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# Potential of Essential Oils

*Edited by Hany A. El-Shemy*





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### Contributors

Alev Önder, Elena Stashenko, Jairo René Martínez, Mozaniel Santana De Oliveira, Marcos Martins Almeida, Marielba De Los Angeles Rodriguez Salazar, Flávia Cristina Seabra Pires, Fernanda Wariss Figueiredo Bezerra, Vânia Maria Borges Cunha, Renato Macedo Cordeiro, Glides Rafael Olivo Urbina, Marcilene Paiva Da Silva, Ana Paula Souza Silva, Rafael Henrique Holanda Pinto, Raul Nunes De Carvalho Junior, Hartati - Soetjipto, Suzan Kantarci Savaş, Efendi Nasibov, Cinzia Barbieri, Patrizia Borsotto, Billmary Zuleyma Contreras-Moreno, Geun Hee Seol, You Kyoung Shin, Maria Paz Arraiza, Avonor Cidral Da Costa Junior, Marta Berrocal-Lobo, Azucena González-Coloma, Maria Fe Andres, José Alfonso Domínguez-Núñez, NAVARRO ROCHA JULIANA, Carlos Calderón - Guerrero

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# Meet the editor



Prof. Hany A. El-Shemy received his two PhD degrees in Biochemistry and Genetic Engineering from the University of Cairo, Egypt and University of Hiroshima, Japan. He became an Assistant Professor at the Biochemistry Department of Cairo University, Egypt in 1996, an Associate Professor in 2002, and a full Professor in 2007. His research interests are in the field of plant biotechnology and medicinal plants (Molecular Biology). He received 2 patents, authored 13 international books, published more than 90 SCI journal papers and 50 conference presentations, and served as a technique committee member as well as a chair in many international conferences and the editor including PLoS ONE journal, BMC Genomics, and Current Issue in Molecular Biology. He also reviewed more than 25 SCI cited journals. He received several awards, including State prize awarded from the Academy of Science, Egypt (2004); Young Arab Researcher prize awarded from Shuman Foundation, Jordan (2005); State Excellence prize from Academy of Science, Egypt (2011); and Cairo University prizes 2007, 2010, 2014. He served as an expert for the African Regional Center of Technology, Dakar, Senegal plus a visiting professor at the Pan African University, African Union, Nairobi, Kenya. He was appointed as an acting vice president of the Academy of Science and Technology from 2013 to 2014, Egypt. Since 2014 to 2017, he was the Dean of the Faculty of Agriculture, Cairo University.



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## Preface

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This book provides an overview of essential oils, and contributors were responsible for the contents of nine chapters.

In the chapters, the authors attempted to review the importance of essential oils and their impacts for medical societies.

Moreover, the book aims to present some issues related to sources, structures, impacts, and uses of essential oils. The biodiversity of essential oils is especially well known from the medical point of view, and therefore, this has been highlighted in this book.

Essential oils are still used in rural areas from natural resources for medical treatment, and some of these uses are explained in this book.

This book will be useful for researchers and other academic staff and will add significant value to the readers.

**Hany A. El-Shemy, Professor**  
Faculty of Agriculture  
Cairo University  
Giza, Egypt



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# Potential of Medicinal Use of Essential Oils from Aromatic Plants

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Mozaniel Santana de Oliveira,  
Marcos Martins Almeida,  
Marielba de Los Angeles Rodriguez Salazar,  
Flávia Cristina Seabra Pires,  
Fernanda Wariss Figueiredo Bezerra,  
Vânia Maria Borges Cunha,  
Renato Macedo Cordeiro,  
Glides Rafael Olivo Urbina, Marcilene Paiva da Silva,  
Ana Paula Souza e Silva,  
Rafael Henrique Holanda Pinto and  
Raul Nunes de Carvalho Junior

Additional information is available at the end of the chapter

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## Abstract

The use of medicinal plants rich in essential oils can represent a viable source for the control of some diseases, being able to constitute a possible therapeutic alternative due to its effectiveness. Essential oils are natural volatile fractions extracted from aromatic plants and formed by classes of substances such as esters of fatty acids, mono and sesquiterpenes, phenylpropanoids, aldehyde alcohols and, in some cases, aliphatic hydrocarbons, among others. Essential oils have been used by mankind for medicinal purposes for several centuries, with reports coming from Ancient Egypt. In this sense, the present work aims to approach the biological activities of essential oils such as antioxidant, anticancer, antiprotozoal, antifungal, antibacterial and anti-inflammatory activities of different plant matrices rich in essential oils.

**Keywords:** natural products, essential oils, medicinal application, biological activity

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## 1. Introduction

The essential oils are formed by volatile substances and generally have low molecular weight, these substances are formed in the secondary metabolism of aromatic plants [1, 2]. However, some natural factors such as physiological variations, environmental conditions, geographic variations, genetic factors and plant evolution can alter the chemical composition of these oils as well as their yield [3].

The extraction of essential oils usually occurs with the use of conventional techniques such as hydrodistillation using a Clevenger type extractor, which is the most widespread technique for the isolation of volatile plant oils [4, 5], however, other extraction techniques are also efficient such as extraction with supercritical CO<sub>2</sub> [6, 7], this type of extraction is a technique considered clean and does not cause change in the chemical structures of the molecules, since it usually works at low operating temperatures [8].

In nature, essential oils play an important role in plants as protection and communication, chemical protections that these secondary metabolites present, also is decisive in plant resistance against pathogens and herbivores [9]. In the communication the plant can use a chemical agent that travels through the atmosphere and activate defensive genes of other plants, such as the methyl jasmonate of *Solanaceae* and *Fabaceae* [10].

In the industry these oils are widely studied, mainly for their potential applications as agents promoting biological activities. The volatile compounds have presented over the years several pharmacological applications, such as antioxidant, anticancer, antiprotozoal, antimicrobial and anti-inflammatory activities [11–15]. In recent work [16] demonstrated that species like *Ocimum basilicum* and *Thymbra spicata* have good antioxidant and antimicrobial activity against *Staphylococcus aureus*, *Streptomyces murinus*, *Micrococcus luteus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Proteus vulgaris*, *Candida albicans* and *Aspergillus niger*. Jeena et al. [17] revealed that ginger oil has significant antioxidant, anti-inflammatory and antinociceptive activities and Xiang et al. [18] evidenced that the essential oils of *Curcuma herbs* have anticarcinogenic actions against LNCaP and HepG2 cells. In this sense, this work aims to approach different biological activities of essential oils that may be important for the maintenance of human health.

## 2. Biological activities of essential oils

### 2.1. Antibacterial and antifungal activity of essential oils (EO)

The antimicrobial action of essential oils is not yet fully understood, but can be attributed to their permeability to the cell wall of microorganisms due to their diverse chemical and synergistic composition. The hydrophobic characteristic of the essential oils acts in the partition of the lipids of the cellular membrane and the mitochondria, making them more permeable, in this way, the critical ions and molecules (lipids, proteins and nucleic acids) are extravasated, leading them to death. EOs generally have less action on gram-positive bacteria than on gram-negative bacteria due to the interaction of the hydrophobic components of the essential oils and the cell membrane [19–21].

Different methods are used to evaluate the antibacterial and antifungal properties. The most used are: the method of disc diffusion of Agar, Minimal Inhibition Concentration (MIC), Minimum Bacteria Concentration (MBC) and Minimum Fungicide Concentration (MFC). Since the use of the disc diffusion method in agar is limited by the hydrophobic nature of essential oils and plant extracts that prevents its uniform diffusion through the agar medium, most authors report the results obtained with MIC and MBC [22].

In recent years, different microbial species of medical interest have been tested, from which encouraging results have emerged. **Table 1** shows data on the antimicrobial activity of essential oils on fungi and bacteria, also showing the main components of essential oil.

The potential antimicrobial activity of essential oils of the *Hedychium coronarium* Koen rhizome from different locations in Eastern India was studied in gram-positive, gram-negative bacteria and fungal strains. The study revealed that the essential oils presented more satisfactory effects to the antifungal action than to the antibacterial activity. In addition, the gram-positive bacteria are more sensitive to oil than gram-negative due to the peptidoglycan layer did not selectively act on essential oil compounds. The antimicrobial action of the essential oils was attributed to its constituents in an isolated way, as well as synergistically, additive or antagonistic to each other [23].

Essential oils isolated from *Nepeta leucophylla*, *Nepeta ciliaris*, *Nepeta clarkei* and *Calamintha umbrosa* showed significant antifungal activity *in vitro* against phytopathogenic fungi responsible for plant diseases. Essential oils have the potential to be used as a possible biofungicide (as an alternative to synthetic products) that may contribute to an increase in the pre and post harvest storage life of food crops [25].

The good results obtained encourage future research aimed at a possible application of these substances in food, pharmaceutical and cosmetology fields. **Table 1** presents the main chemical components of essential oils of several plants with antimicrobial potential.

## 2.2. Antioxidant activity

The interest in the study of the antioxidant substances of essential oils has become more and more intensified and is now indispensable for the prevention of diverse pathologies [27]. In the literature, it is reported the presence of antioxidant activity in several essential oils [28–30].

This property acts at different levels in the microorganism protection and plays a key role in some of the biological activities of essential oils, being able to combat the development of oxidative stress that causes damage to health, increasing the risk of diseases such as Alzheimer's, Parkinson's and inflammation associated with atherosclerosis and rheumatoid arthritis. Some studies point out that these diseases may be consequences of damages caused by free radicals, besides oxygen and reactive nitrogen species that act as mediators of inflammation as messenger molecules. This shows that essential oils can also act as an anti-inflammatory agent [31–33].

Essential oils have great potential in the nutrition industry in view of their antioxidant properties, they are use as feed additives for farm animals, for example, and that may be fundamental to the quality of food products from these animals, since essential oils can improve nutritional value, oxidative stability and increase the shelf life of these products such as meats

Plant source	Main components	Microorganism	*MIC	Reference
<i>Hedychium coronarium</i> Koen.	$\beta$ -Pinene; eucalyptol; linalool; coronarin-E; $\alpha$ -pinene; p-cymene; $\gamma$ -terpinene and 10- <i>epi</i> - $\gamma$ -eudesmol	<i>Candida albicans</i> and <i>Fusarium oxysporum</i>	3.12–400 $\mu$ g/ml	[23]
<i>Laportea aestuans</i> (Gaud)	Methyl salicylate; fenchol; 1,2-cyclohexanedione dioxime; 1,4-octadiene and linalool	<i>E. coli</i> ; <i>S. aureus</i> , <i>B. subtilis</i> ; <i>P. aeruginosa</i> ; <i>K. pneumoniae</i> ; <i>S. typhi</i> ; <i>C. albicans</i> ; <i>R. stolon</i> ; <i>A. niger</i> and <i>P. notatum</i>	50–200 mg/ml	[24]
<i>C. umbrosa</i>	$\beta$ -caryophyllene Germacrene D Spathulenol	<i>F. oxysporum</i> <i>H. maydis</i> <i>A. solani</i>	1500–3000 $\mu$ g/ml	[25]
<i>N. leucophylla</i>	Caryophyllene oxide Iridodial $\beta$ -monoenoil Acetate	<i>F. oxysporum</i> <i>H. maydis</i> <i>A. solani</i>	1000–3000 $\mu$ g/ml	
<i>N. ciliaris</i>	$\beta$ -Caryophyllene $\beta$ -Sesquiphellandrene Caryophyllene oxide	<i>F. oxysporum</i> <i>H. maydis</i> <i>A. solani</i>	1000–3000 $\mu$ g/ml	
<i>N. clarkei</i>	$\beta$ -Sesquiphellandrene Actinidine Germacrene D	<i>F. oxysporum</i> <i>H. maydis</i> <i>A. solani</i>	1000–3000 $\mu$ g/ml	
<i>Juglans regia</i> L.	$\alpha$ -Pinene $\beta$ -Pinene $\beta$ -Caryophyllene germacrene D limonene	<i>S. aureus</i> <i>E. coli</i> <i>S. typhi</i> <i>S. dysenteriae</i> <i>K. pneumonia</i> <i>B. subtilis</i> <i>S. epidermidis</i> <i>P. vulgaris</i> <i>P. aeruginosa</i>	15.62–62.50 $\mu$ g/ ml	[26]

\*Minimum Inhibitory Concentrations.

**Table 1.** Main components of essential oils with antimicrobial potential.

and eggs. In addition, they are often treated as foods to enhance the taste and organoleptic properties, and even has the function of decreasing the process of deterioration of food. The latter is mainly due to its antimicrobial and antioxidants activities [31, 34, 35].

The interest in extracts rich in natural antioxidants has recently increased, especially the antioxidant activity of essential oils. Most of them confirm the assumption that essential oils are promising as natural antioxidants, which can replace synthetic additives such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) that are potentially harmful to

human health [36–38]. In this context, **Table 2** presents some more recent studies found in the literature based on the antioxidant activity of essential oils, highlighting its main constituents and antioxidant performance evaluation methods.

### 2.3. Anticancer activity

Essential oils from aromatic plants have been treated as a product containing anticancer properties because they have the ability to inhibit cell proliferation and decrease the spread of cancer, improving the quality of life of cancer patients and reducing the level of their agony. Mediated therapy with essential oils can be used in combination with conventional therapies in the treatment of cancer (quimioterapia e radioterapia) [44–46].

According to the World Health Organization [47] cancer is a generic term used for a large group of diseases that can affect any part of the body, is characterized by the growth of abnormal cells beyond their usual limits in the body. Other common terms used are malignant tumors and neoplasms, the latter process or stage of the disease is called metastasis. Cancer is a major public health problem and is considered the second leading cause of death worldwide, accounting for 8.8 million deaths by 2015, where nearly 1 in 6 deaths is caused by cancer. Ref. [48] reported that the American Cancer Society reported in the year 2017 approximately 1,688,780 new cases of cancer and 600,920 deaths from cancer in the United States. According to [49–51] the most common causes of cancer death are melanoma, leukemia, followed by lung, liver, prostate, breast, cervical, colorectal, and endometrial cancers.

Plant source	Main constituents	Biological activity	Reference
<i>Pinus</i> ( <i>P. tabulaeformis</i> , <i>P. tabulaeformis</i> f. <i>shekanensis</i> , <i>P. tabulaeformis</i> var. <i>mukdensis</i> , <i>P. tabulaeformis</i> var. <i>umbraculifera</i> , <i>P. henryi</i> and <i>P. massoniana</i> )	$\alpha$ -Pinene, bornyl acetate, $\beta$ -caryophyllene, $\alpha$ -guaiene, germacrene D	<i>Pinus</i> were evaluated for antioxidant potential by three methods (DPPH, FRAP and ABTS)	[39]
<i>Ocimum basilicum</i> L.	Linalool, methyl chavicol, 1,8-cineole	The free radical scavenging activity of the oil was measured by the DPPH method	[40]
<i>Ocimum basilicum</i> , <i>Mentha spicata</i> , <i>Pimpinella anisum</i> and <i>Fortunella margarita</i>	Carvone, methyl chavicol, trans-anethole, limonene	The evaluation of the ability to eliminate the free radicals of the oils was by the DPPH and ABTS methods	[41]
<i>Salvia lavandulifolia</i>	Camphor, 1,8-cineole, camphene, $\alpha$ -pinene	The <i>S. lavandulifolia</i> were evaluated for antioxidant potential by three methods (DPPH, FRAP and ABTS)	[42]
<i>Rosmarinus officinalis</i>	$\alpha$ -Pinene, 1,8-cineole, Camphor	The antioxidant activity was evaluated in 7 samples of rosemary oil based on the measurement of the antioxidant reduction capacity in relation to the DPPH radical	[43]

**Table 2.** Antioxidant activity of essential oils.

The sharp increase in the number of cancer cases can be attributed to eating habits, since foods contain many chemicals such as preservatives and dyes, making people more susceptible to cancer, which can also be accentuated with the use of tobacco and alcohol, chronic infections, exposure to harmful radiation, or due to change in lifestyle and environmental pollution [45, 52]. Previous studies have reported that oxidative stress increases the onset of different chronic diseases, including cancer. Reactive oxygen species (ROS) are highly unstable compounds that have the ability to attack cells and tissues in the human body, followed by destructive effects that lead to the beginning of cancer [46, 53].

Therefore, there has been a recent increase in the use of natural products such as spices and plants to replace or accompany common treatments for cancer because of their high costs, side effects and the development of resistance of patients against anticancer drugs [44, 52].

Thus, essential oils from different aromatic plants have anticancer potential against mouth, breast, lung, prostate, liver, kidney, colon, bone, ovary, pancreas, uterus and brain cancer and even in leukemia, glioblastoma, melanoma [45, 54]. Thus [52] have shown that essential oil extracted from cloves (*Syzygium aromaticum* L.) is an ideal natural source as a chemopreventive agent against breast cancer betulinic acid and other triterpenes, can be indicated as constituents responsible for anticancer properties [55] which determined that the essential oil of eucalyptus (*Pulicaria inuloides*) presented anticancer activity against breast, liver and colorectal/colon cancer due to the abundant presence of citronellol, pulegol and citronelil acetate.

The myrtle essential oil (*Myrtus communis* L.) shows anticancer activity against blood cancer (leukemia) due to the presence of 1,8-cineole, linalool, myrtenyl acetate, and myrtenol [56]. However, [46] have shown that orange peel oil (*Citrus sinensis*) has anticancer properties against colorectal/colon, prostate and lung cancer, with  $\alpha$ -limonene being the predominant chemical constituent. Therefore, the results of studies justify the use of essential oils, as a possible alternative medicine in the treatment of cancer.

Essential oils act in the chemoprevention and suppression of cancer, which involve apoptosis, cell cycle retention, antimetastatic and antiangiogenic, increased levels of reactive oxygen and nitrogen species (ROS/RNS), modulation of DNA repair and others that demonstrate their antiproliferative cancer cell activity [53, 57]. In addition, the lipophilic nature of the EOs allows them to cross cell membranes and enter easily within the cell [45, 54], in **Table 3** we can observe the anticancer activities of different aromatic plants.

#### 2.4. Antiparasitic activity

Current treatment media control most diseases of protozoan origin mainly through chemotherapy, where synthetic drugs are generally used, but they show several side effects of cytotoxicity in humans. Due to the hydrophobic and bioactivities nature of its components, essential oils (EO) can be considered important sources of development of agents against intracellular pathogens such as protozoa, which cause parasitic diseases [64].

The EO of leaves of *Artemisia indica* showed antimalarial activity in vitro, being a prophylactic potential of malaria, which is a disease caused by the protozoan of the genus *Plasmodium*. The oil inhibited at least two recombinant enzymes from the biosynthesis of plasmid fatty acids and showed low cytotoxicity in mammals [65].

Plant source	Main constituents	Biological activity	Reference
<i>Rosa damascena</i>	Nerol, kaempferol and geraniol	Liver cancer, human breast cancer, prostate cancer	[58]
<i>Pulicaria inuloides</i>	4,5-dimetiltiazol-2-il and 2,5-difeniltetrazólio	Breast cancer	[55]
<i>Citrus sinensis</i>	D-Limonene and alcohol perylic (oxygenated monoterpene)	Colorectal/colon cancer, prostate cancer, lung cancer	[46]
<i>Aquilaria crassna</i>	$\beta$ -Caryophyllene, 1-phenanthrenecarboxylic acid, $\alpha$ -caryophyllene and azulene benzenedicarboxylic acid	Colorectal/colon carcinoma, pancreatic cancer	[59]
<i>Myrtus communis</i> L.	1,8-cineole, linalool, myrtenyl acetate and myrtenol	Blood cancer (leukemia)	[56]
<i>Eucalyptus citriodora</i> Hook	Pulegol, citronellol and citronellil acetate	Breast cancer, liver cancer, colorectal/colon cancer	[53]
<i>Cinnamon cassia</i> spp.	Cinnamic aldehyde, cinnamyl aldehyde and tannins	Head and neck cancer	[57]
<i>Syzygium aromaticum</i> L.	Betulinic acid and triterpenes	Human breast cancer	[52]
<i>Trachyspermum ammi</i> L.	$\gamma$ -Terpinene, timol and P-cymene	Liver cancer	[60]
<i>Commiphora myrrha</i>	2-cyclohexen-1-one and 4-ethynyl-4-hydroxy-3,5,5-trimethyl	Liver cancer, cervical cancer	[61]
<i>Salvia officinalis</i>	Hydrocarbons, monoterpene, oxygenated monoterpenes sesquiterpene and diterpenes	Human breast cancer, prostate cancer, kidney cancer	[62]
<i>Tagetes minuta</i> L.	cis- $\beta$ -ocimene, cis-tagetone and trans-tagetenone	Breast cancer, blood cancer (leukemia)	[63]

**Table 3.** Anticancer activity of essential oils.

Another EO that presents the antimalarial effect is that obtained from *Piper aduncum* leaves, with camphor (17.1%), viridiflorol (14.5%) and piperitone (23.7%) being the main components found in this oil [66]. The EO of the leaves of *Aniba canelilla* (HBK) Mez presented a trypanocidal effect, being considered a potential for the natural treatment to trypanosomosis, which is caused by the protozoan *Trypanosoma evansi*, since it proved its action *in vivo*. Its antiprotozoal activity is related to the compounds 1-nitro-2-phenylethane (83.68%) and methyleugenol (14.83%), the latter being slightly more active than the first in the treatment of the disease [67].

The EO of the leaves of *Tetradenia riparia* presented antileishmanial effect *in vivo* and *in vitro*, being effective in the fight against the protozoan of the species *Leishmania (Leishmania) amazonenses*, without showing toxicity to human erythrocytes. The main compound responsible for this therapeutic effect is the 6,7-dehydroroyleanone, which was also tested in isolation and showed a similar effect to the EO [68]. EO from *Lippia alba*, was investigated *in vitro* and *in vivo* assays to evaluate antiparasitic effects and histopathological changes of tambaqui (*Colossoma macropomum*). Concentrations of 1280 and 2560 mg/L showed 100% efficacy after 20 min of oil exposure in (*Anacanthorus spathulatus*, *Notozothecium janauachensis* and *Mymarothecium boegeri*) [69].

The antiparasitic activity of *Lavandula stoechas* oil was investigated in *Leishmania major*, *Leishmania tropica* and *Leishmania infantum*. The evaluation of the antileishmanial activity of *Lavandula stoechas* EO presented a greater effect in comparison to the drug Glucantime. The bioactive compounds present in this oil are: fenchone (31.81%), camphor (29.60%), terpineol (13.14%), menthone (8.96%) and eucalyptol [70].

The anthelmintic activity of *Thymus vulgaris* L. EO was investigated in *in vitro* and *in vivo* tests to evaluate the effect on *Haemonchus contortus* parasites present in the gastrointestinal system of sheep. Thymol is the major compound corresponding to 50.22% of the oil from the *Thymus vulgaris* species. Results showed that EO inhibited 96.4% of egg incubation, 90.8% of larval development and 97% of larval mobility [71]. Other essential oils, their chemical constituents and biological antiparasitic activities are shown in **Table 4**.

## 2.5. Anti-inflammatory activity

Essential oils have complex mixtures of chemicals that are present in different concentrations, these oils are used in medicine to treat a myriad of diseases because they present potential for anti-inflammatory activity [78, 79].

Inflammation is typically a protective mechanism that can be stimulated by a variety of harmful agents, which may be chemical, physical or biological. Living and vascular tissues respond to stimuli that are considered irritating to the body. These irritations can usually be linked to pain, redness (erythema), heat, tumor (edema), tissue loss or organic function [80, 81].

In recent years the anti-inflammatory potential of essential oils and their chemical position has become the object of study of several researchers in the search for new drugs of natural origin [82–84], as well as a study of the synergistic anti-inflammatory effect of the chemical constituents of essential oils and synthetic drugs, showing a possible association between

Plant source	Main constituents	Biological activity	Reference
<i>Chenopodium ambrosioides</i>	Ascaridole, carvacrol and caryophyllene oxide	Antileishmanial, antimalarial and antitrypanosoma	[72]
<i>Cinnamomum verum</i>	(E)-cinnamaldehyde and eugenol	Antitrypanosoma	[73]
<i>Eugenia uniflora</i> L.	Sesquiterpenes, curzerene, $\gamma$ -elemene and trans- $\beta$ -elemenone	Antileishmanial	[74]
<i>Lavandula angustifolia</i>	Borneol, epi- <i>D</i> -muurolol, <i>D</i> -bisabolol, precocene I and eucalyptol	Antischistosomal	[75]
<i>Piper hispidinervum</i> (Piperaceae)	Safrole	Antiamoebicidal	[76]
<i>Teucrium ramosissimum</i>	$\delta$ -Cadinene, $\delta$ -cadinol, $\beta$ -eudesmol, $\gamma$ -gurjunene and cedrene	Antiamoebicidal	[77]

**Table 4.** Anti-parasitic activity of essential oils.

Plant source	Main constituents	Biological activity	Reference
<i>Globba sessiliflora</i> Sims.	$\beta$ -Eudesmol, (E)- $\beta$ -caryophyllene, caryophyllene oxide, T-muurolol	Anti-inflammatory	[91]
<i>Piper glabratum</i>	$\beta$ -Pinene, longiborneol, $\alpha$ -pinene, (E)-caryophyllene	Anti-inflammatory	[84]
<i>Phyllanthus muellerianus</i>	Isoelemicinb, caryophyllene oxide, $\alpha$ -Cadinol, 2-isopropyl benzoic acid	Anti-inflammatory	[92]
<i>Salvia officinalis</i>	1,8-Cineole, camphor, $\beta$ -pinene, E- $\beta$ -caryophyllene	Anti-inflammatory	[93]
<i>Lippia gracilis</i> Schauer	Thymol, carvacrol, p-cymene, $\alpha$ -pinene	Anti-inflammatory and healing activity	[94]
<i>Citrus limon</i>	Limonene, $\beta$ -pinene, $\gamma$ -terpinene, sabinene	Anti-inflammatory	[95]
<i>Cymbopogon citratus</i>	Geranial, neral, $\beta$ -myrcene, geranyl acetate	Anti-inflammatory	[96].
<i>Anethum graveolens</i> L.	$\alpha$ -Phellandrene, limonene, dill ether, $\alpha$ -pinene	Anti-inflammatory	[97]
<i>Citrus aurantium</i> L.	Linalool, linalylacetate, nerolidol, Z,E-farnesol	Anti-inflammatory	[98]
<i>Blumea balsamifera</i> (L.) DC.	Borneol, caryophyllene, ledol, caryophyllene oxide	Anti-inflammatory	[99]

**Table 5.** Anti-inflammatory activity of essential oils.

clinical remedies with natural products as a pharmacological alternative and avoiding adverse reactions caused by synthetic products [85]. In vivo tests performed on rats confirm the potential of these essential oils as natural products, helping to advance research [86, 87].

