed Science. 2002;**50**(6):700-712

[18] Heap I. Herbicide resistant weeds. In: Integrated Pest Management. Springer; 2014. pp. 281-301. DOI: 10.1007/978-94-007-7796-5_12

[19] Soltys D, Krasuska U, Bogatek R, Gniazdowska A. Allelochemicals as bioherbicides—Present and perspectives. In: Herbicides—Current Research and Case Studies in Use. IntechOpen; 2013. pp. 517-542. DOI: 10.5772/56185

[20] Sekutowski T. Alleloherbicydy i bioherbicydy-mitczy rzeczywistość? Journal of Research and Applications in Agricultural Engineering. 2010;**55**(4):84-90

[21] Weber R, Golebiowska H. Wplyw herbicydu Titus 25 WG na zmiennosc plonowania odmian kukurydzy na Dolnym Slasku. Fragmenta Agronomica. 2009;**26**(4):181-188. DOI: 9d0504af-2719-4036-87c9-af984326f6c2

[22] Hussain MI, Reigosa MJ. Allelochemical stress inhibits growth, leaf water relations, PSII photochemistry, non-photochemical fluorescence quenching, and heat energy dissipation in three C3 perennial species. Journal of Experimental Botany. 2014;**62**(13):4533-4545. DOI: 10.1093/jxb/err161

[23] Duke SO. Why have no new herbicide modes of action appeared in recent years? Pest Management Science. 2012;**68**(4):505-512. DOI: 10.1002/ps.2333

[24] Beckie HJ, Tardif FJ. Herbicide cross resistance in weeds. Crop Protection. 2012;**35**:15-28. DOI: 10.1093/jxb/err161

[25] Bhadoria P. Allelopathy: A natural way towards weed management. American Journal of Experimental Agriculture. 2011;**1**(1):7. DOI: 10.9734/ajea/2011/002

[26] Sodaeizadeh H, Hosseini Z. Allelopathy an Environmentally Friendly Method for Weed Control. IntechOpen; 2012. DOI: 10.5772/intechopen.84109

[27] Kato-Noguchi H. Assessment of allelopathic potential of shoot powder of lemon balm. Scientia Horticulturae. 2003;**97**(3):419-423. DOI: 10.1016/S0304-4238(02)00159-0

[28] Xuan T, Tawata S, Khanh T, Chung I. Decomposition of allelopathic plants in soil. Journal of Agronomy and Crop Science. 2005;**191**(3):162-171. DOI: 10.1111/j.1439-037X.2005.00170.

[29] Chaturvedi S, Pandey J, Dhyani V, Guru S, Kaushal R. Phytotoxic potential of Eucalyptus leaf essential oil to control *Parthenium hysterophorus* L. Allelopathy Journal. 2012;**29**(2):315-324

[30] Bitas V, Kim HS, Bennett JW, Kang S. Sniffing on microbes: Diverse roles of microbial volatile organic compounds in plant health. Molecular Plant-Microbe Interactions. 2013;**26**(8):835-843. DOI: 10.1094/MPMI-10-12-0249-CR

[31] Cantrell CL, Dayan FE, Duke SO. Natural products as sources for new pesticides. Journal of Natural Products. 2012;**75**(6):1231-1242. DOI: 10.1021/np300024u

[32] Dayan FE, Owens DK, Duke SO. Rationale for a natural products approach to herbicide discovery. Pest Management Science. 2012;**68**(4):519-528. DOI: 10.1002/ ps.2332

[33] Khalaj M, Amiri M, Azimi M. Allelopathy: Physiological and sustainable agriculture important aspects. International Journal of Plant Production. 2013;**4**(5):950-962. Available from: https://www.cabdirect.org/cabdirect/abstract/20133173144

[34] Cheema ZA, Farooq M, Wahid A. Allelopathy: Current Trends and Future Applications. Springer Science & Business Media; 2012. DOI: 10.1007/978-3-642-30595-5

[35] Dayan FE, Howell JL, Marais JP, Ferreira D, Koivunen M. Manuka oil, a natural herbicide with preemergence activity. Weed Science. 2011;**59**(4):464-469. DOI: 10.1614/WS-D-11-00043.1

[36] Kumar S, Gupta N, Kumar S, Yadav V, Prakash A, Gurjar H. Metabolites in plants and its classification. World Journal of Pharmacy and Pharmaceutical Sciences. 2014;**4**(1):287-305. Available from: https://pdfs.semanticscholar.org/e6bf/8acabc5a5ddbe7ce8a1857e443415faca96e.pdf