The knowledge of the chemical composition and the chemotype of the aromatic plants are important factors in studies of the anti-inflammatory activity, since the concentration of the compounds diverge due to this biological variation, in this way researchers have evaluated both aspects [88, 89]. Evaluating the specific constituents of a particular essential oil may help in understanding the performance of these compounds in the anti-inflammatory action [90].

**Table 5** shows the anti-inflammatory potential of different essential oils.

### 3. Conclusion

Essential oils may play an important role in the maintenance of human health, since they have several biological properties, and may become a natural alternative for the control of several diseases, however, the great majority of published works present the results of these oils based on its chemical composition complex and not only based on a substance, because the biological effects of these oils can be related to a synergism and/or an antagonism between the chemically active substances that are part of its composition.

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## Author details

Mozaniel Santana de Oliveira\*, Marcos Martins Almeida, Marielba de Los Angeles Rodriguez Salazar, Flávia Cristina Seabra Pires, Fernanda Wariss Figueiredo Bezerra, Vânia Maria Borges Cunha, Renato Macedo Cordeiro, Glides Rafael Olivo Urbina, Marcilene Paiva da Silva, Ana Paula Souza e Silva, Rafael Henrique Holanda Pinto and Raul Nunes de Carvalho Junior\*

\*Address all correspondence to: mozaniel.oliveira@yahoo.com.br and raulncj@gmail.com

LABEX/FEA (Faculty of Food Engineering), Program of Post-Graduation in Food Science and Technology, Federal University of Para, Belém, Pará, Brazil

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# Chemical Composition of Essential Oil of Genus *Pimenta* (Myrtaceae): Review

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Billmary Zuleyma Contreras-Moreno

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## Abstract

Myrtaceae Juss., the name derived from the genus *Myrtus communis*, is considered the eighth largest flowering plant family and of considerable importance on the ecological and economic area (by its production of essential oils). The species that belong to Myrtaceae with primarily tropical and subtropical distribution, with a greater diversity in the southern hemisphere, dispersed mainly in the regions of South America, Central America, Asia, East and Southwest of Australia and with a low representation in Africa. The Myrtaceae family includes more than 5500 species and approximately 150 genera, the genus *Pimenta* being one of the representatives of medicinal interest, which comprises 15 species (+6 varieties) located mainly in tropical America. Due to its economic and pharmacological importance, its best known species are *Pimenta dioica* and *P. racemosa*. *Pimenta* species can produce a volatile content of 1–5% from fresh leaves. To date, studies of this genus have been focused mainly on the content of volatile essences, used in formulation of cosmetics, analysis of chemical composition, and biological activities, such as antimicrobial, antioxidant, insecticidal, and anti-inflammatory activity, eugenol being the main compound responsible for their biological potential.

**Keywords:** essential oil, chemical composition, chemotypes, eugenol, *Pimenta*

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## 1. Introduction

Plants are considered as one of the main natural resources of secondary metabolites for medicinal use, due to their biological potential, either to attack deadly diseases, endemics, or diseases that affect living beings, so, according to the World Health Organization, nearly

80% of the population in developing countries use them for their primary health-care needs, either because of cultural tradition or because there are no other options, due to the high cost of medicines for these populations [1].

The diverse nature of chemical compounds produced by species of the family Myrtaceae has allowed to locate it as one of the families of greater medicinal use, since some of its species are used to treat respiratory affections [2–5], to strengthen the gums, pains of tooth [3], gastrointestinal disorders [4, 6], skin conditions and snake bites [4–6], for rheumatic or muscular pain, neuralgia, migraine, nervous system disorders, fevers, diseases of the urinary system, diabetes [2, 4, 6], help in job of childbirth [7], and from the economic point of view by their wood and as a producer of spices and essential oils [8].

Genus *Pimenta*, one of the representatives of this family, comprises 21 species including several varieties, is typical of tropical America [9, 10], is considered of medicinal and economic interest, and is rich in a structural variety of volatile substances such as monoterpenes, sesquiterpenes, and phenylpropenes (present in the essential oils), can generate from fresh leaves, a content of volatile essences between 1 and 5% [11, 12].

Essential oils derived from plants, obtained by hydrodistillation, steam distillation, or by extraction with organic solvents, are complex mixture that may contain between 20 and 100 volatile substances of low-molecular weight belonging to different chemical classes, which are presented as liposoluble liquids at room temperature, generally colorless or pale yellow, light, hydrophobic (soluble in alcohol, non-polar or weakly polar solvents, waxes, and oils), and easily oxidizable by exposure to air, light, and heat [13], and they can be biosynthesized in different parts of the plant anatomy (in the leaves, in the flowers, in the fruits, in the pericarp of the fruit, in the seeds, in the bark, and in the rhizomes, whether they are stored in oil glands, glandular hairs, or dissolved in resins) [13, 14].

Interest in essential oils in recent years is based on the versatility of its use in different industrial areas (pharmaceutical, food, health, cosmetics, and perfumery), not only on the possibility of obtaining aromatic compounds (pleasant odor) but in its application as antioxidants, food preservatives, and medicines, and its application as protectors of crops and plants, incorporating them into the packaging material of the products, being less toxic than the synthetic antioxidants of greater use [14–16] or incorporated in dermocosmetic formulations aimed at the treatment and prevention of skin diseases mediated by oxidative stress [15, 17]. This is the case of essential oil obtained from *Pimenta racemosa* var. *racemosa*, which, for its aroma and antioxidant and antimicrobial activity, has been incorporated in perfumes, creams, formulations of aftershave lotions, soaps and hair treatments, as antifungal treatment for aquarium waters and flavorings of foods and products of confectionery, making it a very valuable ingredient for the cosmetic, pharmaceutical, and food industry [14–25].

Taking into account that essential oils represent a therapeutic alternative in natural products against several pathogens that threaten public health and individual health of patients, it would be interesting to establish for genus *Pimenta*, if the chemical composition of its volatile essences has among their major components chemotypes that can classify the oils of the

different species that constitute it and be responsible for their biological potential. This chapter provides information on all documents on *Pimenta* species reported between 1921 and 2018 with chemical composition of essential oils.

## 2. Myrtaceae family

Myrtaceae Juss., the name derived from the genus *Myrtus communis* [26], which comes from the Mediterranean region [27], is considered within the angiosperms as one of the largest families in the world, occupying the eighth place of flowering plants and of considerable importance in the ecological and economic area (by its production of essential oils), corresponds to the subclass Rosidae and to the order Myrtales [28]; it contains more than 5500 species separated by taxonomists in two subfamilies, Psiloxylloideae and Myrtoideae, 17 tribes and approximately 150 genera [9, 29–31]; its species are often difficult to identify and classify, so a high probability of plants that still remain undescribed is estimated [32].

The species that belong to this family have a primarily tropical and subtropical distribution, with a greater diversity in the southern hemisphere, dispersed mainly in regions of South America, Central America, Asia, east and southwest of Australia, and with a low representation in Africa [8, 9, 16, 33], having mostly shrubs and trees predominantly woody, ericoids, with evergreen leaves. Venezuela has 20 native genera, five genera introduced with several species in cultivation [34], and about 246 species, of which 34 species (+2 varieties) are endemic to the country [35, 36].

This family is very old. It is believed that it originated in the Cretaceous period [31], diversifying widely over time from the most primitive forms of rainy and humid forests to specialized forms in semi-arid, very dry regions, highly influenced by seasonal changes [37]; its diverse nature of chemical compounds produced by species of the family Myrtaceae has allowed to locate it as one of the families of source of substances with pharmacological activities [2–13], as a producer of woods, spices, and essential oils [9].

## 3. Genus *Pimenta*

Genus *Pimenta* Lindley belongs to family Myrtaceae, subfamily Myrtoideae, and to Myrteae tribe, comprises 15 species (+6 varieties) [38], was described by John Lindley in 1821 as the type species "*Pimenta officinalis*." Its name derives from the Latin pigmentum, "color" of the verb to paint, a name destined for spices, in association with the characteristics of the fruit of that type [40, 41]; it is characterized by fragrant shrubs or trees, with opposite leaves and glandular on both sides, simple hairs, more or less conspicuous collector nerve. Inflorescence in multiflora vertices, arranged in the upper armpits or subterminals, can have 3–15 flowers [9, 42]; its distribution is typical from tropical America [9, 38, 42, 43], being the majority of the species, native to the Caribbean and Central America, except the species *P. pseudocaryophyllus*

(Gomes) Landrum LR, which is endemic from Brazil [39, 40, 44, 45]. In Venezuela, it is only represented by *P. racemosa* (Mill.) JW Moore (*P. acris* Kostel) and is distributed in Falcón, Federal District, Lara, Mérida, Nueva Esparta, Táchira, Sucre, and Zulia states [35].

The species of this genus are used in several countries including Barbados, Brazil, China, Cuba, Dominican Republic, England, Haití, India, Kerala, Mangalore, Mexico, Middle East, Taiwan, USA, and Venezuela [45–56], in various areas, whether to build agricultural tools, houses, or living fences because of the resistance of its wood against termites, industrially for the production of condiments, flavors, perfumes, and cosmetics, or in the treatment of various pathologies of traditional medicine such as fever, rheumatism, toothache, abdominal pain, pneumonia, colds, pectoral angina, diarrhea, incontinence, stroke, anti-inflammatory, and analgesic properties [10, 12, 15, 57–59]. Among pharmacological effects reported for different *Pimenta* species include anticancer, antidermatophytic, antihemorrhagic bleeding, anti-inflammatory, antimicrobial, antimutagenic, antinociceptive, antioxidant, antipyretic, central nervous system depressant, cobra venom, hypoglycemic, hypotensive, inhibitor of histone acetyl transferase enzyme, inhibitor of enzyme histidine carboxylase, and insect repellent [10, 12, 15, 59].

Chemistry studies of *Pimenta* species have led to the identification of a variety of secondary metabolites of the type: tannins, phenolic compounds, flavonoids, and a structural variety of volatile substances such as monoterpenes, sesquiterpenes, and phenylpropenes (present in essential oils), which could generate a content of volatile essences from fresh leaves between 1 and 5% [12, 13]. Essential oils of *P. racemosa* can present characteristic, aromatic, and pleasant odors, due to their major components; for example, a lemon smell due to the neral/geranial content (72%), an aniseed odor due to the presence of methylchavicol/methyleugenol (81%), and clove odor due to the presence of chavicol/eugenol (73%) [60].

Furthermore, the best-known species of this genus, due to its economic and pharmacological importance, are *P. dioica* (L.) Merrill and *P. racemosa* (Mill.) J. W. Moore [40, 41].

#### 4. Chemical composition of essential oils of the genus *Pimenta*

Essential oils, also called essences, volatile oils, or etheric oils [13, 61], are from a chemical point of view complex mixtures of volatile substances that comprise between 20 and 100 or more components at various concentrations; in general, there are two or three major compounds, which are in concentrations between 20 and 70% in comparison with the other components of the oil that may be present in lower amounts or even in traces [14]. They are described frequently only as a product of “vegetable raw materials” [61, 62]; these oils are lipophilic, usually odoriferous, yellow pale, or colorless when recently extracted and liquid at room temperature [61]; they are oxidized by exposure to air, light, and heat [13] and produced by the plants as defense mechanism, signaling, or as part of their secondary metabolism [61, 63, 64]; they can be biosynthesized in different parts of the plant anatomy (in the leaves, in the flowers, in the fruits, in the pericarp of the fruit, in the seeds, in the bark, and in the rhizomes, whether stored in glands of oils, glandular hairs, or dissolved in resins) [13, 14], and almost always, they are endowed with aromas pleasant as the case of species from genus *Pimenta* with aromas at lemon, anise, or clove [60].

Plant species (origin)	Part of plant used	Extraction method	Main compounds (area %)	Reference
<i>P. adenoclada</i> (Cuba)	Leaves	Hydrodistillation	Caryophyllene oxide (15.4), $\alpha$ -muurolol (9.4), humulene epoxide II (7.6), trans-sabinol (5.6), $\beta$ -pinene (5.3)	[67]
<i>P. dioica</i> (Jamaica)	Leaves	Steam distillation	Eugenol (66.38–79.24), $\beta$ -caryophyllene (0.97–7.10)	[68]
<i>P. dioica</i> (México)	Berries	Steam distillation	Methyl-eugenol (48.3), myrcene (17.7), eugenol (17.3), $\beta$ -caryophyllene (6.2)	[69]
<i>P. dioica</i> (México)	Berries	Hydrodistillation	Methyl-eugenol (62.7), myrcene (16.5), eugenol (8.3), 1,8-cineole (4.1)	[69]
<i>P. dioica</i> (México)	Berries	Supercritical CO <sub>2</sub> extraction	Methyl-eugenol (67.9), eugenol (14.9), myrcene (6.0), $\beta$ -caryophyllene (5.2)	[69]
<i>P. dioica</i> (Cuba)	Leaves	Hydrodistillation	Eugenol (34.14), 1,8-cineole (14.69), $\alpha$ -humulene (10.12), $\gamma$ -cadinene (5.49)	[70]
<i>P. dioica</i> (Australia)	Leaves	Supercritical CO <sub>2</sub> extraction	Eugenol (77.9), $\beta$ -caryophyllene (5.1), squalene (4.1)	[71]
<i>P. dioica</i> (Australia)	Leaves	Hydrodistillation	Eugenol (45.4), $\beta$ -caryophyllene (8.9), $\alpha$ -cadinol (5.9), $\alpha$ -humulene (5.4)	[71]
<i>P. dioica</i> (Antilles)	Leaves	Commercial (Robert et Fils, Montréal, QC, Canada)	Eugenol (47.78), myrcene (26.76), geraniol (10.40)	[72–73]
<i>P. dioica</i> (Jamaica)	Leaves	Commercial (Kurt Kitzing Co., Wallerstein, Germany, 800675)	Eugenol (76.02), methyl eugenol (7.14), $\beta$ -caryophyllene (6.47)	[19]
<i>P. dioica</i> (Jamaica)	Leaves	Hydrodistillation	Eugenol (79.81–83.68)	[74]
<i>P. dioica</i> (Jamaica)	Berries	Commercial (Oshadhi Ltd., Cambridge, UK)	Eugenol (86.44), $\beta$ -caryophyllene (7.70), methyl eugenol (3.87)	[75]
<i>P. dioica</i> (Jamaica)	Leaves	Commercial (Kurt Kitzing Co., Wallerstein, Germany, 800116)	Eugenol (76.0)	[76]
<i>P. dioica</i>	Berries	Steam distillation	Methyl-eugenol (62.7), eugenol (8.3), 1,8-cineole (4.1)	[77]

Plant species (origin)	Part of plant used	Extraction method	Main compounds (area %)	Reference
<i>P. dioica</i> (Brazil)	Fruits	Hydrodistillation	Eugenol (76.98), $\beta$ -pinene (6.52), limonene (4.09)	[78]
<i>P. dioica</i> (Mexico)	Fruits	Hydrodistillation	Methyl-eugenol (48.7), eugenol (16.3), myrcene (17.1)	[79]
<i>P. dioica</i> (USA)	Leaves	Hydrodistillation	Eugenol (62.1), methyl-eugenol (22.9)	[80]
<i>P. dioica</i> (India)	Leaves	Hydrodistillation	Eugenol (47.80–55.35)	[81]
<i>P. dioica</i> (India)	Leaves	Hydrodistillation	Eugenol (68.4), chavicol (10.4), methyl-eugenol (6.1), 1-octen-3-ol (2.7)	[82]
<i>P. dioica</i> (México)	Leaves	Hydrodistillation	Eugenol (94.86), $\alpha$ -terpineol (2.45)	[83]
<i>P. dioica</i> (Sri Lanka)	Leaves	Hydrodistillation	Eugenol (85.33), $\beta$ -caryophyllene (4.36), 1,8-cineole (4.19)	[84]
<i>P. dioica</i> (India)	Fruits	Commercial (Plant Lipids Ltd. India)	Eugenol (35.42), methyl-eugenol (28.02), $\beta$ -caryophyllene (8.66), $\beta$ -Mirtsen (8.55), 1,8-cyneole (5.62)	[85]
<i>P. guatemalensis</i> (Costa Rica)	Leaf	Hydrodistillation	Eugenol (72.8), $\beta$ -caryophyllene (8.2), terpinolene (3.0).	[86]
<i>P. guatemalensis</i> (Costa Rica)	Fruits	Hydrodistillation	Eugenol (74.7), caryophyllene oxide (3.3).	[86]
<i>P. haitiensis</i> (Dominican Republic)	Leaves	Steam distillation	Methyl-chavicol (11.65–41.10), 1,8-cineole (11.35–16.63), linalool (16.03–17.81), trans-anethol (6.76–8.70), methyl-eugenol (0.61–24.39),	[87]
<i>P. haitiensis</i> (Dominican Republic)	Leaves	Hydrodistillation	Methyl-chavicol (19.94–32.83), 1,8-cineole (17.62–33.14), linalool (15.97–16.32), methyl-eugenol (0–14.95), trans-anethole (4.66–8.50)	[87]
<i>P. jamaicensis</i> (Jamaica)	Leaves	Steam distillation	Eugenol (61.79), 1,8-cineole (43.94–49.43), $\alpha$ -terpineol (0.34–18.02), limonene (10.33), 4-terpineol (6.37–7.17), p-cymene (2.25–10.25), $\beta$ -caryophyllene (5.77)	[88]

Plant species (origin)	Part of plant used	Extraction method	Main compounds (area %)	Reference
<i>P. obscura</i> (Jamaica)	Leaves	Steam distillation	1,8-cineole (16.84–25.11), $\alpha$ -cymene (10.97–11.33), $\alpha$ -terpineol (6.71–8.13), limonene (5.31), $\beta$ -eudesmol (5.29), 4-terpineol (4.92–9.80), $\alpha$ -phellandrene (6.33), Ledol (13.47), palustrol (7.64)	[89]
<i>P. pseudocaryophyllus</i> var. <i>pseudocaryophyllus</i> (Brazil)	Leaves	Hydrodistillation	Geranial (34.26), neral (27.85), linalol (5.18), geraniol (4.82), $\beta$ -caryophyllene (4.40)	[90]
<i>P. pseudocaryophyllus</i> (Brazil)	Leaves (Cardoso isle)	Hydrodistillation	Eugenol (71.9)	[91]
<i>P. pseudocaryophyllus</i> (Brazil)	Leaves (Paranapiacaba)	Hydrodistillation	Methyl-eugenol (94.6)	[91]
<i>P. pseudocaryophyllus</i> (Brazil)	Leaves	Hydrodistillation	Eugenol (92.59)	[92]
<i>P. pseudocaryophyllus</i> (Brazil)	Leaves	Hydrodistillation	Chavibetol (70.9), methyl-eugenol (20.7), $\alpha$ -cymene (2.8)	[93]
<i>P. pseudocaryophyllus</i> (Brazil)	Leaves (Brazilian)	Hydrodistillation	( <i>E</i> )-methyl-isoegenol (78.0–93.6), methyl-eugenol (3.1–18.1)	[11]
<i>P. pseudocaryophyllus</i> (Brazil)	Leaves (São Gonçalo do Abaeté)	Hydrodistillation	Geranial (36.5–47.2), neral (21.4–33.6), $\beta$ -caryophyllene (0–6.1), caryophyllene oxide (0–13.5)	[11]
<i>P. pseudocaryophyllus</i> (Brazil)	Leaves	Hydrodistillation	Chavibetol (50.2–70.9), methyl-eugenol (15.4–20.7)	[94]
<i>P. pseudocaryophyllus</i> (Brazil)	Leaves	Hydrodistillation	Geranial (37.3–46.6), neral (25.8–28.7), spathulenol (0–6.1), caryophyllene oxide (0–5.5), $\beta$ -caryophyllene (0–8.0), Bicyclgermacrene (0–5.7)	[95]
<i>P. pseudocaryophyllus</i> (Brazil)	Leaves	Hydrodistillation	( <i>E</i> )-methyl-isoegenol (5.0–94.3), ( $\beta$ -caryophyllene (8.5–26.6), elemicin (5.8–11.7), $\delta$ -cadinene (0–9.2), $\alpha$ -copaene (0–5.7), ( <i>E</i> )-asarone (0–65.5)	[95]
<i>P. pseudocaryophyllus</i> (Brazil)	Leaves (cital)	Hydrodistillation	Geranial (36.49), neral (27.59), caryophyllene oxide (8.88)	[96]

Plant species (origin)	Part of plant used	Extraction method	Main compounds (area %)	Reference
<i>P. pseudocaryophyllus</i> (Brazil)	Leaves	Hydrodistillation	( <i>E</i> )-methyl-isoeugenol (93.9)	[96]
<i>P. pseudocaryophyllus</i> (Brazil)	Leaves	Commercial (Lazlo Aromatologia Ltda., Brazil)	Eugenol (88.6), $\beta$ -caryophyllene (4.8)	[97]
<i>P. racemosa</i>	Leaves	Steam distillation	Contenido de fenol (65–73)	[98]
<i>P. racemosa</i>	Leaves	Commercial	Eugenol (33.8), myrcene (21.3), 1,8-cineole (9.7), chavicol (8.9)	[99]
<i>P. racemosa</i> (Colombia)	Leaves	Steam distillation	Eugenol (96)	[100]
<i>P. racemosa</i>	Leaves (Bay)	Steam distillation	Eugenol (56.2), chavicol (21.6), myrcene (13.9)	[101]
<i>P. racemosa</i>	Leaves (anise)	Steam distillation	Methyl-eugenol (43.1), methyl-chavicol (31.6), myrcene (12.0)	[101]
<i>P. racemosa</i>	Leaves (lemon)	Steam distillation	Geranial (53.2), neral (32.6)	[101]
<i>P. racemosa</i>	Leaves	Hydrodistillation	Eugenol (56.9), myrcene (18.4), chavicol (12.2)	[101]
<i>P. racemosa</i>	Leaves	Commercial	Eugenol (45.5), myrcene (29.1), chavicol (12.0)	[101]
<i>P. racemosa</i> (Jamaica)	Leaves	Commercial (Kurt Kitzing Co., Wallerstein, Germany, 800116)	Eugenol (45.60), myrcene (24.97), chavicol (9.31)	[20]
<i>P. racemosa</i> (Cuba)	Leaves	Hydrodistillation	terpinen-4-ol (20.7), 1,8-cineole (20.4), eugenol (10.7), chavicol (10.1), $\alpha$ -terpineol (10.0), $\alpha$ -cymene (8.0)	[102]
<i>P. racemosa</i> (Benin)	Leaves	Hydrodistillation	Eugenol (55.7–61.9), myrcene (12.5–22.3), chavicol (8.0–15.3)	[103]
<i>P. racemosa</i> (Jamaica)	Leaves	Commercial (Kurt Kitzing Co. Wallerstein, Germany, 800116)	Eugenol (45.6)	[76]
<i>P. racemosa</i> (Nigeria)	Aerial part	Hydrodistillation	Germacrene D (10.6), $\beta$ -elemene (8.8), germacrene A (7.3), selin-11-en-4- $\alpha$ -ol (6.3), $\delta$ -cadinene (5.9), $\beta$ -caryophyllene (5.8), germacrene B (5.3), $\alpha$ -copaene (5.2)	[22]

Plant species (origin)	Part of plant used	Extraction method	Main compounds (area %)	Reference
<i>P. racemosa</i> (Benin)	Leaves	Hydrodistillation	Eugenol (52.7), myrcene (29.4), chavicol (9.3)	[23]
<i>P. racemosa</i> (USA)	Leaves	Hydrodistillation	Eugenol (64.0), myrcene (14.6)	[80]
<i>P. racemosa</i> (India)	Leaves	Hydrodistillation	Eugenol (72.9–92.9), myrcene (0–9.6), chavicol (0–7.7)	[104]
<i>P. racemosa</i> (Jamaica)	Leaves	Hydrodistillation	Eugenol (64), myrcene (14.6), chavicol (7.7), $\beta$ -caryophyllene (4.9)	[105]
<i>P. racemosa</i> var. <i>racemosa</i> (Dominican Republic)	Leaves	Steam distillation	Eugenol (44.41–68.93), myrcene (0–16.17), chavicol (0–15.51), methyl-eugenol (0–11.88), $\beta$ -caryophyllene (0–7.24)	[106]
<i>P. racemosa</i> var. <i>racemosa</i> (Guadeloupe)	Leaves (bay)	Hydrodistillation	Eugenol (56.1), chavicol (17.1), myrcene (6.4), linalool (6.0)	[60]
<i>P. racemosa</i> var. <i>racemosa</i> (Guadeloupe)	Leaves (lemon)	Hydrodistillation	Geraniol (40.3), neral (31.7), limonene (5.3), myrcene (4.6)	[60]
<i>P. racemosa</i> var. <i>racemosa</i> (Guadeloupe)	Leaves (anise)	Hydrodistillation	Methyl-eugenol (48.1), methyl-chavicol (32.8), myrcene (12.8), linalool (6.0)	[60]
<i>P. racemosa</i> var. <i>racemosa</i> (Benin)	Leaves	Hydrodistillation	Eugenol (52.7), myrcene (26.6), chavicol (6.3)	[107]
<i>P. racemosa</i> var. <i>racemosa</i> (Venezuela)	Leaves	Hydrodistillation	Eugenol (48.7), limonene (13.6), 1,8-cineole (12.7)	[108]
<i>P. racemosa</i> var. <i>racemosa</i> (Venezuela)	Leaves (light oil)	Hydrodistillation	Eugenol (60.4) myrcene (11.7), chavicol (6.0), limonene (5.4), linalool (4.4)	[12, 15, 59]
<i>P. racemosa</i> var. <i>racemosa</i> (Venezuela)	Leaves (heavy oil)	Hydrodistillation	Eugenol (82.9), chavicol (9.3)	[12, 15, 59]
<i>P. racemosa</i> var. <i>grisea</i> (Dominican Republic)	Leaves	Steam distillation	Trans-methyl-isoeugenol (85.08–86.32), methyl-eugenol (0–92.60), geraniol (0–85.52)	[106]
<i>P. racemosa</i> var. <i>grisea</i> (Dominican Republic)	Leaves	Hydrodistillation	4-metoxi-isoeugenol (75.23)	[50, 109]
<i>P. racemosa</i> var. <i>hispaniolensis</i> (Dominican Republic)	Leaves	Steam distillation	Methyl-eugenol (7.08–63.88), methyl-chavicol (5.13–22.61), 1,8-cineole (17.57–37.96), 4-terpineol (16.21–28.98), timol (0–44.02), $\gamma$ -terpinene (0–16.67), $o$ -cymene (0–8.59)	[106]

Plant species (origin)	Part of plant used	Extraction method	Main compounds (area %)	Reference
<i>P. racemosa</i> var. <i>ozua</i> (Dominican Republic)	Leaves	Steam distillation	1,8-cineole (47.24–55.93), 4-terpineol (5.05–15.67), $\alpha$ -terpineol (6.68–15.12), limonene (9.32–30.07)	[106]
<i>P. racemosa</i> var. <i>terebinthina</i> (Dominican Republic)	Leaves	Hydrodistillation	$\alpha$ -Terpineol acetato (27.0), $\alpha$ -terpineol (20.0), 4-metoxi-eugenol (12.6), terpinen-4-ol (5.95)	[50, 109]

**Table 1.** Main compounds of essential oils from genus *Pimenta* (L).

The composition of essential oils contributes significantly to the determination of the pharmacological potential attributed to the plant species (indicated mainly by the major compounds) and is constantly being transformed, due to factors external to the biology of the plants (edaphic or environmental) and/or intrinsic to the biology of plants (physiological and genetic) [14, 65, 66].

Essential oils of *Pimenta* are characterized by the presence of monoterpenes, sesquiterpenes, and phenylpropanoids, and due to medicinal and economic interest, many researchers in different latitudes of the planet have been dedicated to carrying out studies to their chemical composition (**Table 1**), using basically three methods of extraction: steam distillation, hydrodistillation, and supercritical CO<sub>2</sub> extraction, with gas chromatography coupled to mass spectrometry (GC-MS) as analysis technique.

The subsequent text is reflected in **Table 1**; the chemical composition for species of genus *Pimenta* is analyzed by GC-MS and reported in the study consulted from 1921 to the present. All the information collected was organized taking into account plant species, origin, part of plant used, extraction method, and main compounds (area %).

According to the data reported in **Table 1**, the important qualitative and quantitative differences in the chemical composition of the essential oils of genus *Pimenta* can be estimated; the leaves have been the most studied part of the plant, followed by fruits and aerial parts. The conventional technique and the most used was the hydrodistillation using Clevenger apparatus. Of all the known species from genus *Pimenta* in South America, only *P. pseudocaryophyllus*, *P. racemosa*, and *P. dioica* have been collected. GC/MS analysis demonstrated the presence of volatile compounds with a content higher than 20% (area peak), such as eugenol (*P. dioica*, *P. haitiensis*, *P. jamaicensis*, *P. pseudocaryophyllus*, and *P. racemosa*), methyleugenol (*P. dioica*, *P. haitiensis*, *P. pseudocaryophyllus*, *P. racemosa*, *P. racemosa* var. *grisea*, *P. racemosa* var. *hispaniolensis*, and *P. racemosa* var. *racemosa*), 1,8-cineole (*P. dioica*, *P. haitiensis*, *P. jamaicensis*, *P. obscura*, *P. pseudocaryophyllus*, *P. racemosa*, *P. racemosa* var. *hispaniolensis*, *P. racemosa* var. *ozua*, and *P. racemosa* var. *racemosa*), and myrcene (*P. dioica*, *P. racemosa*, *P. racemosa* var. *hispaniolensis*, and *P. racemosa* var. *Racemosa*). It can also be seen that these compounds are mainly derivatives of phenylpropanoids and monoterpenes.

## 5. Conclusions and future perspectives

According to the study, the analysis of the chemical composition of the essential oils of *Pimenta* species collected in 16 countries revealed a high content of phenolic compounds, highlighting eugenol and methyl-eugenol as the major constituents. When comparing the major compounds of the essential oils among the 12 analyzed species of genus *Pimenta*, it is evident that there are variations between different species and between the same species with different origin. In addition, taking into account that eugenol can be considered a chemotaxonomic marker for the species *P. dioica*, *P. haitiensis*, *P. jamaicensis*, *P. pseudocaryophyllus*, and *P. racemosa* and that essential oils with a high content of eugenol exhibit antimicrobial, antioxidant, and insecticide activities, it can be said that the essential oils of the genus *Pimenta* have a therapeutic potential for the treatment of many pathologies. Therefore, the economic importance of essential oils from genus *Pimenta* around the world is unquestionable.

## Author details

Billmary Zuleyma Contreras-Moreno<sup>1,2,3\*</sup>

\*Address all correspondence to: [billmary.contreras@gmail.com](mailto:billmary.contreras@gmail.com)

1 Laboratory of Polymers and Colloids (POLYCOL), Faculty of Engineering, University of Los Andes (ULA), Mérida, Venezuela

2 Laboratory "C" of Natural Products, Research Institute, Faculty of Pharmacy and Bioanalysis, University of Los Andes (ULA), Mérida, Venezuela

3 Natural Products Research Group (GIPRONA), Nucleus University Rafael Urdaneta (NURR), University of Los Andes (ULA), Trujillo, Venezuela

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# Antibacterial Properties of Essential Oil in Some Indonesian Herbs

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Hartati Soetjipto

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## Abstract

The antibacterial activity of essential oil of five Indonesian herbs has been studied. The essential oil produced from different parts of plants (lime, lemon, Surinam cherry, fennel, and toothache plants) were extracted by water steam or hydro distillation and then examined by GCMS. The antibacterial activities of the essential oils were determined by measuring MIC (minimum inhibitory concentration), whereas some bacterial strains were used in this study such as follows: *Staphylococcus aureus* FNCC 0047, *Bacillus subtilis* ATCC 6051, *B. cereus* FNCC 0057, *Escherichia coli* IFO 0091, *Pseudomonas cepacia* FNCC 0063, and *P. aeruginosa* FNCC 0063, respectively. All the five samples used in this experiment have antibacterial activity against Gram-positive and Gram-negative bacteria. Gram-negative bacteria appear more resistant than Gram-positive bacteria. By using MIC measurement the Gram-positive and Gram-negative bacteria showed different sensitivities toward essential oils. Among the material study, the essential oil of *Eugenia uniflora* L (Surinam cherry) showed the highest antibacterial activity.

**Keywords:** antibacterial, essential oil, Indonesian herbs, *Eugenia uniflora*, *Spilanthes paniculata*

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## 1. Introduction

Essential oil has been used since ancient times as perfumery in ritual ceremony and folk medicine by native countries. Together with the time passed, essential oil utilizing has been developed not only in folk medicine but also in food industry as flavoring, cosmetics as fragrance, and additives [1]. These oil have many biological activities especially rich in mono

and sesquiterpene which are known as antimicrobial. Indonesia has around 28,000 plant species and was predicted that more than 7000 species have potency as medicinal plants. Unfortunately just less than 300 species were used in pharmaceutical industry, whereas the rest still need the evidence [2]. One of the important compounds in Indonesian herbs is essential oil, a volatile oil from the plants composed of many phenolic compounds and responsible for strong antibacterial effect.

Essential oil is aromatic oily liquid obtained from different parts of the plants (root, bark, leaf, bud, flower, fruit, and seeds). This oil name bears the name of the plant species from which it is derived. This oil has a sharp smell that is produced as secondary metabolite and variable mixtures of terpenoid, monoterpene (C<sub>5</sub>), sesquiterpene (C<sub>15</sub>), and diterpene (C<sub>20</sub>). Another functional groups present in the molecule formed another molecules such as aldehydes, ketones, acids, lactones, etc. The amount of components varies from approximately 10–100, although usually the main part of the oil is composed of only a few components [3].

As a tropical country, Indonesia is rich with aromatic plants, and it is natural for this country to be one of the essential oil world supplier. Approximately 70 kinds of essential oil were trading in the international market and 40 kinds among them come from Indonesia [4]. There are a lot of methods to produce essential oil, and it can be obtained by expression, enfleurage, solvent extraction, and distillation (hydro distillation and steam distillation). Distillation is most commonly used for commercial production [5]. Plant materials cut in small pieces were placed in distillation apparatus and hydro distilled/steam distilled for 3–6 hours. The hot water or vapors contact with material and bring the essential oil inside the sample, and the next step solvent was evaporated. The system of distillation instrument will cool down and condense vapors to produce essential oil and water mixture. Distillate separation will give essential oil and water.

Although the essential oils have different aroma and big variation, but this oil shows similar physical properties as color and solubility in water, for example essential oil are immiscible with water but quite soluble in most organic solvents. These characters can be used as basic criterion of quality of essential oil.

The increasing demand of essential oil in the world was due to the potential of essential oil in pharmacological therapeutic.

The major problem in antimicrobial chemotherapy is the increasing occurrence of resistance to antibiotic. A lot of essential oils are known to exert antimicrobial activity, but the mechanism of action is often not entirely understood. The overuse of antibiotics is the most important factor contributing to the appearance of many kinds of resistant microbes [6, 7]. The aim of this study was to determine the antibacterial activity of five Indonesian herbs and to analyze the dominant component of each essential oil.

### **1.1. Antibacterial activity of essential oil**

The bioactivity of essential oils has been known since ancient times. This compound has been known to have various bioactivities, including antibacterial, antiviral, anti-inflammatory, antifungal, antimutagenic, anticarcinogenic, and antioxidant, as well as other miscellaneous activities [8, 9].

Essential oil/volatile oil is produced from plant materials and showed an important role in plants by acting as protector of the plants from herbivores, microbial, and insects. On the other side, essential oil also has a role in the pollination and seed distribution because the strong smell of the oil attracts some insects to carry out both processes [10]. *Rosmarinus officinalis* essential oil was reported to possess potential psychostimulant activity [11]. The essential oil of leaves from *E. uniflora*, characterized by sesquiterpenes, has anti-*Leishmania* activity [12]. Essential oil of *Mentha piperita* leaves demonstrated good antiseptic, antibacterial, and antiviral properties [13, 14]. This oil contains a lot of secondary metabolites that can inhibit the growth of microbial and a rich source of biological active compounds [10]. Essential oils with aldehydes or phenols as major components (cinnamaldehyde, citral, carvacrol, eugenol, or thymol) are the most effective, followed by essential oil containing terpene alcohols [15]. Essential oil with ketones or esters ( $\beta$ -myrcene,  $\alpha$ -thujone, or geranyl acetate) possesses a lower activity [16, 17]. Although the major components of essential oil are very important for their biological activity, the minor components play a significant role, as they can strengthen the effects of major components, though antagonistic, and additive effects have also been observed.

Antibacterial activity of essential oil depends on their chemical composition and the amount of each compound. The composition, structure, as well as functional groups of the oils play an important role in determining their antimicrobial activity [18, 19].

The mixture of various chemical substances that belong to different chemical families, including terpenes, aldehydes, alcohols, esters, phenolic, ethers, and ketones, gives the antibacterial activity [20, 21].

In general, essential oil is easier to attack Gram-positive bacteria than Gram-negative bacteria due to the differences of the strength of the cell membrane. The cell wall of Gram-positive bacteria is more simple than Gram-negative bacteria, because the big part of the cell wall is peptidoglycan, and so hydrophobic molecules are able to penetrate the cell. On the contrary, the cell wall of Gram-negative bacteria is more complex, and it has peptidoglycan layer thinner than Gram-Positive bacteria but the peptidoglycan linked to lipopolysaccharide. This is the reason that the cell wall of Gram-negative bacteria stronger than the other and relatively resistant to hydrophobic compounds [22].

Generally, the chemical characterization of many essential oils reveals the presence of only 2–3 major components at a fairly high concentration (20–70%) compared to other components present in trace amounts. Most essential oils are composed of terpenes, terpenoids, and other aromatic and aliphatic constituents with low molecular weights [23]. Essential oil contains a wide series of secondary metabolites that can inhibit or slow the growth of bacteria, yeasts, and molds. The essential oil and their components have a variety of targets, particularly the membrane and cytoplasm, and in certain situations, they completely alter the morphology of the cells [24–26].

## 2. Experiment

### 2.1. Material and methods

Five essential oils were used which are as follows: Lime oil (*Citrus aurantifolia*), Lemon oil (*Citrus limon* (L) Burm), Eugenia oil (*Eugenia uniflora*), Foeniculi oil (*Foeniculum vulgare* Mill),

and *Spilanthes* oil (*S. paniculata*). Microorganisms were obtained from the Laboratory of Microbiology Faculty of Biology Universitas Kristen Satya Wacana. The strains used for the study were *Staphylococcus aureus* FNCC 0047, *Bacillus subtilis* ATCC 6051, *B. cereus* FNCC 0057, *Escherichia coli* IFO0091, *Pseudomonas cepacia* FNCC 0063, and *P. aeruginosa* FNCC 0063, respectively.

All chemical reagents made by E-Merck, Germany, Nutrient Broth, Mueller-Hinton Agar and Tetracycline (PA, Oxoid, England), and paper disk (PA, Whatman, England).

Isolation and physicochemical analysis of essential oil were subjected to steam distillation using clevenger-type apparatus. GCMS analysis of essential oils was done in Laboratory of Organic Chemistry, Faculty of MIPA Universitas Gadjah Mada Yogyakarta.

### 2.1.1. Isolation of essential oil

The essential oils were obtained from the aerial part of plants, such as peel (*C. aurantifolia*), leaves (*E. uniflora*), and flowers (*S. paniculata*), with steam distillation and seeds (*F. vulgare*) with hydrodistillation method. The fresh material was collected from Salatiga area and was identified in Laboratory of Natural Product, Universitas Kristen Satya Wacana.

One kilogram of each plant part was cut into small pieces and subjected to steam distillation apparatus, which is completed with Clevenger apparatus for 6 hours. The next step, essential oils were isolated by extraction of the distillate used diethylether, and its percentage yield was calculated.

### 2.1.2. Direct bioautographic test

The essential oil was evaluated in vitro by thin-layer chromatography (TLC) method. Before using, the plates were activated at 105°C for 10 minutes. The plate of silica gel F254 4 × 10 cm (Merck) as a solid phase and toluene:ethylacetate (93:7) as a mobile phase were used. Afterward, plate was sprayed with bacterial suspension in Mueller-Hinton Broth (MHB), and plate was stored in a water-vapor chamber at 37°C in 24 hours. Iodonitrotetrazolium chloride 5 mg/ml was used to visualize the antibacterial spot [27]. This activity was used as an effort in the beginning for check and recheck after measurement antibacterial activity.

### 2.1.3. Determination of antibacterial activity

The antibacterial activity was detected by minimum inhibitory concentration (MIC) of five essential oils. Bacteria was inoculated to nutrient broth (NB), incubated at 30°C for 24 hours. Inoculum was diluted by using physiological solution (NaCl 0.9%) to match 0.5 Mc Farland standard. The bacterial suspension was diluted and measured by UV-Vis Spectrophotometer to obtain Optical Density (OD) 0.4–0.5 at 550 nm [28].

A paper disk was dropped 20- $\mu$ l essential oil in certain concentration and put the disk in a petri dish with medium content bacteria inside. The petri dish was incubated at 30°C for

24 hours. Inhibition area diameter (IAD) was measured as a middle line start from the clear spot around the disk. The lowest concentration which shows the clear spot around the paper disk is the minimum inhibitory concentration (MIC).

#### 2.1.4. Data analysis

Antibacterial activity data of each plant were analyzed by using randomized completely block design (RCBD) sub-sampling, five treatments, three subsamples, and five replications, whereas the blocks are the analysis time [29].

## 2.2. Result and discussion

### 2.2.1. Essential oil

The percentage yield (rendement) of essential oil obtained from samples less than 3% except Foeniculi oil (3%), and the smallest amount was Spilanthes oil around 0.1% (**Table 1**).

Plant volatiles constitute about 1% of plant secondary metabolites and are mainly represented by terpenoids, phenylpropanoids/benzenoids, fatty acid derivatives, and amino acid derivatives [34]. A lot of monoterpenes demonstrated their potent aromas, and these compounds are known as essential oil composer. Essential oil containing monoterpenes is responsible for the fragrant and biological properties of aromatic and medicinal plants [35].

Essential oil obtained from samples was further analyzed by GCMS. A total of more than 20 compounds were found in each essential oil sample. **Table 2** informed five dominant compounds of each essential oil.

#### 2.2.1.1. *Citrus aurantifolia* Swingle (lime) and *C. limon* (L) Burm

According to the result of this study, the percentage yields of essential oil of the peel of lime and lemon were 0.4 and 0.5%, respectively. Limonene was major component of both Citrus oils, but limonene in lemon oil (43.40%) was higher than lime oil (29.29%). The presence of  $\beta$ -pinene in big amount (24.54%) in lime oil makes the aroma of lime oil quite different from

Scientific name	Plant family	Part used	Rendement (% weight)
<i>Citrus aurantifolia</i> Swingle (Lime) [30]	Rutaceae	peel	0.4
<i>Citrus limon</i> (L) Burm (Lemon) [30]	Rutaceae	peel	0.5
<i>Eugenia uniflora</i> L (Surinam cherry) [31]	Myrtaceae	leaf	0.5
<i>Foeniculum vulgare</i> Mill (Anise) [32]	Apiaceae	seeds	3.0
<i>Spilanthes paniculata</i> Wall (Legetan) [33]	Compositae	flower	0.1

**Table 1.** Plants, families, part used and rendement.

No	Scientific name	Five dominant compounds
1	<i>Citrus aurantifolia</i> Swingle (Lime)	<b>limonena, 29.29%</b> ; $\beta$ -pinene, 24.54%; Terpineol, 2.87%; $\alpha$ -terpineol, 2.84%; $\alpha$ -terpinolene, 1.93%
2	<i>Citrus limon</i> (L) Burm	<b>limonena, 43.40%</b> ; $\beta$ -myrcene, 3.34%; $\alpha$ -terpinolene, 2.50%; geranyl acetate, 2.44%; 2- $\beta$ pinene, 1.38%
3	<i>Eugenia uniflora</i> L (Surinam cherry)	<b>spathulenol, 12.03%</b> ; dodecanol, 11.78%; dodekanal, 4.16%; $\beta$ -elemen, 4.08%; <i>caryophyllene</i> , 2.97%
4	<i>Foeniculum vulgare</i> Mill (Anise)	<b>estragole, 38.51%</b> ; trans-anetol, 29.67%; fenkon(1-1,2,3-trimethyl bicyclic) 2.2.1-2-heptanol, 22.70%; 1-limonena, 2.97%; alpha-pinena 2.18%
5	<i>Spilanthes paniculata</i> Wall (toothache plant)	<b>trans-caryophyllene, 24.19%</b> ; $\beta$ -ocimene, 16.38%; $\beta$ -phellandrene, 10.79%; 1-pentadecene, 9.75%; germacrene, 8.08%

**Table 2.** Five dominant chemical component of essential oil from five Indonesian herbs.

lemon oil. Limonene is the main component of Citrus essential oil. The major compound in the lime essential oil is **limonena (29.29%)**, followed by  $\beta$ -pinene (24.54%), terpineol (2.87%),  $\alpha$ -terpineol (2.84%), and  $\alpha$ -terpinolene (1.93%), whereas for the lemon oil, the major compound also was **limonena (43.40%)**, and followed by  $\beta$ -myrcene (3.34%),  $\alpha$ -terpinolene (2.50%), geranyl acetate (2.44%), and 2- $\beta$  pinene (1.38%).

Ref. [36] found that limonene is the dominant compounds in lime essential oil (49.657%), and this compound was also observed at every stages of maturation, which indicates that limonene could be used as a functional index of ripeness. The peel of Sicilian lemon variety was reported to have d-limonene concentration around 70 and 0.84% of bisabolene [37]. Ref. [38] also found that monoterpenes are the dominant component (86–88.79%) of *Citrus volkameriana* peel oil, and Limonene concentration is able to reach almost 80% depending on the state of ripeness of the fruit.

Oil of lemon is one of the most important flavoring oils, used widely in all kind of beverages, soft drink, baked goods, such as cakes, pastries, gelatin dessert, ice cream, etc. This oil can also be applied in perfumes, toilet waters, *eaux de cologne*, and cosmetics [39]. In Malaysia, the oils from the fruits and the leaves are commercially used as flavors and fragrances, as well as in cooking, perfumery, and medical treatments, especially in aromatherapy [40]. This situation is not different within Indonesia. Citrus oil was used as fragrances and aromatherapy.

#### 2.2.1.2. *Eugenia uniflora* L. (Surinam cherry)

*Eugenia uniflora* L is one of Myrtaceae family, commonly known as Brazilian Cherry tree or “Dewandaru” (Indonesian). It is an aromatic species, and its essential oil has pharmacological properties that are well characterized in the literature as antioxidant and antimicrobial [41]. The yield of *Eugenia* essential oil was obtained in this study was 0.5%, composed of 57 compounds, but the highest compounds are Spathulenol (12.03%) and dodecanol (11.78%), both of them almost in the same amount, then followed by dodekanal

(4.16%),  $\beta$ -elemen (4.08%), and caryophyllene (2.97%). According to the report of [42], five dominant compounds of 16 compounds in the essential oil of *E. uniflora*: caryophyllene (8.812%), spathulenol (7.712%), isolongifolene (6.621%), viridiflorol (5.781%), and alloaromadendrene (5.568%). Ref. [43] also found that the yield of EuEO was 0.3%, 32 components were identified in this oil by GC-MS, constituting 92.65% of the total mixture. EuEO was shown to be rich in oxygenated sesquiterpenes (62.55%) and sesquiterpene hydrocarbons (29.37%). Curzerene was the major constituent (47.3%), followed by  $\gamma$ -elemene (14.25%) and trans- $\beta$ -elemenone (10.4%), (E) caryophyllene (4.33%), and atractylone (2.38%). Spathulenol and viridiflorol are also found in this oil but only in small amount less than 0.2%. Different with [44] indicated atractylone (26.78%) and curzerene (17.96%) as major constituents of *E. uniflora* essential oil. The main constituent of essential oil of *E. uniflora* may vary but the dominant classes are sesquiterpenes. *E. uniflora* has known antihypertensive [45], antitumor [46], and antinociceptive properties [47], and it shows good performance against microorganism. The important issue is that this essential oil is also used in industrial perfumery [48].

### 2.2.1.3. *Foeniculum vulgare* Mill. (fennel)

*Foeniculum vulgare* Mill. (Adas in Indonesian, family Apiaceae) is commercially cultivated in some Indonesian area and also grow wild. The leaves contains essential oil, and it can be eaten as salad and give a warm feeling for the body, beside that the leaves also can be used for accelerate mother's milk, while the seeds are used as an important ingredient in various folklore. In Indonesian herbs, the fennel seed oil is used as one of the components for baby oil massage. Essential oils of the seeds are very famous and are used as flavoring agents in food products for appetizing as digestive aid, liqueurs, bread, cheese, and an ingredient of cosmetics and pharmaceutical products. This seed was also used as classical decoction for nursing babies to prevent flatulence and colic spasms [49–52].