[37] Bhowmik PC. Challenges and opportunities in implementing allelopathy for natural weed management. Crop Protection. 2003;**22**(4):661-671. DOI: 10.1016/S0261-2194(02)00242-9

[38] Jefferson LV, Pennacchio M. Allelopathic effects of foliage extracts from four *Chenopodiaceae* species on seed germination. Journal of Arid Environments. 2003;**55**(2):275-285. DOI: 10.1016/S0140-1963(03)00028-4

[39] Ferguson JJ, Rathinasabapathi B. Allelopathy: How Plants Suppress Other Plants. University of Florida Cooperative Extension Service, Institute of Food and Agricultural Science, EDIS; 2003. Available from: https://books.google.com.my/books/about/Allelopathy.html?id=Vx8JkAEACAAJ&redir_esc=y

[40] Li Z-H, Wang Q, Ruan X, Pan C-D, Jiang D-A. Phenolics and plant allelopathy. Molecules. 2010;**15**(12):8933-8952. DOI: 10.3390/molecules15128933

[41] Kruse M, Strandberg M, Strandberg B. Ecological effects of allelopathic plants—A review. In: NERI Technical Report. Molecules. 2000. p. 315. Available from: file:///c:/Users/allelopathy/Downloads/9780429155666_googlepreview.pdf

[42] Baser KHC, Buchbauer G. Handbook of Essential Oils: Science, Technology, and Applications. CRC Press; 2015. Available from: https://www.crcpress.com/handbook-of-essential-oils-science-technology-and-applications-second/Baser-Buchbauer/p/book/9781466590465

[43] Fornari T, Vicente G, Vázquez E, García-Risco MR, Reglero G. Isolation of essential oil from different plants and herbs by supercritical fluid extraction. Journal of Chromatography. A. 2012;**1250**:34-48. DOI: 10.1016/j.chroma.2012.04.051

[44] Silva SG, da Costa RA, de Oliveira MS, da Cruz JN, Figueiredo PLB, Brasil DDSB, et al. Chemical profile of *Lippia thymoides*, evaluation of the acetylcholinesterase inhibitory activity of its essential oil, and molecular docking and molecular dynamics simulations. PLoS One. 2019;**14**(3):1-17. DOI: 10.1371/journal.pone.0213393

[45] de Oliveira MS, da Cruz JN, Silva SG, da Costa WA, de Sousa SHB, Bezerra FWF, et al. Phytochemical profile, antioxidant activity, inhibition of acetylcholinesterase and interaction mechanism of the major components of the *Piper divaricatum* essential oil obtained by supercritical CO_2. The Journal of Supercritical Fluids.

2019;**145**:74-84. DOI: 10.1016/j.supflu.2018.12.003

[46] Bassolé IHN, Juliani HR. Essential oils in combination and their antimicrobial properties. Molecules. 2012;**17**(4):3989-4006. DOI: 10.3390/molecules17043989

[47] Koul O, Walia S, Dhaliwal G. Essential oils as green pesticides: Potential and constraints. Biopesticides International. 2009;**4**(1):63-84. Available from: http://projects.nri.org/adappt/docs/63-84.pdf

[48] de Oliveira MS, da Costa WA, Pereira DS, Botelho JRS, de Alencar Menezes TO, de Aguiar Andrade EH, et al. Chemical composition and phytotoxic activity of clove (*Syzygium aromaticum*) essential oil obtained with supercritical CO_2. The Journal of Supercritical Fluids. 2016;**118**:185-193. DOI: 10.1016/j.supflu.2016.08.010

[49] Koul O, Walia S. Comparing impacts of plant extracts and pure allelochemicals and implications for pest control. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources. 2009;**4**(049):1-30. DOI: 10.1079/PAVSNNR20094049

[50] Verdeguer M, Blázquez MA, Boira H. Chemical composition and herbicidal activity of the essential oil from a *Cistus ladanifer* L. population from Spain. Natural Product Research. 2012;**26**(17):1602-1609. DOI: 10.1080/14786419.2011.592835

[51] Pattanaik B, Lindberg P. Terpenoids and their biosynthesis in cyanobacteria. Lifestyles. 2015;**5**(1):269-293. DOI: 10.3390/life5010269

[52] Mazid M, Khan T, Mohammad F. Role of secondary metabolites in defense mechanisms of plants. Biochemical Medicine. 2011;**3**(2):232-249. Available from: https://www.cabdirect.org/cabdirect/abstract/20113298981