In this work, the composition of essential oil of fennel seeds obtained by hydro distillation composed of 30 compounds and the highest amount is **Estragole 38.51%**, followed by trans-anetol 29.67%, fenkon(1–1,2,3-trimethyl bicyclic) 2.2.1–2-heptanol 22.70%, 1-limonena 2.97%, and alpha-pinena 2.18%. Ref. [48] report that GC-MS analyzed of fennel seed oil showed that 28 components were identified, and the major components were trans-anethol 68.53% and estragole 10.42%. According to [53], fennel volatile oil is a mixture of many different constituents, and the main ingredients are anethole (40–70%), fenchone (1–20%), and estragole (2–9%). Trans-anetol, estragole, fenkon, alfa-limonena, and pinena are monoterpenoids highly abundant in all the fennel oil [54]. The high concentration of trans-anethol 29.67% is responsible to antibacterial activity. Ref. [55, 56] reported that anethol and its isomers are responsible for antimicrobial activities of fennel oil. Due to antimicrobial activity possessed by essential oil, this oil can be used as antibiotic. Ref. [57] report that the main advantage of natural agents is that they do not enhance the antibiotic resistance, a phenomenon commonly happened in long termed use of synthetic antibiotics.

#### 2.2.1.4. *Spilanthes paniculata* wall (toothache plant)

*S. paniculata* belonging to the family Asteraceae is one of medicinal plant, found in tropical and subtropical countries. There are some species from *Spilanthes*, for example *S. acmella* Murr, *S. calva* D.C, *S. mauritiana*; these plants are rich source of therapeutic compounds. Spilanthal is a powerful compound for local anesthetic, which is contained in the whole aerial part of *Spilanthes*. Tincture of flowers of *Spilanthes* cures toothache and is useful for throat infection and paralysis of the tongue [58]. This compound is an alkyl amide, which is found in nonvolatile phase; on the contrary, essential oil is a volatile oil.

According to this study, the essential oil of fresh flower of *S. paniculata* Wall showed that **Trans-caryophyllene 24.19%** is the dominant compound, followed by  $\beta$ -ocimene 16.38%,  $\beta$ -felandrene 10.79%, 1-pentadecene 9.75%, and **germacrene 8.08%**. This result is quite different with essential oil of the same species from GaoLigong Mountains, China. The essential oil obtained by hydro distillation of *S. paniculata* obtained from Gaoligong Mountains, and China was analyzed by gas chromatography/spectrometry (GC/MS), simultaneously. Main constituents of the oil were found as E-y-cadinene (10.64%),  $\beta$ -**caryophyllene (6.31%)**, thymol (5.55%),  $\beta$ -pinene (5.42%), 1,8-cineole (4.28%), p-cymene (3.56%), and **bicyclgermacrene (3.17%)**. The essential oil was also screened for its antimicrobial properties against various pathogens [59].

A comparison of oil composition of this study with those reported from different places in the world show differences not only in the kind of the compounds but also the percentage content of some of the mayor and minor constituents.

#### 2.2.2. Antimicrobial activity

**Table 3** demonstrated the antimicrobial properties of the five essential oils (lime, lemon, Surinam cherry, fennel, and toothache plants). The strength of antibacterial activity was declared as minimum inhibitory concentration (MIC); Low (L) if the inhibitory area diameter (IAD) less than 0.7 cm, Medium (M) if the IAD 0.7–0.8 cm, and Strong (S) The IAD >0.8 cm [60].

According to **Tables 2** and **3**, essential oil of every plants used in this study had significant antibacterial activities against some of bacterial. The diameters of inhibitory area (IAD) or the diameters of growth inhibition zone were measured including the diameter of disk 6 mm.

The strength of antibacterial activity of the essential oil is presented in **Table 3**.

##### 2.2.2.1. Lime and lemon essential oil

Antibacterial activity for Medium level of Lime and lemon oil showed the similar antibacterial strength against Gram (+) bacteria (1000  $\mu$ g), but for Gram (-) bacteria, the higher concentration was needed. The antibacterial activity of lemon oil was higher than the lime oil, because 3000  $\mu$ g concentration of lemon oil gives strong level and lime oil need 5000  $\mu$ g. The higher the concentration of the essential oil, the lower is the antibacterial activity obtained [61].

Material	Minimum inhibitory concentration (MIC) µg					
	<i>B. cereus</i> FNCC 0057	<i>B. subtilis</i> ATCC 6051	<i>S. aureus</i> FNCC 0047	<i>P. aeruginosa</i> FNCC 0063	<i>P. cepacia</i> FNCC 0063	<i>E. coli</i> 0091 IFO
<i>Citrus aurantifolia</i> Swingle (Lime)	—	1000(M)	—	2000(M)- 4.000(S)	—	3000(M)-5000(S)
<i>Citrus limon</i> (L) Burm	—	1000(M)- 2000(S)	—	1000(M)- 3000(S)	—	1000(M)-3000(S)
<i>Eugenia uniflora</i> L (Surinam cherry)	—	200(M)- 300(S)	—	—	—	300(M)-600(S)
<i>Foeniculum vulgare</i> Mill (fennel)	—	2500(S)	—	—	—	2500(S)
<i>Spilanthes paniculata</i> Wall (toothache plant)	1000(M)- 1500(S)	—	1000(M)- 1500(S)	3000(M)- 4000(S)	—	1000(M)-1500(S)

Note: —, no test; M, Medium (IAD 0.7–0.8 cm); S, strong (IAD > 0.8 cm).

**Table 3.** Minimum inhibitory concentration (MIC), the strength of essential oil antibacterial activity.

Both of essential oils (lime and lemon) showed the pale yellow color, bitter taste, and fresh piquant odor. But the odor of each essential oil (lime and lemon) is not exactly the same. The different of the odor between lime and lemon oil relates the profile of chemical compounds. Although limonene is the dominant compound of both essential oil, but the amount is different and followed by different compounds. Lime oil showed **limonene (29.29%)**,  $\beta$ -pinene (24.54%), Terpineol (2.87%),  $\alpha$ -terpineol (2.84%),  $\alpha$ -terpinolene (1.93%), whereas lemon oil showed **limonene (43.40%)**,  $\beta$ -myrcene (3.34%),  $\alpha$ -terpinolene (2.50%), geranyl acetate (2.44%), and 2- $\beta$  pinene (1.38%). Essential oil of lime and lemon can inhibit both Gram bacteria (positive and negative) [62]. The major components of lime essential oil proved to be  $\beta$ -pinene (12.6%), limonene (53.8%),  $\gamma$ -terpinene (16.5%), terpinolene (0.6%),  $\alpha$ -terpineol (0.4%), and citral (2.5%), which are very likely responsible for the good antimicrobial activity, in particular on Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, and *Staphylococcus epidermidis*) [63]. These oils are rich with limonene and other compounds belonging cyclic monoterpene hydrocarbon family. The cyclic monoterpene hydrocarbon family is considered to accumulate in the microbial plasma membrane and thus causes a loss of membrane integrity and dissipation of the proton motive force [64]. Carvacrol and citral (another terpenes and terpenoids) also demonstrated the occurrence of sub-lethal injury in the outer and cytoplasmic membranes [65, 66], pointing out the membrane disruption as a mechanism of inactivation by these compounds. However, the precise targets of terpenes and terpenoids are not yet completely understood [67].

#### 2.2.2.2. *Eugenia uniflora* L (Surinam cherry) essential oil

The essential oil was collected from leaf; the oil has yellowish white color, unidentified odor, and the aroma is quite difficult to express it. This oil also shows good antibacterial activity because in low concentration give medium to strong level MIC in 200–600 µg. The

profile of essential oil composed of **spathulenol (12.03%)**, dodecanol (11.78%), dodekanal (4.16%),  $\beta$ -elemen (4.08%), and *caryophyllene* (2.97%). The main constituent of this essential oil has big variation; there are no specific compounds in high amount. Antibacterial activity of the essential oil of *E. uniflora* showed the medium level at 200  $\mu\text{g}$  and strong level at 300  $\mu\text{g}$  against gram positive bacteria, whereas 300  $\mu\text{g}$  for medium level and 600  $\mu\text{g}$  for strong level against gram negative bacteria. Five major components of this oil have key role to show the antibacterial activity. Spathulenol, caryophyllene, alpha/beta pinene, humulene, and eugenol contributed to antibacterial activity of the essential oils [68–70]. It is also possible that the minor components might be involved in some type of synergism with the other active compounds [71]. Essential oil of several species of *Eugenia* also demonstrated antibacterial activity. Beta-Caryophyllene, spathulenol, 5-hydroxy calamenene, Bisabolene, caryophyllene, Farnesol, Selinene, Germacrene, and elemene  $\beta$ -elemen are important compounds in *Eugenia* oil [72].

#### 2.2.2.3. *Foeniculum vulgare* Mill (fennel) essential oil

This essential oil of *Foeniculum vulgare* Mill obtained from the seeds is colorless to pale yellow with a powerful sweet odor, which is the characteristic aroma of anethol. This essential oil showed antibacterial activity in strong level at 2500  $\mu\text{g}$  against Gram positive and Gram negative bacteria. The main compound in this essential oil obtained from this study is estragole (38.51%). Estragole is a phenylpropene, a plant secondary metabolite that has antibacterial activity. The high concentration of trans-anethol (29.67%), also responsible to antibacterial activity, anethol, and its isomers are responsible for antimicrobial activities of fennel oil [55, 73, 74].

The GC–MS analysis of essential oils of *Foeniculum vulgare* (fennel) showed the occurrence of trans-anethole, methylchavicol, limonene, and fenchone. This oil exhibited the lowest MIC values of 0.062 and 0.031%(v/v) against *E. coli* and *S. typhimurium* [75]. For augmenting wound healing, Limonene and fenchone were reported can increase collagen synthesis and decrease the number of inflammatory cells during wound healing and may be useful for treating skin wounds [76].

#### 2.2.2.4. *Spilanthes paniculata* wall (toothache plant) essential oil

The essential oil was obtained from toothache plant leaf; the oil has a yellowish color. This essential oil showed antibacterial activity in medium level at 1000  $\mu\text{g}$  and strong level at 1500  $\mu\text{g}$  against Gram positive, whereas negative test bacteria except against *P. aeruginosa* FNCC 0063 (4000  $\mu\text{g}$ ). The presence of trans-caryophyllene in the oil makes it potentially useful for antifungal, antimycotic, and antimicrobial properties [77, 78].

Genus *Spilanthes* is one of the oil-rich genera belonging to the family Asteraceae, although only a few species have been explored for their essential oils [79]. The composition of the essential oil is very variable, suggesting the existence of a high number of chemotypes. From the flower heads of *S. acmella*, volatile constituents were characterized [80]. In the same plant, the presence of a mixture of C22 to C35 hydrocarbons was also reported [77, 81]. Seven components from the essential oil have been identified, including the sesquiterpene caryophyllene oxide,

caryophyllene, limonene, and myrcene as significantly dominating compounds of the essential oil from the inflorescences of *S. calva* DC [82].

### 2.2.3. Discussion

All the essential oil samples used in this study indicated broad antibacterial spectrum because of its show antibacterial activity against Gram-positive and negative bacteria. These data conform with [83, 84] that essential oil exhibits antibacterial activity against a large number of Gram-positive and Gram-negative bacteria. It has been observed that the mode of action of essential oil is based on their ability to disrupt cell wall and cytoplasmic membrane, leading to lysis and leakage of intracellular compounds [3]. The disturbance of the cell membrane will disturb many vital processes such as energy conversion, nutrient processing, the synthesis of structural macromolecules, and the secretion of growth regulators [85]. Essential oils of various plants were reported to cause increased bacterial cell membrane permeability, leading to the leakage of cellular components and loss of ions [86, 87].

The strength of antibacterial activity of essential oil is able to be a basic potent to reduce antibiotic consuming, although many antibiotics are available for treating various bacterial pathogens. The increased multidrug resistance has led to the increased severity of diseases caused by bacterial [88]. The use of several antibacterial agents at higher doses may cause toxicity in human, so that the researcher needs to explore alternative new molecules against bacterial strains. Plant essential oils are potential candidates as antibiotic/antibacterial agents. The main advantage of natural agents is that they do not enhance the antibiotic resistance, a phenomenon commonly happened in long termed use of synthetic antibiotics [57].

## 3. Conclusions

In conclusion, all the five samples used in this experiment have antibacterial activity against gram positive and negative bacteria. Gram-negative bacteria appear more resistant than Gram-positive bacteria. Gram-positive and Gram-negative bacteria showed different sensitivities to essential oil. Among these samples antibacterial activity of *E. uniflora* is stronger than the others (300 µg and 600 µg, strong level against Gram-positive and negative bacteria, respectively). *S. paniculata* show the same response either to Gram-positive or Gram-negative bacteria (1500 µg), except for *P. aeruginosa* FNCC 0063 (4000 µg). Antibacterial activity of *C. aurantifolia* Swingle (Lime) is weaker than the other especially against *E. coli* (5000 µg). Five essential oils of aromatic Indonesian herbs in this study are potential candidates as antibiotic/antibacterial agents, can be applied as flavoring and preservative agents in cosmetic and food industry.

## Conflict of interest

The authors declare that there is no conflict of interest.

## Author details

Hartati Soetjipto

Address all correspondence to: hartati.sucipto@staff.uksw.edu

Department of Chemistry, Faculty of Science and Mathematics, Universitas Kristen Satya Wacana, Salatiga, Central Java, Indonesia

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# The Expression of Biodiversity in the Secondary Metabolites of Aromatic Plants and Flowers Growing in Colombia

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Elena Stashenko and Jairo René Martínez

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## Abstract

A network of research groups has carried out a bioprospective study of Colombia's vegetal biodiversity, with focus on aromatic plants. This chapter presents results on the chromatographic analysis of flower fragrances and essential oils obtained from vegetal material collected in botanical expeditions to various Colombian regions. Essential oils and flower fragrances are composed of volatile substances that differ greatly in polarity, functional groups, and relative amounts. The study of these complex mixtures requires special sampling and analysis techniques, described in this chapter. The large chemical diversity of the essential oil and flower fragrance constituents is a formidable characterization challenge. Typically, the number of essential oil components surpassed 50. It was rare to find an essential oil composition in which a single substance was present with a relative amount above 50%.

**Keywords:** essential oil, flower scent, gas chromatography, HS-SPME, tropical

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## 1. Introduction

The Research Center for Agroindustrialization of Aromatic and Medicinal Tropical Vegetal Species, CENIVAM, is a multidisciplinary research network of groups from Colombian public and private universities that joined efforts to study Colombia's agricultural biodiversity, with focus on aromatic plants. Under permit from Colombia's Environment Ministry, botanical expeditions were organized to obtain vegetal material from different regions in the country. A primary taxonomical identification made by the researchers in the field was subsequently replaced by the assessment made at Colombia's National Herbarium, where exsiccatæ of all studied materials have been deposited. The vegetal material was dried, chopped, and either distilled or macerated,

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to obtain essential oils and extracts, respectively. Samples of these secondary metabolites were sent to several collaborating groups for the characterization of their biological activity. High-resolution chromatographic and mass spectrometric techniques were utilized in the chemical characterization of the essential oils and extracts. The combined knowledge of chemical composition and biological activity serves as the basis for the sustainable use of the biodiversity in the development of new consumer products for the cosmetics, hygiene, food, and pharmaceutical industries. Pilot essential oil production units have been implemented in some municipalities (Socorro, Sucre, and Barbosa) in the state of Santander. Farmers associations have been trained on good agricultural practices, post-harvest treatment of the vegetal material, and operation of stills designed at Universidad Industrial de Santander, UIS, for the rural essential oil extraction under either hydrodistillation or steam distillation. Thanks to these pilot units, farmers have begun production of essential oils of *Cymbopogon nardus*, *C. martinii*, and *Lippia origanoides*. New developments have started to extend the cultivation and essential oil production to additional species, such as *Cananga odorata*, *Pogostemon cablin*, *Vanilla planifolia*, *Lippia alba*, and *Rosmarinus officinalis*.

This chapter presents results from essential oil and flower fragrance analysis. Flowers maintained in CENIVAM's experimental garden were sampled both *in vivo* and *in vitro* to characterize their volatile compounds. The complex combination of volatile compounds emitted by flowers depends on the plant species, its habitat, phenological state, propagation strategy, time of day, circadian rhythm, climate, and many more variables.

## 2. Study of tropical flowers volatile compounds

The study of natural products includes a very interesting area: isolation and analysis of floral fragrances, which can be monitored both *in vivo* and *ex vivo* [1]. The study of volatile secondary metabolites, emanated from flowers, is important in many areas of the biological and chemical sciences, in agriculture, for the pest control, in the study of plant-insect interactions, allelopathy, in analytical sciences (sample preparation and chromatography), in the pharmaceutical, in perfumes and cosmetics, flavors and fragrances industries, among others.

Floral fragrances are complex mixtures, product of the metabolism of a flowering plant; they are composed of hundreds of molecules of different biochemical origin, with different physicochemical characteristics (polarity, volatility, and solubility); they contain various functional groups (hydrocarbons, alcohols, aldehydes, ketones, acids, esters, ethers, etc. ), and can be found in diverse concentrations (from parts per trillion, ppt, to parts per million, ppm). They are predominantly lipophilic substances, with molecular weight less than 300 Da, non-polar or moderately polar, and with high vapor pressure. The human nose can be more sensitive than a chromatographic detection system, to some floral fragrance substances, present at the trace level; therefore, it is necessary to carry out the extraction and concentration processes of the floral fragrance in such a way that its components are detectable and can be identified. This constitutes a very big analytical challenge. Nowadays, this challenge is solved by applying different strategies: headspace extraction techniques (*headspace*), distillation methods, extractive solvents, and an active surface, *i.e.*, adsorption/thermal desorption processes using adsorbents with different physical characteristics [2, 3].

For the instrumental analysis of volatile fractions and extracts, gas chromatography (GC) is used in one-dimensional (1D) or two-dimensional (2D) versions (GCxGC), in capillary columns of different polarities, using universal detection systems (flame ionization detector (FID); mass selective detector (MSD), in *full scan* mode), or selective detection, e.g., selective nitrogen and phosphorus detector (NPD), flame photometric detector (FPD), to register nitrogenous or sulfur compounds, respectively, and very specific detection systems such as electroantennography, electronic nose, and so on [4]. High-resolution mass spectrometry detection systems (HR-TOF, Orbitrap) are nowadays an excellent alternative for exact mass determination (elemental composition analysis), selective and very sensitive detection of secondary metabolites in complex floral scent mixtures.

Many and very diverse compounds have been detected and identified in floral fragrances. More than 1700 have been recorded in a diverse group of flowers studied [5]. The main families of chemical compounds found in floral scents include hydrocarbons (saturated, cyclic and olefinic); terpenes, basically, monoterpenoids and some sesquiterpenoids; benzenoids and phenylpropanoids, the oxygenated compounds of mixed nature, e.g., alcohols, aldehydes, ethers, esters (fatty acid derivatives), and substances that contain heteroatoms, such as sulfur or nitrogen.

The basic biological function of the floral fragrance is to promote or facilitate cross-pollination, which is a vital process in the life cycle of most angiosperm plants. The knowledge of the floral fragrance chemical composition is important to understand plant-insect interaction, the chemical strategies not only to attract the pollinators but also to deter the herbivores and to face the pathogens, to adapt to different abiotic stresses; to study the biochemical pathways of secondary metabolism in a plant, its adaptability and biological evolution. Also, it is of practical interest to know the floral composition as a source of inspiration to create new fragrances and odorous mixtures, which are used in the cosmetics, perfumes, personal hygiene products, or aromatherapy industries.

The floral fragrances of diverse plants, despite of having a different smell, could contain many common compounds. Among these, the terpenoids are a large group: monoterpene (ocimenes, phellandrenes, carenes, terpinenes, limonene, and *p*-cymene), sesquiterpenes (caryophyllenes, farnesenes, bisabolenes, cadinenes, cubebenes, elemenes, germacrene, and their structural isomers), and their oxygenated analogues (caryophyllene oxide, farnesol alcohols, nerolidol, and their esters), and some irregular terpenes. Among the most frequent oxygenated monoterpenes one can find alcohols: linalool, geraniol, nerol, and their acetates; ketones: carvone, menthone, verbenone; and aldehydes: citral (geranial and neral), and their oxides. Another family in the floral fragrance is made up of hydrocarbons, aliphatics,  $C_1$ - $C_{30}$  (more frequently,  $C_{13}$ - $C_{21}$  hydrocarbons, and olefinics, including some cycloparaffins). These substances, together with the fatty acids,  $C_{12}$ - $C_{22}$ , are part of the wax protective layer that lines the petals of many flowers, a lot of fatty acid derivatives (alcohols, ketones, ethers, esters, and lactones) could predominate in the floral scents of some flowers. A distinctive odoriferous note in the floral fragrance is due to the presence in its mixtures of compounds that contain sulfur or nitrogen atoms, probably originating from the metabolism of amino acids; among these volatile secondary metabolites, there are compounds with nitro group, indoles, oximes, nitriles, anthranilates, and sulfides, among the most common.

We studied the chemical composition of volatile fractions of 30 tropical plants; their floral scents were monitored by *in vivo* solid-phase micro-extraction (SPME), exposing the fiber, coated with Carboxen/poly(dimethylsiloxane) (CAR/PDMS) or Carboxen/divinylbenzene/poly(dimethylsiloxane) (CAR/DVB/PDMS) to the flower, mostly at its anthesis stage, for 20–30 minutes. The on-fiber collected volatiles immediately were desorbed into the injection port of a gas chromatograph coupled to mass spectrometer (GC-MS). **Table 1** contains the results of these analyses; the presence of diverse groups (monoterpenoids, sesquiterpenoids, benzenoids, oxygenated compounds, and fatty acid derivatives, as well as sulfur- and nitrogen-containing compounds, very characteristic for some plants (*Cananga odorata*, *Sansevieria guineensis*, *Erythroxylum coca*, *Moringa oleifera*, *Stapelia gigantea*) can be observed. The plants have been cultivated in the experimental plots of the Research Center CENIVAM (Bucaramanga, Colombia). Although diverse volatile fractions may contain common compounds, they are mostly different in their chemical composition.

## 2.1. Methods for floral fragrance isolation

Before proceeding to collect the volatile flowers, it is important to establish if their monitoring will be done *in vivo* (in the field) or *ex vivo*. The experimental setup for each purpose will be different. Some extraction techniques are not applicable in the field for *in vivo* flower monitoring. It is also important to have prior knowledge about the concentration of volatiles that the flower emits since the extraction and concentration system used will depend on the volatile fraction quantity. The bouquet of volatile substances produced by the flowers can have from 1 to more than 100 compounds but generally contains 20–60 different substances. The concentration of volatiles emitted ranges from low picogram levels to more than 30 µg/L [6].

Some methods of collecting floral volatiles can have an automated design that allows monitoring for 24 hours or longer periods. However, most extraction techniques make a momentary capture, a “snapshot” of the floral volatiles emitted [7]. The extraction methods of the flower volatile secondary metabolites can be divided into three large categories, namely: (I) Headspace techniques (*headspace*, HS) in static or dynamic modes; (II) Distillation techniques, among them steam distillation, water-steam distillation, hydro-distillation, hydro-distillation assisted by microwave radiation, and (III) Extractive techniques, using solvents of different nature, *e.g.*, fats (maceration, *enfleurage*, obtaining ointments), non-polar solvents (hydrocarbons, obtaining concretes), polar solvents (alcohols, obtaining absolutes), and supercritical fluids (mainly, CO<sub>2</sub>). Headspace techniques are used in two different “formats”: *static headspace* (S-HS) and *dynamic headspace*, *e.g.*, purge and trap (P&T). Today, the most popular technique for the analysis of floral fragrances is solid-phase micro-extraction (SPME), operated in the *headspace* mode (HS-SPME). This method of extraction on a polymeric adsorbent, which covers a fused silica fiber combines the high selectivity of extraction, which is achieved with the choice of the polymer, its chemical nature and thickness, and the optimization of sampling conditions (volumes of the material and headspace, temperatures, pre-equilibrium, and fiber exposure times, sample agitation modes, additives, etc.), with the concentration of analytes on the fiber. The extraction and simultaneous concentration of the sample are processes that distinguish the SPME technique and make it very advantageous compared to other methods [8].

Species	Family	Chemical composition
<i>Thunbergia grandiflora</i>	Acanthaceae	<p><b>Monoterpenoids:</b> <i>trans</i>-<math>\beta</math>-ocimene, linalool.</p> <p><b>Benzenoids:</b> methyl salicylate.</p> <p><b>Oxygenated compounds:</b> acetaldehyde, 3-octanone, 1-octen-3-one, 3-octanol, 1-octen-3-ol, lauryl acetate.</p> <p><b>Sulfur and nitrogen compounds:</b> dimethyl sulfide, benzyl nitrile. <b>Hydrocarbons:</b> <i>n</i>-pentadecane.</p>
<i>Cananga odorata</i>	Annonaceae	<p><b>Monoterpenoids:</b> <math>\alpha</math>-pinene, <math>\beta</math>-pinene, <math>\beta</math>-myrcene, limonene, 1,8-cineole, <i>trans</i>-<math>\beta</math>-ocimene, terpinolene, <i>allo</i>-ocimene, linalool, <math>\alpha</math>-terpineol, geranyl acetate, geraniol, nerol.</p> <p><b>Sesquiterpenoids:</b> <math>\alpha</math>-cubebene, <math>\alpha</math>-ylangene, <math>\alpha</math>-copaene, <math>\beta</math>-copaene, <i>trans</i>-<math>\beta</math>-elemene, <i>trans</i>-<math>\beta</math>-caryophyllene, <i>cis</i>-muurolo-3,5-diene, <i>trans</i>-muurolo-3,5-diene, <math>\alpha</math>-humulene, <math>\alpha</math>-muurolene, <math>\gamma</math>-muurolene, germacrene D, <math>\alpha</math>-farnesene, <math>\alpha</math>-cadinene, <math>\gamma</math>-cadinene, <math>\delta</math>-cadinene, cadina-1,4-diene, calamenene.</p> <p><b>Benzenoids:</b> <i>p</i>-methyl anisole, benzaldehyde, methyl benzoate, ethyl benzoate, methyl salicylate, 3,4-dimethoxy-toluene, 2-ethyl phenyl acetate, anethole, benzyl acetate, <i>p</i>-cresol, cinnamyl acetate, methyl isoeugenol. <b>Oxygenated compounds (fatty acid derivatives):</b> 3-methyl 3-butenyl acetate, 3-methyl 2-butenyl acetate, hexyl acetate, <i>cis</i>-3-hexenyl acetate.</p> <p><b>Sulfur- and nitrogen-containing compounds:</b> phenyl acetonitrile, 4-methyl benzaldoxime, 2-phenyl-1-nitroethane, benzyl nitrile, methyl anthranilate, indole.</p>
<i>Aristolochia ringens</i>	Aristolochiaceae	<p><b>Sesquiterpenoids:</b> <math>\alpha</math>-farnesene.</p> <p><b>Benzenoids:</b> benzaldehyde, methyl benzoate, methyl salicylate, benzyl acetate, benzyl alcohol, 3-phenyl propyl acetate, benzyl isovalerate, eugenol, methyl eugenol, <i>trans</i>-cinnamaldehyde, methyl anisate, methyl <i>trans</i>-cinnamate, benzyl tiglate, cinnamyl acetate, benzyl benzoate, benzyl salicylate.</p> <p><b>Oxygenated compounds (fatty acid derivatives):</b> methyl acetate, ethyl acetate, 2-isopentyl acetate, penten-1-yl acetate, 3-hexenyl acetate, hexyl acetate, heptyl acetate, octyl acetate, nonyl acetate, decyl acetate, decenyl acetate, undecyl acetate, lauryl acetate, dodecyl acetate, tetradecenyl acetate, hexanol, heptanol, octanol, 1-octen-3-ol, nonanol, decenol, dodecenol, octanal, nonanal, decanal, dodecanal, tridecanal, pentadecanal, 2-pentyl furan, methyl decanoate, methyl myristate.</p> <p><b>Sulfur- and nitrogen-containing compounds:</b> dimethyl sulfide, dimethyl pyrazine, methoxy-dimethyl pyrazine.</p> <p><b>Hydrocarbons:</b> heptadiene, tetradecadiene, methyl tridecane, <i>n</i>-pentadecane.</p>
<i>Sansevieria guineensis</i>	Asparagaceae	<p><b>Monoterpenoids:</b> limonene, 1,8-cineole, carvone, <math>\alpha</math>-terpineol. <b>Sesquiterpenoids:</b> <i>cis</i>, <i>cis</i>-farnesol.</p> <p><b>Benzenoids:</b> benzyl alcohol, methyl benzoate, ethyl benzoate, benzyl salicylate, eugenol, methyl eugenol, methyl isoeugenol, benzyl benzoate. <b>Oxygenated compounds (fatty acid derivatives):</b> 2-methyl-butan-1-ol, tridecanal, pentadecanal.</p> <p><b>Sulfur- and nitrogen-containing compounds:</b> methyl anthranilate.</p>

Species	Family	Chemical composition
<i>Polianthes tuberosa</i>	Asparagaceae	<p><b>Monoterpenoids:</b> limonene, <i>cis</i>-limonene oxide, <i>trans</i>-limonene oxide, linalool, nerolidol.</p> <p><b>Benzenoids:</b> 2-phenyl ethanol, 3-hexenyl benzoate.</p> <p><b>Sulfur- and nitrogen-containing compounds:</b> benzyl nitrile.</p>
<i>Plumeria rubra</i>	Apocynaceae	<p><b>Monoterpenoids:</b> limonene, <i>cis</i>-limonene oxide, <i>trans</i>-limonene oxide, linalool.</p> <p><b>Sesquiterpenoids:</b> nerolidol.</p> <p><b>Benzenoids:</b> 2-phenyl ethanol, 3-hexenyl benzoate.</p> <p><b>Sulfur- and nitrogen-containing compounds:</b> benzyl nitrile.</p>
<i>Stapelia gigantea</i>	Apocynaceae	<p><b>Monoterpenoids:</b> <math>\alpha</math>-pinene, <math>\beta</math>-pinene, <math>\alpha</math>-phellandrene, <math>\Delta^3</math>-carene, <math>\beta</math>-phellandrene, <i>trans</i>-<math>\beta</math>-ocimene, <i>cis</i>-<math>\beta</math>-ocimene, <i>allo</i>-ocimene, <i>neo-allo</i>-ocimene, 1,3,8-<i>p</i>-menthatriene, <i>trans</i>, <i>trans</i>-2,6-dimethyl-1,3,5,7-octatetraene, <i>p</i>-cymenene, 2,6-dimethyl-1,3,5,7-octatetraene.</p> <p><b>Benzenoids:</b> anisole, methoxy benzene.</p> <p><b>Oxygenated compounds (fatty acid derivatives):</b> butanoic acid, pentanoic acid, 3-methyl hexanoic acid.</p> <p><b>Sulfur- and nitrogen-containing compounds:</b> dimethyl disulfide, dimethyl trisulfide, methoxy phenyl oxime.</p>
<i>Veitchia merrillii</i>	Arecaceae	<p><b>Monoterpenoids:</b> limonene, 1,8-cineole, <i>trans</i>-<math>\beta</math>-ocimene, <i>cis</i>-<math>\beta</math>-ocimene, linalool oxide, linalool.</p> <p><b>Benzenoids:</b> methyl salicylate, 2-phenyl ethanol.</p>
<i>Cannabis indica</i>	Cannabaceae	<p><b>Monoterpenoids:</b> <math>\alpha</math>-pinene, <math>\alpha</math>-thujene, camphene, <math>\beta</math>-pinene, <math>\Delta^3</math>-carene, <math>\beta</math>-myrcene, <math>\alpha</math>-phellandrene, <math>\beta</math>-phellandrene, <math>\alpha</math>-terpinene, limonene, <i>cis</i>-<math>\beta</math>-ocimene, <math>\gamma</math>-terpinene, <i>trans</i>-<math>\beta</math>-ocimene, <i>allo</i>-ocimene, terpinolene, <i>p</i>-cymenene, linalool, limonen-4-ol, fenchol, <math>\alpha</math>-terpineol.</p> <p><b>Sesquiterpenoids:</b> <math>\alpha</math>-ylangene, <i>cis</i>-<math>\alpha</math>-bergamotene, <math>\alpha</math>-santalene, <i>trans</i>-<math>\beta</math>-bergamotene, <i>trans</i>-<math>\beta</math>-caryophyllene, <i>allo</i>-aromadendrene, <i>cis</i>-<math>\beta</math>-farnesene, <math>\alpha</math>-humulene, <math>\alpha</math>-selinene, <math>\beta</math>-selinene, <math>\gamma</math>-selinene, <math>\alpha</math>-bulnesene, valencene, bicyclogermacrene, <i>trans</i>, <i>trans</i>-<math>\alpha</math>-farnesene, <math>\delta</math>-amorphene, selina-3,7(11)-diene.</p>
<i>Ipomoea horsfalliae</i>	Convolvulaceae	<p><b>Sesquiterpenoids:</b> <math>\alpha</math>-cubebene, <math>\beta</math>-cubebene, <math>\alpha</math>-copaene, <math>\beta</math>-copaene, <i>trans</i>-<math>\beta</math>-elemene, <i>trans</i>-<math>\beta</math>-caryophyllene, <i>trans</i>-muurolo-3,5-diene, <i>trans</i>-muuro-4 (14), 5-diene, <math>\gamma</math>-muuroloene, germacrene D, dauca-5,8-diene, <math>\alpha</math>-selinene, <math>\beta</math>-selinene, bicyclogermacrene, <math>\delta</math>-cadinene, <math>\gamma</math>-cadinene, <i>cis</i>-calamenene, caryophyllene oxide.</p> <p><b>Benzenoids:</b> 2-phenyl ethanol.</p> <p><b>Oxygenated compounds (fatty acid derivatives):</b> acetaldehyde, butanal, 2-propenal, 2-methyl butanal, 3-methyl butanal, ethanol, 1-penten-3-one, hexanal, <i>cis</i>-2-penten-1-al, 1-penten-3-ol, 3-methyl-1-butanol, 2-pentyl furane, pentanol, 3-octanone, 1-octen-3-one, <i>cis</i>-2-pentenol, hexanol, <i>cis</i>-3-hexenol.</p> <p><b>Sulfur- and nitrogen-containing compounds:</b> dimethyl sulfide.</p>

Species	Family	Chemical composition
<i>Erythroxyllum novogranatense</i>	Erythroxylaceae	<p><b>Monoterpenoids:</b> <math>\beta</math>-myrcene, <math>\alpha</math>-phellandrene, <math>\alpha</math>-pinene, <math>\Delta^3</math>-carene, <math>\beta</math>-phellandrene, <i>trans</i>-<math>\beta</math>-ocimene, <i>cis</i>-<math>\beta</math>-ocimene, <i>allo</i>-ocimene, <i>neo-allo</i>-ocimene, 1,3,8-<i>p</i>-menthatriene, linalool, <i>cis</i>-linalool oxide, linalool.</p> <p><b>Sesquiterpenoids:</b> <math>\alpha</math>-farnesene.</p> <p><b>Benzenoids:</b> benzaldehyde, methyl benzoate, benzeneacetaldehyde, methyl salicylate, ethyl salicylate, benzyl alcohol, 2-phenyl ethanol. <b>Oxygenated compounds (fatty acid derivatives):</b> propan-2-one, 2-methyl furane, butanal, propanol, butan-2-one, 2-methyl butanal, 3-methyl butanal, ethanol, 3-buten-2-one, pentanal, hexanal, 1-penten-3-ol, <i>cis</i>-2-pentenol, 2-<i>trans</i>-hexanal, heptanal, 2-methyl-1-butanol, 3-methyl-1-butanol, 3-<i>cis</i>-hexenol, nonanol, bovolide.</p> <p><b>Sulfur- and nitrogen-containing compounds:</b> dimethyl sulfide, 2-methyl butane nitrile, 3-methyl butane nitrile, 1-methyl-1H-pyrrole, 2-phenyl nitroethane, ecgonidine methyl ester, quinoline, benzene acetonitrile, 6-methyl-2-pyridinecarboxaldehyde.</p>
<i>Brownea macrophylla</i>	Fabaceae	<p><b>Oxygenated compounds (fatty acid derivatives):</b> hexanol, 2-heptanone, 2-heptanol, 2-nonanol, 3-octanol.</p>
<i>Perilla frutescens</i>	Lamiaceae	<p><b>Monoterpenoids:</b> perillene, linalool, perilla aldehyde.</p> <p><b>Sesquiterpenoids:</b> <math>\alpha</math>-cedrene, <i>trans</i>-<math>\alpha</math>-bergamotene, <i>trans</i>-<math>\beta</math>-elemene, <math>\beta</math>-cedrene, <i>trans</i>-<math>\beta</math>-caryophyllene, <math>\alpha</math>-humulene, <i>trans</i>-<math>\beta</math>-farnesene, <math>\alpha</math>-himachalene, <math>\alpha</math>-selinene, <math>\beta</math>-selinene, <i>trans</i>, <i>trans</i>-<math>\alpha</math>-farnesene, cuparene.</p> <p><b>Benzenoids:</b> benzaldehyde, methyl salicylate.</p> <p><b>Oxygenated compounds (fatty acid derivatives):</b> <i>trans</i>-2-hexenal, <i>cis</i>-3-hexenol, 3-octanol, <i>trans</i>-2-hexen-1-ol, 1-octen-3-ol.</p>
<i>Plectranthus amboinicus</i>	Lamiaceae	<p><b>Monoterpenoids:</b> <math>\Delta^3</math>-carene, <math>\gamma</math>-terpinene, <i>p</i>-cymene, carvacrol. <b>Sesquiterpenoids:</b> <i>trans</i>-<math>\alpha</math>-bergamotene, <i>trans</i>-<math>\beta</math>-caryophyllene, <math>\alpha</math>-humulene.</p> <p><b>Oxygenated compounds (fatty acid derivatives):</b> 1-octen-3-ol.</p>
<i>Persea americana</i>	Lauraceae	<p><b>Monoterpenoids:</b> <math>\alpha</math>-pinene, <math>\alpha</math>-thujene, <math>\beta</math>-pinene, <math>\beta</math>-phellandrene, <math>\beta</math>-myrcene, limonene, 1,8-cineole, 4,8-dimethyl-1,3,7-nonatiene, <i>trans</i>-linalool oxide, <i>cis</i>-linalool oxide, linalool.</p> <p><b>Sesquiterpenoids:</b> <i>trans</i>-<math>\beta</math>-caryophyllene.</p> <p><b>Benzenoids:</b> benzeneacetaldehyde.</p> <p><b>Oxygenated compounds (fatty acid derivatives):</b> acetaldehyde, butan-2-one, ethanol, pentan-3-one, 1-penten-3-ol, hexanal, 4-pentenal, 2-pentenal, 3-hexenal, 1-penten-3-ol, 3-methyl-1-butanol, <i>trans</i>-2-hexenal, hexanol, <i>cis</i>-3-hexenol, <i>trans</i>, <i>trans</i>-2,4-hexadienal, <i>trans</i>-2-hexen-1-ol.</p> <p><b>Sulfur- and nitrogen-containing compounds:</b> benzeneacetonitrile, indole.</p> <p><b>Hydrocarbons:</b> tridecane.</p>

Species	Family	Chemical composition
<i>Gossypium barbadense</i>	Malvaceae	<p><b>Monoterpenoids:</b> <math>\alpha</math>-pinene, camphene, <math>\beta</math>-pinene, sabinene, <math>\beta</math>-myrcene, <math>\alpha</math>-phellandrene, <math>\beta</math>-phellandrene, <math>\alpha</math>-terpinene, limonene, <i>cis</i>-<math>\beta</math>-ocimene, <i>trans</i>-<math>\beta</math>-ocimene, <math>\gamma</math>-terpinene, <i>p</i>-cymene, <i>allo</i>-ocimene, <i>neo-allo</i>-ocimene, 1,3,8-<i>p</i>-menthatriene, bornyl acetate.</p> <p><b>Sesquiterpenoids:</b> <math>\alpha</math>-cubebene, <math>\alpha</math>-copaene, <i>trans</i>-<math>\beta</math>-caryophyllene, aromadendrene, <math>\gamma</math>-gurjunene, <math>\alpha</math>-bulnesene, <math>\alpha</math>-humulene, <math>\gamma</math>-muurolene, viridiflorene, <math>\alpha</math>-muurolene, bicyclogermacrene, <math>\gamma</math>-cadinene, <i>cis</i>-calamenene.</p> <p><b>Oxygenated compounds (fatty acid derivatives):</b> acetaldehyde, ethanol, 1-penten-3-ol.</p> <p><b>Sulfur- and nitrogen-containing compounds:</b> dimethyl sulfide.</p>
<i>Medinilla myriantha</i>	Melastomataceae	<p><b>Monoterpenoids:</b> italicene, <i>cis</i>-thujopsene.</p> <p><b>Sesquiterpenoids:</b> <math>\beta</math>-cedrene.</p> <p><b>Benzenoids:</b> <i>p</i>-methyl anisole, benzaldehyde.</p> <p><b>Oxygenated compounds (fatty acid derivatives):</b> hexanal, heptanal, 3-octanone, hexyl acetate, octanal, 1-octen-3-one, <i>cis</i>-3-hexenyl acetate, 6-methyl-5-heptene-2-one, hexanol, <i>cis</i>-3-hexenol, 3-octanol, nonanal, 1-octen-3-ol, heptanol, octanol, nonanol, <i>cis</i>-3-nonen-1-ol, 2-undecanone.</p>
<i>Moringa oleifera</i>	Moringaceae	<p><b>Monoterpenoids:</b> <math>\alpha</math>-pinene, camphene, 4,8-dimethyl-1,7-nonadiene, <math>\beta</math>-pinene, sabinene, <math>\beta</math>-myrcene, <math>\alpha</math>-phellandrene, <math>\alpha</math>-terpinene, 2,3-dehydro-1,8-cineole, limonene, <math>\beta</math>-phellandrene, 1,8-cineole, <i>cis</i>-<math>\beta</math>-ocimene, <math>\gamma</math>-terpinene, <i>trans</i>-<math>\beta</math>-ocimene, <i>p</i>-cymene, terpinolene, <i>p</i>-cymenene, thujone, <i>cis</i>-sabinene hydrate, <i>cis</i>-linalool oxide, terpinene-4-ol, linalool, <i>trans</i>-sabinene hydrate, <math>\alpha</math>-terpineol, citronellol, nerol, <i>trans</i>-<math>\beta</math>-ionone. <b>Sesquiterpenoids:</b> <i>cis</i>-<math>\beta</math>-farnesene, <math>\alpha</math>-farnesene, <i>trans</i>-nerolidol. <b>Benzenoids:</b> benzaldehyde, methyl benzoate, benzyl acetate, benzyl alcohol, <i>cis</i>-methyl cinnamate, <i>trans</i>-methyl cinnamate, <i>trans</i>-ethyl cinnamate, <i>trans</i>-cinnamyl acetate.</p> <p><b>Oxygenated compounds (fatty acid derivatives):</b> acetaldehyde, ethanol, 2-ethyl furane, pentan-3-one, 1-penten-3-one, butyl acetate, hexanal, 3-methyl-2-butenal, 3-buten-3-ol, 3-methyl-butan-1-ol, <i>trans</i>-2-hexenal, 2-penten-1-yl acetate, 2-pentyl furane, octan-3-one, <i>n</i>-hexyl acetate, octanal, <i>cis</i>-3-hexenyl acetate, <i>trans</i>-3-hexenyl acetate, 6-methyl-5-penten-2-one, heptyl acetate, 2-heptenyl acetate, 1-octen-3-ol, heptanol, octanal, nonyl acetate, 2,6-nonadienal, nonanol, 2-nonen-1-ol, 2,6-nonadien-1-ol, lauryl acetate.</p> <p><b>Sulfur- and nitrogen-containing compounds:</b> carbonyl sulfide, carbon disulfide, dimethyl sulfide, <i>sec</i>-butyl nitrile, 3-methyl butanenitrile, 2-propyl iso-thiocyanate, 1-butyl iso-thiocyanate, 2-butyl iso-thiocyanate, benzeneacetonitrile, benzyl nitrile, benzyl iso-thiocyanate.</p>
<i>Cattleya mendelii</i>	Orchidaceae	<p><b>Monoterpenoids:</b> <math>\alpha</math>-pinene, <math>\beta</math>-myrcene, limonene, <i>cis</i>-<math>\beta</math>-ocimene, <i>trans</i>-<math>\beta</math>-ocimene <i>p</i>-cymene, linalool oxide, linalool, terpinene-4-ol.</p> <p><b>Sesquiterpenoids:</b> <math>\alpha</math>-cubebene, <math>\beta</math>-cubebene, <math>\alpha</math>-copaene, aromadendrene, <math>\alpha</math>-muurolene, <math>\gamma</math>-muurolene, germacrene D, <i>trans</i>, <i>trans</i>-<math>\alpha</math>-farnesene, <math>\gamma</math>-cadinene, <i>cis</i>-nerolidol.</p> <p><b>Benzenoids:</b> benzaldehyde, benzyl acetate, methyl salicylate, 2-phenyl ethanol.</p> <p><b>Oxygenated compounds (fatty acid derivatives):</b> 3-octanone, isopentyl acetate, isopentyl butyrate.</p>

Species	Family	Chemical composition
<i>Cattleya trianae</i>	Orchidaceae	<p><b>Sesquiterpenoids:</b> <math>\beta</math>-bourbonene, <i>trans</i>-<math>\beta</math>-caryophyllene.</p> <p><b>Benzenoids:</b> benzaldehyde, methyl benzoate, methyl salicylate, 2-phenyl ethanol, benzyl alcohol, methyl cinnamate.</p> <p><b>Oxygenated compounds (fatty acid derivatives):</b> nonanal, octanal.</p>
<i>Vanilla planifolia</i>	Orchidaceae	<p><b>Monoterpenoids:</b> <math>\alpha</math>-pinene, <math>\beta</math>-pinene, <math>\beta</math>-myrcene, limonene, 1,8-cineole, <i>cis</i>-<math>\beta</math>-ocimene, <i>trans</i>-<math>\beta</math>-ocimene, <i>p</i>-cymene, terpinolene, <i>allo</i>-ocimene, 1,3,8-<i>p</i>-menthatriene, perillene, <i>cis</i>-limonene oxide, <i>trans</i>-limonene oxide, <i>cis</i>-salvene, <i>p</i>-cymenene, <i>cis</i>-epoxy-ocimene, myrtenal, terpinene-4-ol, <i>cis</i>-dihydrocarvone, <i>trans</i>-dihydrocarvone, limonen-4-ol, piperitone, borneol, 2,6-dimethyl-1,5,7-octatriene, carvone, dihydrocarveol, nerol, geraniol, <i>cis</i>-dihydrocarvone oxide, <i>trans</i>-dihydrocarvone oxide, <i>cis</i>-carveol, <i>trans</i>-carveol, carvacrol,</p> <p><b>Sesquiterpenoids:</b> <math>\alpha</math>-patchoulene, <i>trans</i>-salvene, guaiacol.</p> <p><b>Benzenoids:</b> 2-phenyl ethanol, 4-methyl phenol.</p> <p><b>Oxygenated compounds (fatty acid derivatives):</b> 1-(furan-2-yl) pentan-2-one, butanol, hexanol, octanol, nonanol.</p> <p><b>Sulfur- and nitrogen-containing compounds:</b> methoxy phenyl oxime.</p> <p><b>Hydrocarbons:</b> <i>trans</i>-2,4-undecadiene.</p>
<i>Vanilla pompona</i>	Orchidaceae	<p><b>Monoterpenoids:</b> <i>cis</i>-geranyl acetone.</p> <p><b>Benzenoids:</b> 4-methyl phenol.</p> <p><b>Oxygenated compounds (fatty acid derivatives):</b> <i>cis</i>-3,7-dimethyl octa-2,6-dienal, nonanal, 6-methyl-5-hepten-2-one, hexanol, <i>cis</i>-3-hexen-1-ol, 2-ethylhexan-1-ol.</p> <p><b>Sulfur- and nitrogen-containing compounds:</b> methoxy phenyl oxime.</p> <p><b>Hydrocarbons:</b> <i>trans</i>-2-methyl-2-pentene.</p>
<i>Passiflora edulis</i>	Passifloraceae	<p><b>Monoterpenoids:</b> <i>cis</i>-<math>\beta</math>-ocimene, <i>trans</i>-<math>\beta</math>-ocimene, <i>p</i>-cymene, <i>cis</i>-4,8-dimethyl-1,3,7-nonatriene, 1,3,8-<i>p</i>-menthatriene, <i>p</i>-cymenene, <i>trans</i>, <i>trans</i>-2,6-dimethyl-1,3,5,7-octatetraene.</p> <p><b>Sesquiterpenoids:</b> <math>\beta</math>-gurjunene, <i>trans</i>-<math>\beta</math>-caryophyllene, aristolene, farnesol, <i>cis</i>-calamenene.</p> <p><b>Benzenoids:</b> benzaldehyde, 1-methoxy 4-methyl benzene, anisole, 4-ethyl resorcinol, 2-methoxy-4-methyl-1-(1-methylethyl) benzene, methyl benzoate, 3-hexen-1-yl benzoate, 1,2-dimethoxy benzene, 1,4-dimethoxy benzene, methyl salicylate, <i>p</i>-methoxy phenethyl alcohol, 3,5-dimethoxy toluene, 2-methoxy phenol, butyl benzoate, benzyl alcohol, 2-phenyl ethanol, methyl eugenol, anisaldehyde, methyl 2-methoxybenzoate, methyl 4-methoxybenzoate, <i>cis</i>-3-hexenyl benzoate, 1,2,4-trimethoxy benzene, benzyl tiglate, 1,3,5-trimethoxy benzene, eugenol,</p> <p>3,4-dimethoxyphenol, <i>p</i>-anisyl alcohol, 2-(4-methoxyphenyl) ethanol, 4-methoxy phenol, benzyl benzoate.</p> <p><b>Oxygenated compounds (fatty acid derivatives):</b> propanal, 2-propanone, 2-hydroxy acetic acid, propanol, 2-butanone, 1-penten-3-one, <i>trans</i>-2-pentenal, <i>trans</i>-2-hexenal, 3-hydroxy-2-butanone, hexanol, <i>cis</i>-3-hexenol, <i>trans</i>-2-hexen-1-ol, acetic acid, <i>trans</i>, <i>trans</i>-2,4-heptadienal, octanol, dodecyl acetate, 2-hexyl hexanoic acid, benzyl <i>trans</i>-2-butenate.</p> <p><b>Sulfur- and nitrogen-containing compounds:</b> indole.</p>