[53] Withers ST, Keasling JD. Biosynthesis and engineering of isoprenoid small molecules. Applied Microbiology and Biotechnology. 2007;**73**(5):980-990. DOI: 10.1007/s00253-006-0593-1

[54] Xu M, Galhano R, Wiemann P, Bueno E, Tiernan M, Wu W, et al. Genetic evidence for natural product mediated plant–plant allelopathy in rice (*Oryza sativa*). New Phytologist. 2012;**193**(3):570-575. DOI: 10.1111/j.1469-8137.2011.04005

[55] Ghasemzadeh A, Ghasemzadeh N. Flavonoids and phenolic acids: Role and biochemical activity in plants and human. Journal of Medicinal Plants Research. 2011;**5**(31):6697-6703. DOI: 10.5897/JMPR11.1404

[56] Maeda H, Dudareva N. The shikimate pathway and aromatic amino acid biosynthesis in plants. Annual Review of Plant Biology. 2012;**63**:73-105. DOI: 10.1146/annurev-arplant-042811-105439

[57] Köksal M, Zimmer I, Schnitzler J-P, Christianson DW. Structure of isoprene synthase illuminates the chemical mechanism of teragram atmospheric carbon emission. Journal of Molecular Biology. 2010;**402**(2):363-373. DOI: 10.1016/j.jmb.2010.07.009

[58] Cheng AX, Lou YG, Mao YB, Lu S, Wang LJ, Chen XY. Plant terpenoids: Biosynthesis and ecological functions. Journal of Integrative Plant Biology. 2007;**49**(2):179-186. DOI: 10.1111/j.1744-7909.2007.00395

[59] Varshney JG, Sondhia S. Introduction to Herbicides. 2008. Available from: https://www.google.com/#q=Introduction+to+herbicides+Book

[60] Gunsolus JL, Curran WS. Herbicide mode of action and injury symptoms.

Urbana. 2007;**51**(61801):217-333. Available from: http://appliedweeds.cfans.umn.edu/sites/appliedweeds.cfans.umn.edu/files/herbicide_mode_of_action_and_injury_symptoms.pdf

[61] Schmidt A, Wächtler B, Temp U, Krekling T, Séguin A, Gershenzon J. A bifunctional geranyl and geranylgeranyl diphosphate synthase is involved in terpene oleoresin formation in Picea abies. Plant Physiology. 2010;**152**(2):639-655. DOI: 10.1104/pp.109.144691

[62] Wink M. Biochemistry of plant secondary metabolism. In: Annual Plant Reviews. Vol. 40. John Wiley & Sons; 2015. Available from: https://www.nhbs.com/biochemistry-of-plant-secondary-metabolism-book

[63] Azimova SS, Glushenkova AI, Vinogradova VI. Lipids, Lipophilic Components and Essential Oils from Plant Sources. Springer Science & Business Media; 2011. DOI: 10.1007%2F978-0-85729-323-7

[64] Poonpaiboonpipat T, Pangnakorn U, Suvunnamek U, Teerarak M, Charoenying P, Laosinwattana C. Phytotoxic effects of essential oil from *Cymbopogon citratus* and its physiological mechanisms on barnyardgrass (*Echinochloa crus-galli*). Industrial Crops and Products. 2013;**41**:403-407. DOI: 10.1016/j.indcrop.2012.04.057

[65] Kaur S, Singh HP, Mittal S, Batish DR, Kohli RK. Phytotoxic effects of volatile oil from *Artemisia scoparia* against weeds and its possible use as a bioherbicide. Industrial Crops and Products. 2010;**32**(1):54-61. DOI: 10.1016/j.indcrop.2010.03.007

[66] Mutlu S, Atici O, Esim N. Bioherbicidal effects of essential oils of *Nepeta meyeri* Benth. on weed spp. Allopathy Journal. 2010;**26**(2):291-300. Available from: https://acgpubs.org/doc/2018080720472751-RNP-EO_1304-027.pdf

[67] Vaid S. Phytotoxicity of citronellol against *Amaranthus viridis* L. International Journal of Engineering and Applied Sciences. 2015;**2**:94-96. Available from: https://www.neliti.com/publications/257763/phytotoxicity-of-citronellol-against-amaranthus-viridis-l

[68] Verdeguer M, García-Rellán D, Boira H, Pérez E, Gandolfo S, Blázquez MA. Herbicidal activity of *Peumus boldus* and *Drimys winterii* essential oils from Chile. Molecules. 2011;**16**(1):403-411. DOI: 10.3390/molecules16010403