Species	Family	Chemical composition
<i>Gardenia augusta</i>	Rubiaceae	<p><b>Monoterpenoids:</b> <math>\beta</math>-myrcene, limonene, <i>cis</i>-<math>\beta</math>-ocimene, <i>trans</i>-<math>\beta</math>-ocimene, <i>allo</i>-ocimene, <i>p</i>-cymene, linalool oxide, linalool, nerol, geraniol, cyperene.</p> <p><b>Sesquiterpenoids:</b> <math>\alpha</math>-<i>cis</i>-bergamotene, <math>\beta</math>-elemene, <math>\beta</math>-copaene, <i>trans</i>-<math>\beta</math>-caryophyllene, <i>cis</i>-muurola-3,5-diene, <math>\alpha</math>-humulene, <math>\beta</math>-farnesene, germacrene D, <math>\alpha</math>-muurolene, <math>\alpha</math>-selinene, <math>\alpha</math>-farnesene, <math>\alpha</math>-cadinene, <math>\delta</math>-cadinene, <math>\gamma</math>-cadinene, <i>cis</i>-calamenene, <i>epi</i>-<math>\alpha</math>-muurolol, <math>\alpha</math>-cadinol. <b>Benzenoids:</b> benzaldehyde, methyl salicylate, 2-phenyl ethanol. <b>Oxygenated compounds (fatty acid derivatives):</b> <i>trans</i>-2-hexenal, 2-pentyl furane, hexanol, <i>cis</i>-3-hexenol, 2-hexen-1-ol, 2-octenal, <i>trans</i>-2-octen-1-ol, <i>cis</i>-3-nonen-1-ol.</p> <p><b>Sulfur- and nitrogen-containing compounds:</b> benzyl nitrile, indole.</p> <p><b>Hydrocarbons:</b> tridecane, tetradecane, hexadecane.</p>
<i>Coffea arabica</i>	Rubiaceae	<p><b>Monoterpenoids:</b> <math>\alpha</math>-pinene, <math>\beta</math>-myrcene, limonene, 1,8-cineole, <i>cis</i>-<math>\beta</math>-ocimene, <math>\gamma</math>-terpinene, <i>trans</i>-<math>\beta</math>-ocimene, <i>p</i>-cymene, <i>p</i>-cymen-8-ol, perillene, <i>p</i>-cymenene, linalool oxide, nerol oxide, linalool, neral.</p> <p><b>Benzenoids:</b> methyl benzoate, phenyl acetaldehyde, ethyl benzoate. <b>Oxygenated compounds (fatty acid derivatives):</b> propanal, 2-methyl propanal, 2-propenal, propanol, 2-butanone, 2-methyl-butenal, 3-methyl butanal, 2-pentanal, 3-pentanone, 1-penten-3-one, hexanal, <i>tert</i>-butyl alcohol, <i>trans</i>-2-methyl-2-butenal, <i>cis</i>-2-penten-1-al, 2-methyl-2-hexenone, heptanal, 2-methyl-1-butanol, 2-hexenal, 2-pentyl furane, 2-hexanol, 2-octanone, <i>cis</i>-2-penten-1-ol, <i>trans</i>-2-heptenal, 5-methyl-2-hexanol, 6-methyl-5-hepten-2-one, heptanol, 2,4-heptadienal, decanal, 2-nonenal, octanol, nonanol.</p> <p><b>Sulfur- and nitrogen-containing compounds:</b> dimethyl sulfide, <i>o</i>-methyl oxime 2-propanone, 2-methyl butyl nitrile, 3-methyl butyl aldoxime.</p> <p><b>Hydrocarbons:</b> tridecane, tetradecane, pentadecane, 1-pentadecene, 7-pentadecene, hexadecane, 3-hexadecene, 7-hexadecene, heptadecane.</p>
<i>Posoqueria longiflora</i>	Rubiaceae	<p><b>Monoterpenoids:</b> <i>trans</i>-<math>\beta</math>-ocimene, <math>\gamma</math>-terpinene, <i>allo</i>-ocimene, linalool.</p> <p><b>Sesquiterpenoids:</b> <math>\alpha</math>-cubebene, <math>\beta</math>-bourbonene, <math>\beta</math>-copaene, <i>trans</i>-<math>\beta</math>-elemene, <i>trans</i>-<math>\beta</math>-caryophyllene, caryophyllene oxide, <math>\alpha</math>-humulene, <math>\alpha</math>-amorphene, germacrene D, <math>\alpha</math>-muurolene, <i>trans</i>, <i>trans</i>-<math>\alpha</math>-farnesene, <math>\gamma</math>-cadinene, cubebene, <i>cis</i>-calamene.</p> <p><b>Benzenoids:</b> benzaldehyde, methyl benzoate, phenyl acetaldehyde, ethyl benzoate, benzyl acetate, methyl salicylate, benzyl alcohol, benzyl isovalerate, 2-phenyl ethanol, isopentyl benzoate, 2-methoxy-phenol, <i>trans</i>-methyl cinnamate, <i>cis</i>-3-hexyl benzoate, eugenol.</p> <p><b>Oxygenated compounds (fatty acid derivatives):</b> isopentyl acetate, 1-penten-3-ol, 4-pentenyl acetate, 2-pentyl furane, 3-methyl-2-butenyl acetate, hexyl acetate, <i>cis</i>-3-hexyl acetate, <i>trans</i>-2-heptanal, <i>trans</i>-2-hexenyl acetate, hexanol, <i>cis</i>-3-hexenol, nonanal, <i>trans</i>-2-octenal, 1-octen-3-ol, octyl acetate, <i>trans</i>, <i>trans</i>-2,4-heptadienal, octanol, nonyl acetate, nonanol, <i>cis</i>-3-nonen-1-ol.</p> <p><b>Sulfur- and nitrogen-containing compounds:</b> <i>cis</i>-3-methyl butyl aldoxime, <i>trans</i>-3-methyl butyl aldoxime, benzyl nitrile.</p> <p><b>Hydrocarbons:</b> dodecane.</p>

Species	Family	Chemical composition
<i>Brugmansia suaveolens</i>	Solanaceae	<p><b>Monoterpenoids:</b> <math>\alpha</math>-thujene, <math>\alpha</math>-pinene, 6-methyl hept-5-en-2-one, sabinene, <math>\beta</math>-pinene, <math>\beta</math>-myrcene, <math>\alpha</math>-terpinene, limonene, 1,8-cineole, <i>cis</i>-<math>\beta</math>-ocimene, <i>trans</i>-<math>\beta</math>-ocimene, <i>p</i>-cymene, <math>\alpha</math>-terpinolene, <i>allo</i>-ocimene, citronellal, <i>p</i>-cymenene, <i>cis</i>-sabinene hydrate, linalool, terpinen-4-ol, citronellol, neral, geraniol, geranial.</p> <p><b>Sesquiterpenoids:</b> <i>trans</i>-<math>\beta</math>-caryophyllene, <i>trans</i>-<math>\beta</math>-farnesene, <math>\alpha</math>-terpineol, <i>trans</i>, <i>trans</i>-farnesol, farnesal, <i>trans</i>-nerolidol.</p> <p><b>Benzenoids:</b> benzaldehyde, methyl benzoate, methyl salicylate, benzyl alcohol, 2-phenyl ethanol, 4-methoxy benzaldehyde, benzyl benzoate, benzyl salicylate.</p> <p><b>Oxygenated compounds (fatty acid derivatives):</b> hexanal, <i>cis</i>-3-hexen-1-ol, hexanol, nonanal, decanal.</p> <p><b>Sulfur- and nitrogen-containing compounds:</b> Indole.</p> <p><b>Hydrocarbons:</b> <i>n</i>-dodecane, <i>n</i>-pentadecane.</p>
<i>Datura metel</i>	Solanaceae	<p><b>Monoterpenoids:</b> linalool.</p> <p><b>Oxygenated compounds (fatty acid derivatives):</b> 3-pentanone, 1-penten-3-ol, 3-methyl-1-butanol, 2-pentyl furane, hexanol, <i>cis</i>-3-hexenol, 4-methyl hexanol.</p>
<i>Petrea volubilis</i>	Verbenaceae	<p><b>Monoterpenoids:</b> <math>\beta</math>-myrcene, <math>\Delta^3</math>-carene, limonene, <i>cis</i>-<math>\beta</math>-ocimene, <i>trans</i>-<math>\beta</math>-ocimene, <i>p</i>-cymene, <math>\gamma</math>-terpinolene, 4,8-dimethyl-1,3,7-nonatriene, <i>allo</i>-ocimene, linalool, <math>\alpha</math>-terpineol, nerol, geraniol, geranyl acetate. <b>Sesquiterpenoids:</b> <math>\alpha</math>-copaene, <i>trans</i>-<math>\beta</math>-caryophyllene, farnesol, <i>trans</i>, <i>trans</i>-<math>\alpha</math>-farnesene.</p> <p><b>Benzenoids:</b> benzaldehyde, methyl salicylate, 2-phenyl ethanol, <i>cis</i>-3-hexenyl benzoate.</p> <p><b>Oxygenated compounds (fatty acid derivatives):</b> <i>trans</i>-2-hexenal, 3-octanone, octanal, 1-octen-3-one, <i>cis</i>-2-penten-1-ol, hexanol, 3-penten-2-ol, <i>cis</i>-3-hexenol, 3-octanol, <i>trans</i>-2-hexen-1-ol, hexyl 2-methyl butanoate, 1-octen-3-ol, <i>cis</i>-3-hexenyl butanoate, <i>cis</i>-3-hexenyl 2-methyl butanoate, <i>cis</i>-3-hexenyl pentanoate, octanol, 1-nonen-3-ol, nonanol, <i>cis</i>-3-hexenyl angelate, <i>cis</i>-3-nonen-1-ol.</p> <p><b>Hydrocarbons:</b> 3-methyl pentadecane.</p>
<i>Hedychium coronarium</i>	Zingiberaceae	<p><b>Monoterpenoids:</b> <math>\alpha</math>-pinene, <math>\beta</math>-pinene, <math>\beta</math>-myrcene, 1,8-cineole, <i>cis</i>-<math>\beta</math>-ocimene, <i>trans</i>-<math>\beta</math>-ocimene, <i>allo</i>-ocimene, <math>\gamma</math>-terpinene, terpinolene, linalool, <i>trans</i>-<i>p</i>-2-menthen-1-ol, terpinene-4-ol, <math>\alpha</math>-terpineol, <i>cis</i>-jasnone.</p> <p><b>Sesquiterpenoids:</b> <i>trans</i>-<math>\beta</math>-caryophyllene, caryophyllene oxide, <i>trans</i>, <i>trans</i>-<math>\alpha</math>-farnesene, <i>trans</i>, <i>trans</i>-farnesol.</p> <p><b>Benzenoids:</b> methyl benzoate, 2-methylbutyl benzoate, benzyl benzoate.</p> <p><b>Oxygenated compounds (fatty acid derivatives):</b> <i>cis</i>-3-hexenol.</p> <p><b>Sulfur- and nitrogen-containing compounds:</b> 1-nitro-2-methyl butane, <i>cis</i>-2-methyl butyl aldoxime, <i>trans</i>-2-methyl butyl aldoxime, 3-methyl butyl aldoxime, <i>trans</i>-3-methyl butyl aldoxime, phenyl acetonitrile, indole. <b>Hydrocarbons:</b> nonane.</p>

**Table 1.** Volatile compounds isolated by *in vivo* HS-SPME from 30 tropical flowers, grown in Colombia.

The headspace methods provide information on the chemical composition of the volatile fractions; distillation techniques, on essential oils, distillates or condensates while extractive methods (solvents, supercritical CO<sub>2</sub>), on the chemical composition of mixtures that may include substances of low-volatility, and higher molecular mass (> 400 Da), which in general are called extracts. The compositions of these mixtures can be differentiated not only quantitatively but also qualitatively. As mentioned above, in condensates and extracts will prevail "heavier" compounds, fatty acids, long-chain paraffinic hydrocarbons, their alcohols or aldehydes while in the volatile fractions, low-molecular-weight compounds are found, which eventually can "scape" during the distillation, in the depressurization stage (SFE-CO<sub>2</sub>) or during the concentration of the extracts.

The chemical composition of the volatile fraction of flowers depends both on intrinsic (genetic) factors of the species and on extrinsic, environmental factors [9]. The habitat, the environment where the plant grows, the conditions (temperature, humidity, light, type of soil, micronutrients, etc.) in which floral secondary metabolites are monitored, will affect the qualitative and quantitative composition of the volatile fraction emitted and collected. For this reason, it is very important during the collection of floral volatiles to maintain control, continually monitoring conditions. Many external factors will affect the production of flower volatiles. These include changes in temperature, humidity, increase or decrease in light energy, among others. Some stress conditions (water, light, and nutrition) can notably alter the generation of floral volatiles or even suppress their production [10].

Some aspects of the study of floral fragrances should contemplate the state of development of the flower [11]. The flowers of the ylang-ylang tree (*Cananga odorata* Hook Fil. and Thomson, genuine form, Annonaceae family) are important raw material for obtaining essential and absolute oils, which are valuable ingredients in many perfumes, soaps, shampoos, and lotions. In the tree, the flower remains several weeks while it matures, it starts as a very small, green flower, which then increases in size, staying several days green, and then turns yellow, large, with brown spots. In the same tree, it is common to find flowers in different degrees of maturation, along with the fruits that carry seeds, through which this plant species spread. In the ylang-ylang tree, the composition of flower volatiles varies markedly with its state of maturity. In small, green flowers, 10 times fewer components are recorded, than in a yellow, mature flower. In the mature, yellow and fully developed ylang-ylang flowers, 16–4 times more light-oxygenated substances are found (*p*-methylanisole, benzyl alcohol, 1,8-cineole, methyl and ethyl benzoate and salicylate, linalool, nerol, geraniol, benzyl acetate, anethole, cinnamyl acetate, and others) and heavier oxygenated substances (sesquiterpenols, farnesal, farnesol, nerolidol and their acetates, cedrol, benzyl benzoate and benzyl salicylate, others), than in green, small flowers that begin their development. The nitrogen-containing compounds, phenyl acetonitrile, 4-methylbenzaldoxime, indole, 2-phenyl-1-nitroethane, and methyl anthranilate, only appear in mature, large and yellow flowers [12].

The composition of the secondary metabolites in the floral emission also varies according to the part of the flower from which the volatiles are extracted. In the petals of the ylang-ylang flowers, oxygenated compounds (oxygenated monoterpenes, benzenoids, and phenylpropanoids) prevail while in the ovaries (central part of the flower, small and compact) monoterpene and sesquiterpene hydrocarbons abound [13]. The relative percentage composition

of the families of compounds present in the ylang-ylang flowers depends on the extraction method: steam distillation or simultaneous solvent distillation-extraction (SDE) allow mixtures of secondary metabolites to be obtained, rich in light oxygenated compounds (50–60%), and in heavy oxygenated compounds (18–20%) while extraction with supercritical fluid, SFE-CO<sub>2</sub>, isolates extracts, rich in aliphatic hydrocarbons (C<sub>n</sub> > 20) and terpenes, nitrogen-containing compounds, and even some fatty acids (C<sub>14</sub>-C<sub>18</sub>).

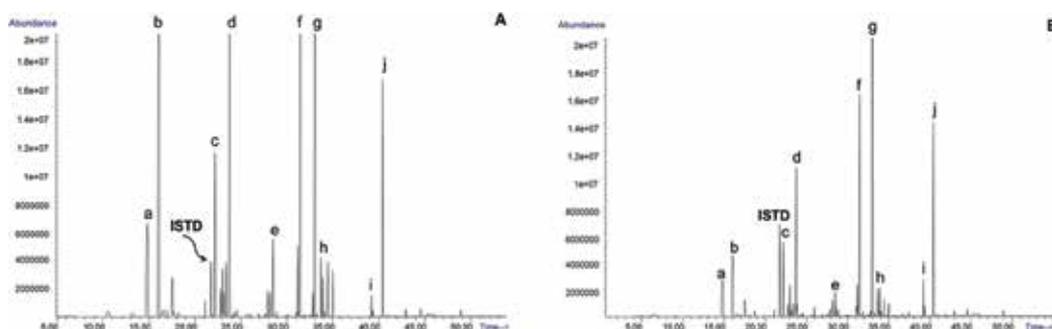
The profile of volatile compounds emitted by the flower also depends on the time of day; the insects that pollinate it can be diurnal or nocturnal, and from this, the kinetics of emanation of fragrant compounds and the type of volatile emitted by the flower will also depend, which vary, for most of the flowers, with the time of the day (circadian rhythm), and according to the biological function they fulfill. For example, in ylang-ylang flowers, the amount of nitrogenous substances changes during the day: it is maximum at dawn, decreases afternoon, and increases again in the afternoon and evening hours.

The flower fragrance of *Brugmansia suaveolens* (Solanaceae family) follows a clear circadian rhythm: the emission of volatiles increases in the evening and reaches its maximum at nine o'clock at night; then, the volatile emission slowly begins to decrease; in the morning and during the day, the flowers almost do not smell, although they attract massively the bees. It is interesting to note that some flowers change their fragrance after they have been pollinated; this happens with the flowers of some orchids (*Ophrys sphegodes*) [14].

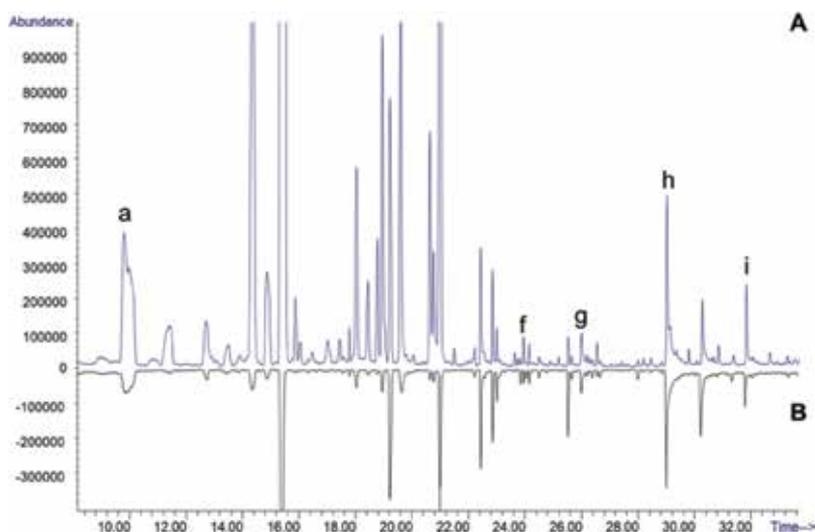
Notorious changes can be observed (**Figure 1**) in *Vanilla pompona* (Orchidaceae family) volatile fraction isolated by HS-SPME from flowers after their pollination.

In carrion flower *Stapelia gigantea* (Apocynaceae family), which emitted fetid, nasty, and badly smelling volatiles (dimethyl disulfide, dimethyl trisulfide, butanoic acid, 3-methyl butanoic acid, hexanoic acid), the number of volatiles diminished after the oviposition of the green bottle fly (*Lucilia sericata*) had occurred (**Figure 2**).

Distinct parts of the flower fulfill different biological roles in it; for example, to protect from herbivores or to attract pollinators, to call for natural enemies or to increase or diminish



**Figure 1.** Chromatographic profiles (GC–MS, EI, 70 eV, DB-WAX column, 60 m) of *Vanilla planifolia* flower volatiles, isolated by *in vivo* HS-SPME (CAR/PDMS) at: (A) 7 a.m. before pollination and (B) 7 p.m. after pollination. Main compounds found in vanilla floral scent: (a)  $\beta$ -myrcene; (b) limonene; (c) *trans*-epoxy myrcene; (d) *trans*-limonene oxide; (e) *trans*-dihydrocarvone; (f) carvone; (g) *trans*-carvone oxide; (h) *trans*-carveol; (i) *p*-methyl phenol; and (j) *diepoxy* limonene. Internal standard (ISTD) – *n*-tetradecane.



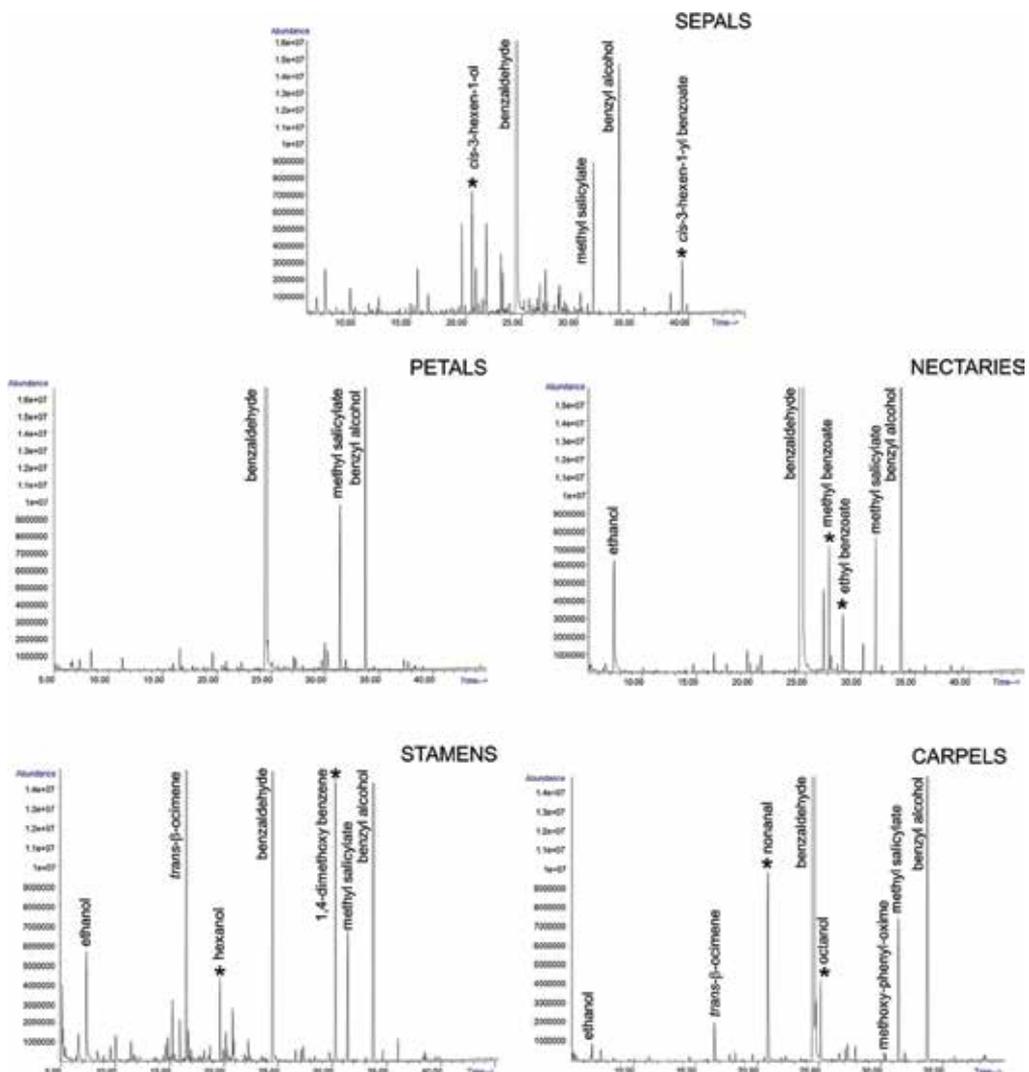
**Figure 2.** Chromatographic profiles (GC-MS, EI, 70 eV, DB-WAX column, 60 m) of volatiles isolated from *Stapelia gigantea* carrion flower, by *in vivo* HS-SPME (CAR/PDMS): (A) during oviposition and (B) after oviposition of the green bottle fly *Lucilia sericata* (Insecta: Diptera: Calliphoridae). Main compounds found in carrion flower odor are as follows: (a) dimethyl disulfide; (b) (3*E*-, 5*E*-)-2,6-dimethyl-1,3,5,7-octatetraene; (c) *trans*- $\beta$ -ocimene; (d) dimethyl trisulfide; (e) 2,6-dimethyl-1,3,5,7-octatetraene (isomer); (f) butanoic acid; (g) 3-methyl butanoic acid; (h) methoxy phenyl oxime; and (i) hexanoic acid.

flower temperature or transpiration. **Figure 3** shows chromatographic profiles (HS-SPME/GC/MS) of volatiles emitted from distinct parts of passion fruit (*Passiflora edulis*) flower, where volatile compounds differ qualitatively or quantitatively; some of these volatile metabolites are unique to each part of the flower.

## 2.2. Chromatographic analysis of floral fragrances

The substances that make up the volatile fraction isolated from flowers are of low-molecular-weight (<300 Da) and are mixtures of components with different polarity and concentration. Thanks to the volatile nature of these compounds, their analysis is done by gas chromatography (GC). Due to the complexity of some mixtures of volatiles isolated from flowers and the presence in them of isomeric substances (geometrical, positional, stereoisomers), it is recommended to make their analysis in capillary fused-silica columns, preferably long, of 50–60 m, with internal diameters (DI) of 0.25, 0.22, or 0.20 mm. The smaller internal diameters, although they allow to increase the resolution, eventually, can also compromise the sensitivity. Columns with the thickness of the stationary phase ( $d_f$ ) equal to or greater than 0.25  $\mu\text{m}$  are used, so that the shape of peaks, their separation and the sensitivity, necessary for their reproducible detection, are adequate.

Generally, for the injection of the sample ( $T^\circ$  of the injector, usually, of 230–250 $^\circ\text{C}$ ), the split ratio of 1:30 can be used, but when the concentrations of some components of interest are low, it is convenient to inject in splitless mode. When the splitless injection mode is used, to decrease the “dispersion” or the widening of the peaks of very volatile substances, the



**Figure 3.** Chromatographic profiles (GC–MS, EI, 70 eV, DB-WAX column, 60 m) of volatiles, obtained by HS-SPME (CAR/PDMS) from distinct parts of passion fruit flower (*Passiflora edulis*). Each part of the flower (sepals, petals, nectaries, stamens or carpels) possesses a “diagnostic”, unique volatile compounds (\*), found only in this part of the flower.

injection can be done in the *pulsed splitless* mode; it is when the inlet pressure of the carrier gas, during the transfer of the sample by the liner, increases by 2–3 times. Considering the presence of some thermolabile substances (esters, oximes), the *on-column* injection or the temperature-programmed injection (PTV, programmed-temperature vaporizer), could be a suitable alternative.

For the analysis of the volatile fractions, the initial column temperature of 35–50°C would be advisable; the nature of the sample (volatile compounds) does not require that the final temperature of the column be high; 200–250°C will be sufficient to elute the most retained

components. The heating speed of the column is a function of its length: the longer it is, the slower the column must be heated, 3–4°C/min, but if shorter columns are used, e.g., 30 m, one could increase the temperature of the column more rapidly, at a rate of 5–10°C/min. Of course, the process of programming the temperature in the column is optimizable, depending on the complexity of the mixture of substances to be analyzed (number of components, isomerism or structural similarity), its nature (polarity, molecular weight), the column dimensions (L, DI), the type of the stationary phase (polarity), and its thickness ( $d_p$ ).

For the analysis of the floral volatile fraction, two columns are used in combination: one with the polar stationary phase, poly(ethylene glycol) (e.g., INNOWAX, DB-WAX, HP-20 M, and others) and the other, with the non-polar stationary phase, poly(dimethyl siloxane) (HP-1, Ultra 1, DB-1, BP-1, and others) or 5% -phenyl poly(dimethyl siloxane) (HP-5, DB-5, Ultra 2, CPSil 5, BP-5, and others).

Enantioselective gas chromatography takes advantage of the fact that the enantiomers have different retention times when compounds that can form adducts are inserted in the stationary phase whose stability is a function of the three-dimensional (3D) form of the analyte. The cyclodextrins with their cone geometry with cavity of different size have turned out to be very effective chiral agents, constituting inclusion complexes that allow the discrimination of isomers according to their shape. The fragrances of jasmine (*Jasminum grandiflorum*) and other flowers (*Osmanthus fragrans*, *Boronia megastigma*) contain a mixture of methyl jasmonate stereoisomers. Wilfred König reported the separation of all isomers by means of preparative gas chromatography in which he used columns packed with cyclodextrins [15]. This allowed to confirm the estimate made by Acree and Barnard, that the methyl (+)-*epi*-jasmonate isomer has an odor threshold about 500 times lower than that of the major isomer, methyl (-)-jasmonate [16].

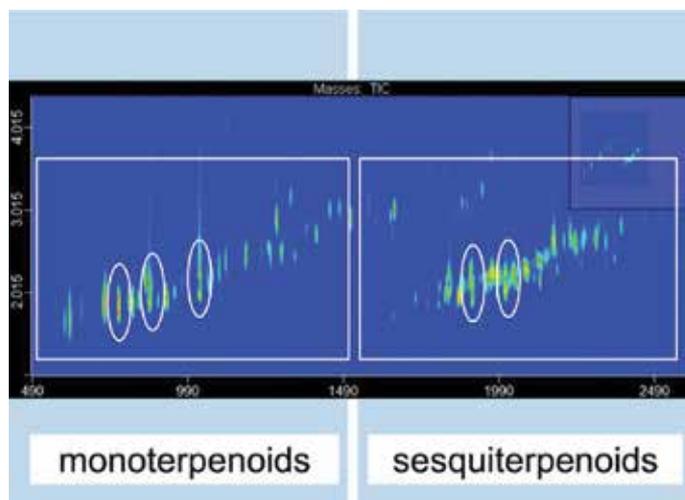
The aldehydes and lilac alcohols are oxygenated monoterpenes found in plant species of many families. Lamiaceae (*Origanum vulgare*) [17], Orchidaceae (*Platanthera* sp.) [18], Rosaceae (*Prunus padus*) [19], and Rubiaceae (*Cephalanthus occidentalis*) are some examples of these families. Each of the lilac molecules has three chiral carbons, which gives rise to eight stereoisomers of the aldehyde and eight stereoisomers of the lilac alcohol. Dötterl and colleagues [20] managed to separate all the isomers of the aldehyde and seven isomers of the lilac alcohol, by means of a two-dimensional gas chromatography system in which a 30 m-capillary column with stationary phase of 5%-phenyl poly(dimethyl siloxane) was bound by means of a T-valve to a mass selective detector, and another capillary column of 30 m with a stationary phase formed by phenyl-poly(dimethyl siloxane) (70%) and a cyclodextrin derivative (30%). Each column had an independent oven. This system was modified to convert it into micro-preparative chromatography. The output of the second column was connected to a flow divider that allowed to directing a part of the effluent toward an FID, and the other part toward a stirring bar covered with PDMS, to absorb the separated analytes. This modification allowed to collecting the isomers that were then used in electroantennography experiments in which antennas of different insects were used to examine if there was any selective response for any of the isomers. It was found that the antennae of the *Hadena bicruris* moth responded to the eight isomers of aldehyde lilac, but they were more sensitive to some isomers than to others.

The most common detection system for comparative analysis and for the quantification of compounds in the volatile fractions isolated from flowers is the flame ionization detector

(FID), a simple, robust system with an acceptable sensitivity, and a wide dynamic range. Selective detectors, such as the nitrogen and phosphorus detector (NPD) and the flame photometric detector (FPD), are very useful tools for the selective detection of nitrogenous and sulfur compounds, very common in floral fragrances.

The most important and widely used detection system in the analysis of volatile mixtures is the mass selective detector; its combination with capillary gas chromatography (GC-MS) is a perfect instrument to achieve separation and identification (presumptive or confirmatory) of components present in a mixture. The ionization mode most used for the analysis of volatile substances is the impact with electrons (EI) of 70 eV-energy. The EI mass spectra contain a lot of information because in the spectrum signals of numerous ionized fragments appear, which form a unique combination that allows to differentiate one molecule from the other, even if they are isomers.

The mass ( $m/z$ ) of fragments (ions or ion-radicals) and their relative abundances, which make up the fragmentation pattern, are the guide to differentiate the structures. The linear retention indexes (LRI) measured experimentally in the polar and non-polar columns are compared with those recorded in the literature or in databases [21–24]. Probably, the greatest progress in the analysis of complex mixtures, such as essential oils or volatile fractions isolated from flowers, has been done with the introduction of comprehensive chromatography GC  $\times$  GC [25–27]. The use of two orthogonal columns (non-polar and polar) linked through a modulator, and the use of a low-resolution (quadrupole, linear time-of-flight time, and TOF) or high-resolution (HR TOF) mass spectrometers, allow to have a complete picture on the number, and quantity of components in a mixture since some analytes can co-elute in one of the columns typically used in one-dimensional chromatography; but the use of two orthogonal



**Figure 4.** GC $\times$ GC chromatogram (TIC) of *Cannabis indica* female flower volatiles, obtained with high-resolution time-of-flight analyzer (HRTOF-MS) and cryogenic dual jet/loop modulator. 1D – First column: Rxi-5MS, 30 m, L, 250  $\mu$ m, DI, 0,25  $\mu$ m, d<sub>r</sub>. 2D – Second column: Rxi-17Sil MS, 2 m, L, 250  $\mu$ m, DI, 0,25  $\mu$ m, d<sub>r</sub>. Modulation time: 5 s. Two groups of terpenoids, *that is*, monoterpenoids and sesquiterpenoids are clearly distinguished in the chromatogram. Compounds co-eluted in the 1D column are separated in the 2D column (areas of co-eluted peaks are circled).

columns avoids this problem. In addition, GCxGC allows “classifying” the substances by families, such as can be observed in **Figure 4**, which shows two groups of substances, monoterpenes and sesquiterpenes, in the scent emitted by *Cannabis* female flowers.

### 3. Essential oil composition

Essential oils were obtained by steam distillation. High-resolution gas chromatography coupled to mass spectrometry was used for component identification. Relative amounts of essential oil constituents were calculated from the peak areas of chromatograms obtained with gas chromatography with flame ionization detection. Linear retention indices were determined on polar (Carbowax) and non-polar (DB-5) capillary chromatographic columns. Tentative compound identification was based on the comparison of retention indices with published values, and the comparison of mass spectra with those of databases [21–24]. **Table 2** presents the main constituents found in the gas chromatographic analysis of essential oils isolated from plant material collected in botanical expeditions carried out by CENIVAM.

Species	Composition
<b>Aristolochiaceae family</b>	
<i>Aristolochia anguicida</i>	$\alpha$ -Ylangene (10%), <i>trans</i> - $\beta$ -caryophyllene (27%), bicyclogermacrene (8%), $\alpha$ -humulene (3%), $\beta$ -farnesene (5%).
<i>Aristolochia ringens</i>	$\beta$ -Bourbonene (4%), $\beta$ -elemene (10%), <i>trans</i> - $\beta$ -caryophyllene (15%), <i>trans</i> -muurola-4(14),5-diene (17%), curzerene (23%), bicyclogermacrene (9%).
<b>Asteraceae family</b>	
<i>Achyrocline alata</i>	$\alpha$ -Pinene (3%), <i>p</i> -cymene (3%), thymol (24%), <i>trans</i> - $\beta$ -caryophyllene (14%), thymyl acetate (2%).
<i>Achyrocline satureioides</i>	$\alpha$ -Pinene (7%), <i>trans</i> - $\beta$ -caryophyllene (25%), $\gamma$ -muurolene (9%), $\gamma$ -cadinene (8%), caryophyllene oxide (13%).
<i>Ageratina</i> aff. <i>Popayanensis</i>	Thymol (26%), carvacrol (37%), $\delta$ -cadinene (1%).
<i>Ambrosia arborescens</i>	Chrysanthenone (14%), $\beta$ -cubebene (4%), 2-ethyliden-6-methyl-heptadienal (4%), $\gamma$ -curcumene (19%), <i>ar</i> -curcumene (8%), germacrene D (9%).
<i>Ambrosia peruviana</i>	$\gamma$ -Curcumene (14%), <i>ar</i> -curcumene (25%), $\beta$ -bisabolene (18%), spathulenol (5%), phytol (5%).
<i>Austro eupatorium inulifolium</i>	$\alpha$ -Pinene (2%), <i>trans</i> - $\beta$ -caryophyllene (10%), germacrene D (17%), spathulenol (5%), caryophyllene oxide (4%).
<i>Baccharis</i> cf. <i>nitida</i>	$\alpha$ -Eudesmol (17%), squalene (1%), spathulenol (1%), caryophyllene oxide (1%).
<i>Baccharis decussata</i>	<i>trans</i> - $\beta$ -Caryophyllene (17%), germacrene D (9%), <i>trans</i> -nerolidol (10%), premnaspirodiene (6%), $\gamma$ -amorphene (6%).
<i>Baccharis latifolia</i>	$\alpha$ -Pinene (3%), limonene (8%), kessane (4%), viridiflorol (4%), <i>cis</i> -cadin-4-en-7-ol (5%), $\beta$ -eudesmol (9%).
<i>Baccharis trinervis</i>	<i>trans</i> - $\beta$ -Caryophyllene (20%), <i>trans</i> - $\beta$ -guaiano (19%), viridiflorol (12%), germacrene D (14%), $\alpha$ -humulene (3%).

Species	Composition
<i>Bidens reptans</i>	<i>p</i> -Cymene (3%), $\beta$ -copaene (3%), germacrene D (3%), caryophyllene oxide (3%), 1-phenyl-hepta-1,3,5-triene (2%).
<i>Calea glomerata</i>	$\alpha$ -Zingiberene (27%), germacrene D (11%), <i>trans</i> - $\beta$ -caryophyllene (7%), <i>ar</i> -curcumene (5%), limonene (3%).
<i>Calea prunifolia</i>	1,8-Cineole (4%), borneol (6%), <i>trans</i> - $\beta$ -farnesene (3%), <i>ar</i> -curcumene (16%), $\alpha$ -zingiberene (14%).
<i>Calea sessiliflora</i>	$\alpha$ -Zingiberene (35%), germacrene D (17%), <i>ar</i> -curcumene (13%), viridiflorol (3%), $\beta$ -sesquiphellandrene (4%).
<i>Chromolaena peltia</i>	Caryophyllene oxide (5%), $\beta$ -amyrin (6%), germacrene D (3%), <i>trans</i> - $\beta$ -caryophyllene (1%), squalene (6%).
<i>Condylium cuatrecasii</i>	$\Delta^2$ -Carene (7%), $\Delta^3$ -carene (37%), $\beta$ -phellandrene (3%), <i>trans</i> - $\beta$ -caryophyllene (7%), liguloxide (3%).
<i>Conyza bonariensis</i>	$\alpha$ -Pinene (8%), $\beta$ -pinene (7%), $\alpha$ -phellandrene (4%), cyclofenchene (4%), isoelemicin (4%), caryophyllene oxide (5%), 1,3,5-trimethoxy-3-methyl-propenyl benzene (4%).
<i>Ichthyothere terminalis</i>	$\alpha$ -Pinene (4%), sabinene (40%), $\beta$ -pinene (3%), terpinen-4-ol (7%), <i>trans</i> - $\beta$ -caryophyllene (3%)
<i>Parthenium hysterophorus</i>	<i>trans</i> - $\beta$ -Caryophyllene (12%), limonene (12%), germacrene B (6%), <i>p</i> -cymene (6%), caryophyllene oxide (6%).
<i>Simsia fruticulosa</i>	$\alpha$ -Thujene (11%), $\alpha$ -pinene (12%), $\beta$ -myrcene (4%), $\alpha$ -copaene (4%), spathulenol (4%).
<i>Stevia aff. Lucida</i>	$\alpha$ -Pinene (25%), camphene (16%), $\beta$ -pinene (11%), $\alpha$ -phellandrene (12%), <i>p</i> -cymene (5%), limonene (12%).
<i>Stevia ovata</i>	<i>trans</i> - $\beta$ -Caryophyllene(10%), germacrene D (8%), bicyclgermacrene (5%), <i>trans</i> -nerolidol (19%), germacrene D-4-ol (4%), caryophyllene oxide (4%), guaialol (4%).
<i>Tagetes caracasana</i>	<i>cis</i> - $\beta$ -Ocimene (12%), dihydrotagetone (16%), <i>allo</i> -ocimene (2%), <i>cis</i> -tagetone (58%), <i>trans</i> -ocimene (2%).
<i>Tagetes heterocarpa</i>	<i>cis</i> - $\beta$ -Ocimene (3%), dihydrotagetone (13%), <i>cis</i> -tagetone (16%), <i>cis</i> - $\beta$ -ocimene (6%), <i>trans</i> -ocimene (12%), spathulenol (5%).
<i>Tagetes zipaquirensis</i>	$\beta$ -Myrcene (5%), <i>trans</i> - $\beta$ -ocimene (12%), dihydrotagetone (42%), 6,7-epoxy myrcene (13%), <i>trans</i> -tagetone (3%), <i>cis</i> -tagetone (3%).
<i>Verbesina centroboyaca</i>	$\beta$ -Myrcene (8%), $\alpha$ -humulene (4%), germacrene D (7%), germacrene D-4-ol (4%), hinesol (4%), valerianol (12%).
<i>Wedelia calycina</i>	Germacrene D (15%), $\beta$ -phellandrene (14%), $\beta$ -pinene (14%), $\alpha$ -pinene (20%), $\alpha$ -phellandrene (9%).
<b>Boraginaceae family</b>	
<i>Cordia curassavica</i>	$\alpha$ -Copaene (17%), <i>trans</i> - $\beta$ -caryophyllene (22%), germacrene D (18%), <i>trans</i> - $\beta$ -guaiene (8%), $\alpha$ -pinene (6%).
<b>Burseraceae family</b>	
<i>Protium heptaphyllum</i>	<i>trans</i> - $\beta$ -Caryophyllene (20%), $\alpha$ -humulene (9%), $\gamma$ -cadinene (13%), caryophyllene oxide (20%), germacrene D (3%).
<b>Chloranthaceae family</b>	
<i>Hedyosmum racemosum</i>	$\alpha$ -Pinene (6%), sabinene (21%), $\beta$ -pinene (9%), 1,8-cineole (6%), <i>trans</i> -4-thujanol (5%).

Species	Composition
<b>Euphorbiaceae family</b>	
<i>Croton ferrugineus</i>	<i>trans</i> - $\beta$ -Caryophyllene (37%), dilapiol (23%), germacrene D (13%), <i>cis</i> -chrysantenyl acetate (7%), bicyclogermacrene (3%).
<b>Fabaceae family</b>	
<i>Dalea cliffortiana</i>	Methyleugenol (69%), <i>trans</i> - $\beta$ -caryophyllene (15%), $\alpha$ -humulene (1%), germacrene D (5%), caryophyllene oxide (3%).
<b>Chenopodiaceae family</b>	
<i>Chenopodium ambrosioides</i>	$\alpha$ -Terpinene (21%), <i>p</i> -cymene (15%), ascaridole (47%), <i>iso</i> -ascaridole (12%).
<b>Lamiaceae family</b>	
<i>Eriope crassipes</i>	6-Methyl-5-hepten-2-one (5%), $\alpha$ -cubebene (3%), $\alpha$ -copaene (3%), methyl citronellate (3%), $\alpha$ -muurolene (3%).
<i>Hyptis brachiata</i>	$\alpha$ -Copaene (5%), <i>trans</i> - $\beta$ -caryophyllene (11%), germacrene D (16%), 7- <i>epi</i> - $\alpha$ -selinene (5%), caryophyllene oxide (4%).
<i>Hyptis brachiata</i>	Caryophyllene oxide (2%), <i>trans</i> -nerolidol (2%), $\alpha$ -humulene (2%), carvacrol (5%).
<i>Hyptis colombiana</i>	Germacrene D (22%), <i>trans</i> - $\beta$ -caryophyllene (34%), caryophyllene oxide (14%), $\alpha$ -copaene (5%), bicyclogermacrene (3%).
<i>Hyptis dilatata</i>	<i>trans</i> - $\beta$ -Caryophyllene (26%), $\Delta^3$ -carene (4%), $\alpha$ -gurjunene (10%), camphor (8%), palustrol (9%).
<i>Hyptis mutabilis</i>	$\beta$ -Elemene (6%), <i>trans</i> - $\beta$ -caryophyllene (6%), germacrene D (12%), curzerene (30%), $\beta$ -bourbonene (2%).
<i>Hyptis pectinata</i>	$\beta$ -Bourbonene (8%), <i>trans</i> - $\beta$ -caryophyllene (15%), germacrene D (18%), bicyclogermacrene (7%), caryophyllene oxide (5%).
<i>Hyptis suaveolens</i>	Sabinene (2%), $\alpha$ -phellandrene (2%), fenchone (7%), <i>trans</i> - $\beta$ -caryophyllene (13%), germacrene D (3%).
<i>Lepechinia betonicifolia</i>	$\alpha$ -Pinene (8%), $\beta$ -pinene (7%), limonene (10%), 1,8-cineole (8%), <i>trans</i> - $\beta$ -caryophyllene (7%), $\gamma$ -curcumene (7%), $\alpha$ -zingiberene (8%), ledol (8%).
<i>Lepechinia bullata</i>	$\beta$ -Pinene (13%), $\alpha$ -copaene (4%), <i>trans</i> - $\beta$ -caryophyllene (21%), $\alpha$ -humulene (7%), caryophyllene oxide (17%).
<i>Lepechinia conferta</i>	$\alpha$ -Pinene (5%), $\beta$ -pinene (10%), <i>p</i> -cymene (17%), palustrol (8%), $\alpha$ -cedrene (4%).
<i>Lepechinia salviifolia</i>	$\alpha$ -Pinene (3%), $\beta$ -pinene (10%), $\Delta^3$ -carene (5%), limonene (21%), <i>trans</i> - $\beta$ -caryophyllene (6%), ledol (4%).
<i>Lepechinia vulcanolica</i>	$\alpha$ -Pinene (8%), 3-octenol (9%), limonene (19%), <i>trans</i> - $\beta$ -caryophyllene (9%), germacrene D (10%), palustrol (7%).
<i>Marsypianthes chamaedrys</i>	$\alpha$ -Copaene (5%), <i>trans</i> - $\beta$ -caryophyllene (4%), spathulenol (6%), caryophyllene oxide (15%), humulene II epoxide (5%).
<i>Mentha suaveolens</i>	<i>cis</i> - $\beta$ -Ocimene (4%), 1-octen-3-yl acetate (4%), piperitenone oxide (52%), nepetalactone (6%), germacrene D (10%).
<i>Minthostachys mollis</i>	Menthone (7%), pulegone (6%), <i>cis</i> -piperitone epoxide (30%), piperitenone oxide (26%), <i>trans</i> - $\beta$ -caryophyllene (5%), germacrene D (6%).
<i>Minthostachys septentrionalis</i>	Piperitone epoxide (59%), piperitone oxide (13%), <i>trans</i> - $\beta$ -caryophyllene (9%), germacrene D (1%), $\alpha$ -humulene (2%).

Species	Composition
<i>Ocimum americanum</i>	Linalool (23%), estragole (63%), <i>cis</i> - $\alpha$ -bisabolene (4%).
<i>Ocimum campechianum</i>	Methyleugenol (54%), 1,8-cineole (3%), <i>trans</i> - $\beta$ -caryophyllene (13%), $\alpha$ -humulene (3%), germacrene D (3%).
<i>Ocimum tenuiflorum</i>	Eugenol (22%), $\beta$ -elemene (23%), <i>trans</i> - $\beta$ -caryophyllene (23%), $\alpha$ -humulene (3%), germacrene D (5%).
<i>Perilla frutescens</i>	Perilla ketone (48%), 1-octen-3-ol (32%), linalool (6%), 3-octanone (5%), 3-octanol (3%).
<i>Plectranthus amboinicus</i>	Carvacrol (13%), <i>trans</i> - $\beta$ -caryophyllene (1%) $\alpha$ -amirine (38%), viminalol (21%), estigmast-4-en-3-one (10%).
<i>Salvia aratocensis</i>	<i>trans</i> - $\beta$ -Caryophyllene (5%), $\gamma$ -cadinene (7%), 1,10-di- <i>epi</i> -cubenol (12%), <i>epi</i> - $\alpha$ -cadinol (16%).
<i>Salvia aratocensis subsp. suratensis</i>	<i>trans</i> - $\beta$ -Caryophyllene (5%), <i>cis</i> - $\beta$ -farnesene (2%), $\gamma$ -cadinene (10%), 1- <i>epi</i> -cubenol (16%), <i>epi</i> - $\alpha$ -cadinol (22%).
<i>Salvia rubriflora</i>	<i>trans</i> - $\beta$ -Caryophyllene (13%), $\alpha$ -farnesene (9%), spathulenol (8%), caryophyllene oxide (5%).
<i>Salvia sagittata</i>	<i>cis</i> -Pinocamphone (6%), linalool acetate (5%), $\alpha$ -terpinyl acetate (24%), <i>trans</i> - $\beta$ -caryophyllene (6%), palustrol (7%), curzerenone (10%).
<i>Satureja aff. Andrei</i>	Limonene (2%), <i>p</i> -mentha-3,8-diene (4%), <i>cis</i> -pulegol (6%), pulegone (23%), <i>trans</i> - $\beta$ -caryophyllene (7%).
<b>Myrtaceae family</b>	
<i>Calycolpus moritzianus</i>	1,8-Cineole (20%), $\alpha$ -terpineol (6%), <i>trans</i> - $\beta$ -caryophyllene (8%), guaiol (5%), $\gamma$ -eudesmol (7%).
<i>Psidium sartorianum</i>	1,8-Cineole (16%), terpinen-4-ol (11%), $\alpha$ -terpineol (13%), <i>trans</i> - $\beta$ -caryophyllene (20%), $\beta$ -pinene (3%).
<b>Piperaceae family</b>	
<i>Piper auritum</i>	Safrol (9%), myristicin (5%).
<i>Piper bogotense</i>	$\alpha$ -Pinene (9%), $\alpha$ -phellandrene (14%), <i>p</i> -cymene (4%), limonene (5%), linalool (5%), <i>trans</i> -sesquisabinene hydrate (14%)
<i>Piper bremedeyeri</i>	$\alpha$ -Pinene (20%), $\beta$ -pinene (32%), limonene (4%), $\beta$ -elemene (4%), <i>trans</i> - $\beta$ -caryophyllene (6%), germacrene D (4%).
<i>Piper carpunya</i>	<i>p</i> -Cymene (11%), 1,8-cineole (11%), safrol (12%), methyleugenol (5%), cumunyl acetate (5%)
<i>Piper cf. divaricatum</i>	$\alpha$ -Pinene (11%), $\beta$ -pinene (5%), $\alpha$ -phellandrene (6%), 1,8-cineole (18%), linalool (15%), <i>trans</i> - $\beta$ -caryophyllene (8%).
<i>Piper cf. subflavum</i>	Apiol (27%), dillapiol (1%), <i>trans</i> - $\beta$ -caryophyllene (1%), $\delta$ -cadinene (1%), <i>cis</i> -calamenene (1%).
<i>Piper marginatum</i>	$\alpha$ -Phellandrene (11%), limonene (8%), $\beta$ -elemene (4%), <i>trans</i> - $\beta$ -caryophyllene (11%), bicyclogermacrene (4%).
<i>Piper medium</i>	$\beta$ -Phellandrene (22%), germacrene D (12%), <i>trans</i> - $\beta$ -caryophyllene (6%), bicyclogermacrene (4%), <i>trans</i> - $\beta$ -elemene (3%).
<b>Scrophulariaceae family</b>	
<i>Achetaria bicolor</i>	<i>trans</i> -Pinocarveol (8%), pinocarvone (6%), $\alpha$ -humulene (18%), humulene II epoxide (5%).
<b>Turneraceae family</b>	
<i>Turnera diffusa</i>	Drima-7,9(11)-diene (23%), valencene (6%), $\beta$ -selinene (6%), viridiflorene (7%), dihydrokaranone (15%).