[69] Batish DR, Singh HP, Kaur M, Kohli RK, Singh S. Chemical characterization and phytotoxicity of volatile essential oil from leaves of *Anisomeles indica* (Lamiaceae). Biochemical Systematics and Ecology. 2012;**41**:104-109. DOI: 10.1016/j.bse.2011.12.017

[70] Zhang J, An M, Wu H, Liu DL, Stanton R. Chemical composition of essential oils of four Eucalyptus species and their phytotoxicity on silverleaf nightshade (*Solanum elaeagnifolium* Cav.) in Australia. Plant Growth Regulation. 2012;**68**(2):231-237. DOI: 10.1007/s10725-012-9711-5

[71] Amri I, Mancini E, De Martino L, Marandino A, Lamia H, Mohsen H, et al. Chemical composition and biological activities of the essential oils from three *Melaleuca* species grown in Tunisia. International Journal of Molecular Sciences. 2012;**13**(12):16580-16591. DOI: 10.3390/ijms131216580

[72] Ismail A, Lamia H, Mohsen H, Bassem J. Herbicidal potential of essential oils from three Mediterranean trees on different weeds. Current Bioactive Compounds. 2012;**8**(1):3-12. DOI: 10.2174/157340712799828197.s

[73] Kekeç G, Mutlu S, Alpsoy L, Sakçali MS, Atici Ö. Genotoxic effects of catmint (*Nepeta meyeri* Benth.) essential oils on some weed and crop plants. Toxicology and Industrial Health. 2013;**29**(6):504-513. DOI: 10.1177/0748233712440135

[74] Rassaeifar M, Hosseini N, Asl NHH, Zandi P, Aghdam AM. Allelopathic effect of eucalyptus globulus essential oil on seed germination and seedling establishment of *Amaranthus blitoides* and *Cyndon dactylon*. Trakia Journal of Sciences. 2013;**11**(1):73-81. Available from: http://tru.uni-sz.bg/tsj/vol11N1_2013/M.Rasaeifar.pdf

[75] Taban A, Saharkhiz MJ, Hadian J. Allelopathic potential of essential oils from four *Satureja* spp. Biological Agriculture and Horticulture. 2013;**29**(4):244-257. DOI: 10.1080/01448765.2013.830275

[76] Ulukanli Z, Karaborklu S, Bozok F, Burhan ATES, Erdogan S, Cenet M, et al. Chemical composition, antimicrobial, insecticidal, phytotoxic and antioxidant activities of Mediterranean *Pinus brutia* and *Pinus pinea* resin essential oils. Chinese Journal of Natural Medicines. 2014;**12**(12):901-910. DOI: 10.1016/S1875-5364(14)60133-3

[77] Ahluwalia V et al. Chemical analysis of essential oils of *Eupatorium adenophorum* and their antimicrobial, antioxidant and phytotoxic properties. Journal of Pest Science. 2014;**87**(2):341-349. DOI: 10.1007/s10340-013-0542-6

[78] Saad LMMG, Abdelgaleil SAM. Allelopathic potential of essential oils isolated from aromatic plants on *Silybum marianum*. Global Advanced Research Journal of Agricultural Science. 2014;**3**:289-297. Available from: http://garj.org/full-articles/allelopathic-potential-of-essential-oils-isolated-from-aromatic-plants-on-silybum-marianum-l.pdf?view=inline

[79] Marichali A, Hosni K, Dallali S, Ouerghemmi S, Ltaief HBH, Benzarti S, et al. Allelopathic effects of *Carum carvi* L. essential oil on germination and seedling growth of wheat, maize, flax and canary grass. Allelopathy Journal. 2014;**34**(1):81

[80] Kashkooli AB, Saharkhiz MJ. Essential oil compositions and natural herbicide activity of four *Denaei thyme* (*Thymus daenensis* Celak.) ecotypes. Journal of Essential Oil Bearing Plants. 2014;**17**(5):859-874. DOI: 10.1080/0972060X.2014.884946

[81] Pinheiro PF, Costa AV, Alves TDA, Galter IN, Pinheiro CA, Pereira AF, et al. Phytotoxicity and cytotoxicity of essential oil from leaves of *Plectranthus amboinicus*, carvacrol, and thymol in plant bioassays. Journal of Agricultural and Food Chemistry. 2015;**63**(41):8981-8990. DOI: 10.1021/acs.jafc.5b03049

[82] Arora K, Batish DR, Singh HP, Kohli RK. Allelopathic potential of the essential oil of wild marigold (*Tagetes minuta* L.) against some invasive weeds. Journal of Environmental and Agricultural Sciences. 2015;**3**:56-60. DOI: 10.212.34.21/handle/32116/2360

[83] Almarie AA, Mamat AS, Wahab Z, Rukunudin IH. Chemical composition and phytotoxicity of essential oils isolated from Malaysian plants. Allelopathy Journal. 2016;**37**(1):55-69. Available from: http://www.allelopathyjournal.org/archives/?Year=2016&Vol=37&Issue=1&Month=1

[84] Almarie A, Mamata A, Rukunudina I. Chemical composition and herbicidal effects of *Melaleuca bracteata* F. Muell. essential oil against some weedy species. International Journal of Scientific and Engineering Research. 2016;**7**(1):507-512. Available from: https://pdfs.semanticscholar.org/9ae2/a079b6635323187bb7beaa714c792039c245.pdf

[85] Almarie AA, Mamat AS, Wahab Z. Allelopathic potentil of *Cymbopogon citratus* against differentweed species. Indian Research Journal of Pharmacy and Science. 2016;**3**(1):324-330. Available from: https://irjps.in/journal/124.pdf

[86] Grichi A, Nasr Z, Khouja ML. Phytotoxic effects of essential oil from *Eucalyptus lehmanii* against weeds and its possible use as a bioherbicide. Bulletin of Environment, Pharmacology and Life Sciences. 2016;**5**:17-23. Available from: https://pdfs.Semanticscholar.org/1533/99a42ea2797f4d42e3746d176d999afc29f5.pdf

[87] Grichi A, Nasr Z, Khouja ML. Phytotoxic effects of essential oil from *Eucalyptus cinerea* and its physiological mechanisms. Journal of New Sciences. 2016;**15**

[88] Saharkhiz MJ, Zadnour P, Kakouei F. Essential oil analysis and phytotoxic activity of catnip (*Nepeta cataria* L.). American Journal of Essential Oils and Natural Products. 2016;**4**(1):40-45. Available from: http://www.essencejournal.com/vol4/issue1/pdf/3-2-12.1.pdf

[89] Amri I, Hanana M, Jamoussi B, Hamrouni L. Essential oils of *Pinus nigra* JF Arnold subsp. laricio Maire: Chemical composition and study of their herbicidal potential. Arabian Journal of Chemistry. 2017;**10**:S3877-S3882. DOI: 10.1016/j.arabjc.2014.05.026

[90] Chotsaeng N, Laosinwattana C, Charoenying P. Herbicidal activities of some allelochemicals and their synergistic behaviors toward *Amaranthus tricolor* L. Molecules. 2017;**22**(11):1841. DOI: 10.3390/molecules22111841

[91] Ibáñez M, Blázquez M. Phytotoxicity of essential oils on selected weeds: Potential hazard on food crops. Plants. 2018;7(4):79. DOI: 10.3390/plants7040079

[92] Benchaa S, Hazzit M, Abdelkrim H. Allelopathic effect of *Eucalyptus citriodora* essential oil and its potential use as bioherbicide. Chemistry & Biodiversity. 2018;**15**(8):e1800202. DOI: 10.1002/cbdv.201800202

[93] Gurgel ESC, de Oliveira MS, Souza MC, da Silva SG, de Mendonça MS, da Silva Souza Filho AP. Chemical compositions and herbicidal (phytotoxic) activity of essential oils of three *Copaifera* species (Leguminosae-Caesalpinoideae) from Amazon-Brazil. Industrial Crops and Products. 2019;**142**:111850. DOI: 10.1016/j.indcrop.2019.111850

Edited by Mozaniel Santana de Oliveira, Wanessa Almeida da Costa and Sebastião Gomes Silva

Essential oils have been used for centuries by communities all over the world in various areas and for various purposes. These include uses in medicine, flavoring, perfumery, cosmetics, insecticides, fungicides, and bactericides, among others. They are natural and biodegradable substances, generally nontoxic or with low toxicity to humans and other animals. Therefore, constant research in these areas represents an alternative for new and more efficient drugs with less side effects as well as obtaining new products and supplies. This book provides a comprehensive overview of the diverse applications of essential oils in a variety of human activities with a focus on the most important evidence-based developments in the various fields of knowledge.

Published in London, UK

IntechOpen

ISBN 978-1-83962-699-9