Species	Composition
<b>Verbenaceae family</b>	
<i>Dalea coerulea</i>	$\alpha$ -Phellandrene (6%), <i>p</i> -cymene (8%), $\beta$ -phellandrene (13%), piperitone (5%), $\alpha$ -caracolene (7%), spathulenol (5%).
<i>Lantana boyacana</i>	$\alpha$ -Pinene (5%), sabinene (11%), 1,8-cineole (14%), <i>trans</i> - $\beta$ -caryophyllene (9%), $\alpha$ -humulene (6%).
<i>Lantana fucata</i>	$\beta$ -Phellandrene (4%), <i>trans</i> - $\beta$ -caryophyllene (14%), $\alpha$ -zingiberene (6%), germacrene D (10%), $\delta$ -cadinene (3%), caryophyllene oxide (7%).
<i>Lippia alba</i>	Limonene (30%), carvone (50%), piperitone (3%), piperitenone (6%), bicyclosesquiphellandrene (4%).
<i>Lippia americana</i>	<i>trans</i> - $\beta$ -caryophyllene (21%), germacrene D (12%), $\delta$ -cadinene (6%), caryophyllene oxide (13%), $\beta$ -cubebene (5%).
<i>Lippia canescens</i>	<i>trans</i> - $\beta$ -caryophyllene (27%), $\alpha$ -humulene (12%), caryophyllene oxide (8%), limonene (7%), <i>p</i> -cymene (6%).
<i>Lippia micromera</i>	<i>p</i> -Cymene (13%), $\gamma$ -terpinene (9%), thymol methyl ether (25%), thymol (27%), $\gamma$ -terpinene (9%).
<i>Lippia organoides</i>	$\gamma$ -Terpinene (6%), <i>trans</i> - $\beta$ -caryophyllene (7%), $\alpha$ -humulene (4%), caryophyllene oxide (2%), <i>p</i> -cymene (10%).

**Table 2.** Main constituents of essential oils of species collected in botanical outings.

#### 4. Biological activity of essential oils

One of the most important research lines of the CENIVAM Project is related to the study of different biological activities of essential oils. Bioactivity assays against *Trypanosoma cruzi* (epimastigotes and amastigotes), *Leishmania chagasi* (promastigotes and amastigotes), Vero cell assays, and THP cells were carried out at the Research Center for Tropical Illnesses, CINTROP. About 48% of the essential oils examined were active against *T. cruzi*. The essential oils were also tested against *L. chagasi* (promastigotes and amastigotes) and 19% were active [28, 29]. The virucidal activity of some essential oils was studied at the CINTROP, against the dengue (serotype 2) [30–33], and yellow fever [31, 34] viruses. Of the essential oils tested, respectively, 83 and 66% were active against this type of virus, constituting this result in a very interesting contribution, especially considering that there is not much data in the literature on the activity of essential oils against this type of virus. The essential oils of two chemotypes of *Lippia organoides* (Fam. Verbenaceae) were in vitro potent agents against dengue and yellow fever viruses, which warrant the future study of the mechanism of their antiviral action.

Different CENIVAM groups, among them, in the Research Center for Biomolecules, CIBIMOL [35, 36], Environmental and Computational Chemistry of the University of Cartagena [37, 38], Chemistry and Biology of the Universidad del Norte, in Barranquilla [39] measured the antioxidant activity of essential oils by different techniques, e.g., lipid oxidation, measurement of secondary end products of lipoxidation, thiobarbituric acid reactive substances (TBARS) test, and free radical trapping tests (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid, ABTS + and 2,2-diphenyl-1-picrylhydrazyl, DPPH). Grosso modo, about 40% of the analyzed samples were active in different tests of antioxidant activity.

The study of the cytotoxic activity (acute toxicity, LC50) of the essential oils was carried out by several groups. Cecilia Mesa et al. examined the activity against *Candida krusei* and *Aspergillus*

*fumigatus* [40]. *Lippia origanoides* and *L. alba* have several chemotypes, and these have been the subject of detailed study to check that their cytotoxicity does not prevent their use in some topical pharmaceutical applications [41, 42]. The group of Environmental and Computational Chemistry has used the *Artemia franciscana* assay to test for acute toxicity [43]. More than 30% of all samples analyzed in these trials did not have any degree of toxicity. In the same group, anti-quorum sensing activities, teratogenic and antigenotoxic effects, and the insect repellent activity of essential oils were studied [44, 45].

Insect repellency is an interesting biological activity that leads to rather soon implementation of essential oils as active ingredients of commercial products. Olivero et al. have examined the potential application of essential oils to repel insects of importance to food storage [46–49]. Another application of insect repellence is the prevention of diseases for which *Aedes aegypti* is the vector [50–52]. More than 50% of the tested essential oils and pure terpenes proved to be good insect repellents (56 and 80%, respectively).

The assays of the anti-genotoxic and chemopreventive activity carried out at the CIBIMOL-UIS group demonstrated a DNA protective effect of the essential oils of several chemotypes of *Lippia alba* and *Lippia origanoides* (Fam. Verbenaceae) [53–56].

Several bacterial strains have been employed in assays of essential oil antibacterial activity [57, 58]. Due to their carvacrol and thymol content, *L. origanoides* oils have shown important antibacterial activity [59]. Antimycobacterial activity, which is of interest in tuberculosis research, has received special attention by CENIVAM researchers [60, 61]. It has been determined in oils from the state of Santander [62].

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## Conflict of interest

The authors declare that they have no conflict of interest with this chapter contents.

## Author details

Elena Stashenko\* and Jairo René Martínez

\*Address all correspondence to: [elena@tucan.uis.edu.co](mailto:elena@tucan.uis.edu.co)

Research Center of Excellence CENIVAM, Universidad Industrial de Santander,  
Bucaramanga, Colombia

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# **A Fuzzy Rule Based Approach to Geographic Classification of Virgin Olive Oil Using T-Operators**

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Suzan Kantarcı-Savaş and Efendi Nasibov

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## **Abstract**

Olive oil is an important agricultural food product. Especially, protected designation of origin (PDO) and protected geographic indications (PGI) are useful to protect the intellectual property rights of the consumers and producers. For this reason, the importance of the geographic classification increases to trace geographical indications. This chapter suggests a geographical classification system for the virgin olive oils. This system is formed on chemical parameters. These parameters include fuzziness. Novel proposed system constructs the rules by using fuzzy decision tree algorithm. It produces rules over fuzzy ID3 algorithm. It uses fuzzy entropy on the fuzzified data. The reasoning procedure depends on weighted rule-based system and is adapted into the fuzzy reasoning handled with different T-operators. Fuzzification is performed with fuzzy *c*-means algorithm for the olive oil data set. The cluster numbers of each variable are selected based on partition coefficient validity criteria. The model is examined by using different decision tree approaches (C4.5 and standard version fuzzy ID3 algorithm) and FID3 reasoning method with eight different T-operators. Also, the conclusions are supported by statistical analysis. Experimental results support that the weights have important manner on fuzzy reasoning method for the geographic classification system.

**Keywords:** fuzzy decision tree, fuzzy rule, T-operators, geographic classification, olive oil

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## **1. Introduction**

Geographic indications are very important signs used on products. Their aim is to specify geographical origin of the product and follow the qualities. There are two kinds of geographical indications, protected designation of origin (PDO) and protected geographic indications (PGI). These indications are generally used for agricultural products. Olive oil has crucial

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manner among these agricultural food products. It is necessary to observe the properties of olive oil produced from different kinds of regions or different types of olive varieties. Geographical classification problem investigates the relationship among the chemical and sensorial parameters for each region.

Nowadays, machine learning discipline and chemical data structures come together with the information age. Machine learning is interested in the design and development of algorithms for computers. It aims to observe the relationships among the data structure and to make knowledge mining without assumptions. There are several machine learning algorithms to search the knowledge.

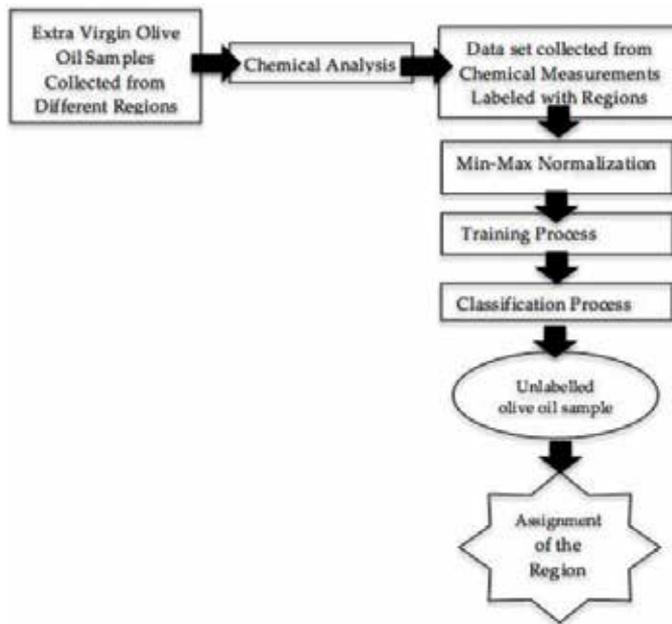
Decision trees as machine learning tasks, are most commonly used in machine learning discipline. There are several types of decision tree algorithms such as ID3, C4.5, CART, etc. Nowadays, fuzzy logic is adapted into decision tree algorithms to handle the uncertainty. The decision trees adapted with fuzzy logic are called as fuzzy decision tree [1–3]. It consists of nodes for testing attributes, edges for branching by test values of fuzzy sets, and leaves for deciding class according to class membership.

The chemical measurements have also uncertainty [4–8]. In this study, geographical classification problem uses chemical measurements. This study aims to propose an improved methodological approach for the classification of olive oil samples based on fuzzy ID3 classification approach.

This novel proposed system constructs the rules by using fuzzy decision tree algorithm. Its reasoning procedure is based on weighted rule-based system adapted into the fuzzy reasoning handled with different T-operators. The model is examined by using different decision tree approaches (C4.5 and standard version fuzzy ID3 algorithm) and FID3 reasoning method with eight different T-operators. This study is examined on 101 virgin olive oil samples collected from four different regions (North Aegean, South Aegean, Mediterranean, and South East) by using measurements of chemical parameters. Min-max normalization was applied into the dataset. The nonparametric methods were preferred for the statistical analysis because of the data structure. Leave-one-out procedure was performed in order to measure the performances of the algorithms. The Friedman aligned rank test and pairwise comparisons were performed to evaluate fuzzy reasoning method based on different T-operators. And, the comparison between unweighted and weighted fuzzy reasoning approaches was done. The rest of the paper is organized as follows: Section 2 presents the geographical classification problem definition and related works. The preliminaries such as fuzzification, fuzzy ID3 algorithm, and fuzzy rule-based classification system are given in Section 3. Experimental study on unweighted and weighted fuzzy rule-based approach to Geographic Classification of Virgin Olive Oil Using T-Operators is given in Section 4, and finally, the conclusion is represented in Section 5.

## 2. Geographic classification problem

Geographic classification problem aims to find the region for an unassigned olive oil sample. This problem comes to exist to support the traceability of denominated protected origin policy for olive oil samples. Especially, the definition of a methodology is an important issue for



**Figure 1.** Geographical classification problem scheme for olive oil.

Turkey. In literature, it is seen that the scholars generally prefer to study on the classification of olive oils [9, 10]. Principal component analysis, linear discriminant, probabilistic neural networks, and classification binary tree were preferred techniques to evaluate the parameters [9, 10]. Back propagation artificial neural networks (BP-ANN) is also used to solve [11] this kind of problem. In [12], the adulteration in olive oil was defined by near-infrared spectroscopy and using chemometric techniques such as principal component analysis, partial least squares regression (PLS), and applied methods for data pretreatments such as signal detection correction. Principal component analysis and SIMCA classification model [13] are other methods to support the geographic classification problem given in **Figure 1**.

### 3. Preliminaries

We briefly explain fuzzy logic and fuzzy c-means algorithm as fuzzification tool. Also, we review briefly fuzzy ID3 builder combined with fuzzy rule-based classification and its reasoning method. We give information about T-operators and we suggest fuzzy ID3 weighted reasoning method approach via different types of T-operators in subsections.

#### 3.1. Fuzzy logic and fuzzy c-means algorithm as fuzzification tool

In 1965, fuzzy set theory was first proposed in [14]. A fuzzy subset of the universe of discourse  $U$  is described by a membership function  $\mu_v(V) : U \rightarrow [0, 1]$ , which represents the degree to which  $u \in U$  belongs to the set  $v$ . Each value defines by a membership degree. The transformation process into membership degrees for each term of fuzzy variables is called as fuzzification.

In literature, there are many types of membership functions, triangular membership functions, trapezoidal membership functions, Gaussian membership functions, etc. [15]. In general, triangular membership functions are preferred. Otherwise, fuzzy c-means (FCM) algorithm, which was suggested in [16] and it was improved in [17], can be used for the transformation of membership degrees for each term of fuzzy variables. This algorithm is a kind of clustering algorithm. This clustering algorithm aims to reach a fuzzy C partition matrix  $U$ . The objective function  $J_m$  is minimized as follows for fuzzy partition (Eq. (1)):

$$J_m(U, v) = \sum_{k=1}^n \sum_{i=1}^c (\mu_{ik})^m (d_{ik})^2 \quad (1)$$

where

$$d_{ik} = d(x_k, v_i) = \left[ \sum_{j=1}^p (x_{kj} - v_{ij})^2 \right]^{1/2}, k = 1, \dots, n; i = 1, \dots, c \quad (2)$$

and,  $\mu_{ik}$  is explained as the membership degree of the  $k$ th data point in  $i$ th class. Dimensionality of the data space is indicated by 'p'. The parameter  $m \in (1, \infty)$  demonstrates sharpness of the fuzzification process. In Eq. (2),  $d_{ik}$  indicates any distance measure (usually the Euclidean distance) between  $k$ th data point and  $i$ th cluster center in  $p$  dimensional space. Then,  $v_i$  displays  $i$ th cluster center. Eq. (3) calculates each of the clusters centers for each class:

$$v_{ij} = \frac{\sum_{k=1}^n \mu_{ik}^m x_{kj}}{\sum_{k=1}^n \mu_{ik}^m}, i = 1, 2, \dots, c; j = 1, 2, \dots, p. \quad (3)$$

Membership degrees are calculated according to the Eq. (4):

$$\mu_{ik} = \frac{1}{\sum_{z=1}^c \left( \frac{\|x_k - v_i\|}{\|x_k - v_z\|} \right)^{\frac{2}{m-1}}}, i = 1, 2, \dots, c; k = 1, \dots, n \quad (4)$$

Validity indicators are used in order to determine the number of clusters (c) [18–20]. One of them is partition coefficient formulized as below (Eq. (5)):

$$V_{PC} = \frac{1}{n} \sum_{i=1}^c \sum_{j=1}^n \mu_{ij}^2 \quad (5)$$

whereas optimal cluster number is determined by the calculation of  $\max(V_{PC}, U, c)$ . Each cluster number represents the number of fuzzy linguistic term for each fuzzy variable.

### 3.2. Fuzzy rule-based classification system (FRBCS)

Fuzzy rule-based classification system (FRBCS) is very useful for the solution of classification problems. In real life, they have been applied into the different kinds of problems, such as image processing [21], medical problems [22], etc.

There is a class  $C_j$  from a preassigned class set  $C = \{C_1, C_2, \dots, C_M\}$  to an object, which is a part of a certain feature space  $x \in S^N$  and a classifier is to realize an assignment for an appropriate class,  $(D = S^N \rightarrow C)$  [23].

In general, the classifier includes a set of fuzzy rules. It can be a neural network, a decision tree, fuzzy decision tree etc. If the classifier produces a set of fuzzy rules, the system is called a fuzzy rule-based classification system (its acronym is FRBCS).

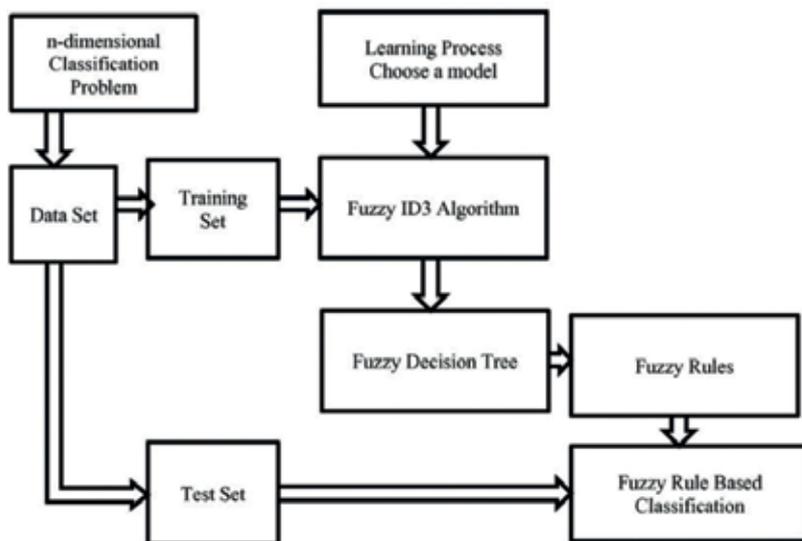
The antecedents of fuzzy rules defined by fuzzy variables provide computational flexibility. Using a set of training samples and a classifier solves a classification problem. The model provides the class of a new sample. The scheme of classification problem with fuzzy ID3 algorithm combined with fuzzy rule-based classification system is summarized in **Figure 2** as follows.

In this study, it is seen that fuzzy interactive dichotomizer 3 (fuzzy ID3) algorithm is preferred as a classifier. This algorithm generate rules, fuzzy ID3 algorithm constructs a tree in learning process. Fuzzy entropy is applied to find the attributes, which has the maximum information whereas minimum uncertainty. Each path of the tree shows the rules. Each leaf node has rule weight (RW) for each class.  $RW_j$  represents  $j$ th rule's weight handled from fuzzy confidence value  $CF_j$  which equals to  $RW_j$ . After the rules induction, fuzzy rule-based reasoning is performed to handle the classification task.

In literature, there are three definitions for fuzzy rules [23]. In this study, the following type of rules is used for the experiments constructed from the fuzzy decision trees.

*Fuzzy rules with a class and a certainty degree in the consequent* [24].

$$R_k : \text{If } x_1 \text{ is } A_1^k \text{ and } \dots \text{ and } x_N \text{ is } A_N^k \text{ then } Y \text{ is } C_j \text{ with } r_k$$



**Figure 2.** A classification problem with fuzzy ID3 algorithm combined with FRBC.

where  $r_k$  is the certainty degree of the classification in the class  $C_j$  for a pattern belonging to the fuzzy substance restricted by the fuzzy antecedent.

### 3.3. Fuzzy interactive dichotomizer 3

Fuzzy decision tree is the adaptation of decision tree structure with fuzzy logic. There are many types of decision tree algorithms, which are adapted with fuzzy logic to construct a fuzzy decision tree. A tree is generated and the decision rules are achieved by using each path from the root to the leaves of the tree. Fuzzy interactive dichotomizer 3 (Fuzzy ID3) defined in [2] is widely used as a classification tree builder algorithm. It is the adaptation of ID3 algorithm proposed by Quinlan in [25] with fuzzy logic. One of the important advantages is to deal with crisp and fuzzy variables defined by the user. This algorithm separates the data set according to a data attribute, which is selected by using a measure called as information gain based on fuzzy entropy. It seeks the attributes, which has the information with the highest degree of resolution.

Let a training set consists of  $p$  samples,  $x_p = (x_{p1}, \dots, x_{pn})$  be the  $p$ th sample of the training set where  $x_{pi}$  is the value of the  $i$ th attribute ( $i = 1, 2, \dots, n$ ) of the  $p$ th training sample. Each sample belongs to a class shown as  $y_p \in C = \{C_1, C_2, \dots, C_m\}$ , where  $m$  is the number of classes of the problem [26]. Assume there are  $N$  labeled fuzzified patterns and  $n$  attributes  $A = \{A_1, A_2, \dots, A_n\}$ . For each  $k$  assume that ( $1 \leq k \leq n$ ). The attribute  $A_k$  takes  $m_k$  values of fuzzy subsets ( $A_{k1}, A_{k2}, \dots, A_{km_k}$ ).  $C$  denotes the classification target attribute, taking  $m$  values  $C_1, C_2, \dots, C_m$ . The symbol  $M(\cdot)$  is used to denote the cardinality of a given fuzzy set, that is, the sum of the membership values of the fuzzy set [2, 26].

The induction process of fuzzy ID3 is given as follows:

**Step 1:** Produce a root node, which contains a set of all data. Each data is fuzzified, and each membership degree equals to 1 for all data for the initialization.

**Step 2:** The attribute for each internal node is selected by using the following steps:

*Step 2a:* Compute its relative frequencies with respect to class  $C_j$  ( $j = 1, 2, \dots, m$ ) for each linguistic label  $A_{ki}$  ( $i = 1, 2, \dots, m_k$ ),

$$p_{ki}(j) = \frac{M(A_{ki} \cap C_j)}{M(A_{ki})} \quad (6)$$

*Step 2b:* Compute its fuzzy classification entropy for each linguistic label  $A_{ki}$  ( $i = 1, 2, \dots, m_k$ ):

$$Entr_{ki} = - \sum_{j=1}^m p_{ki}(j) \log(p_{ki}(j)) \quad (7)$$

*Step 2c:* Compute the average fuzzy classification entropy ( $E_k$ ) of each attribute.

$$E_k = \sum_{i=1}^{m_k} \frac{M(A_{ki})}{\sum_{j=1}^{m_k} M(A_{kj})} Entr_{ki} \quad (8)$$

*Step 2d:* Select the attribute (*Attr*) that maximizes the gain information ( $G_k$ ) [27].

$$Attr = \max_{1 \leq k \leq n} (G_k), \text{ where } G_k = E_k - Entr_{ki} \quad (9)$$

*Step 2e:* Assign the selected attribute as the root node and the linguistic labels as candidate branches of the tree.

**Step 3:** Pick out one branch to analyze. Remove the branch if it is containing nothing. If the branch is nonentity, calculate the relative frequencies via (Eq. (6)) of all objects within the branch into each class. If the relative frequency of each class is above the given threshold  $\theta_r$ , or all the attributes have been expanded for this branch, stop the branch as a leaf. Otherwise, select the attribute from among those, which have not been extended yet in this branch with the smallest average fuzzy classification entropy (Eq. (9)) as a new decision node for the branch and add its linguistic labels as candidates branches to analyze. At each leaf, each class will have its relative frequency [27].

**Step 4:** Repeat Step 3 while there are branches to analyze. If there are no candidate branches then the decision tree is totaled [27].

*The rule structure generated from each branch of the fuzzy decision tree.*

After the fuzzy decision tree induction, the rules are generated from each branch. Each branch behaves as path. The rule  $R_j$  is given as follows [27]:

**Rule  $R_j$ :** If  $x_1$  is  $A_{j1}$  and ... and  $x_n$  is  $A_{jn}$  then  $Class = C_j$  with  $RW_j$ , where  $R_j$  is the label of the  $j$ th rule.  $x = (x_1, x_2, \dots, x_n)$  is an  $n$ -dimensional pattern vector. This vector is used to represent the example.  $A_{ji}$  is a fuzzy set.  $C_j \in C$  is the class label, and  $RW_j$  is the rule weight. In fuzzy decision tree, at each leaf node has rule weights. These rule weights are founded via the relative frequency for each class (as given in Step 3) [27].

### 3.4. Fuzzy reasoning method based on T-operators

Fuzzy reasoning method (FRM) is defined as an inference procedure. This inference procedure aims to achieve an assignment from a set of fuzzy if then rules. It makes the combination between the information of the rules fires and the pattern to be classified. This ability of FRM supports the generalization capability of the classification system [25]. We will analyze this idea in this section according to the following structure. In this section, the adaptation of the general model of fuzzy reasoning is represented with the classical FRM. After that, we talk about a general model of reasoning that involves different possibilities as reasoning methods, we suggest eight alternative FRMs as some particular new proposals, which are adapted with the general reasoning model. Finally, in the last section, we present the experiments carried out, displaying the advantageous behavior of the alternative proposed reasoning methods.

#### 3.4.1. General model of fuzzy reasoning

Let  $x_p = (x_{p1}, \dots, x_{pn})$  be the  $p$ th example of the training set, which is composed of  $P$  examples, where  $x_{pi}$  is the value of the  $i$ th attribute ( $i = 1, 2, \dots, n$ ) of the  $p$ th sample. Each example

belongs to class  $y_p \in C = \{C_1, C_2, \dots, C_m\}$ , where  $m$  is the number of classes of the problem. It is assumed that  $x_p$  is a novel example to be classified FID3 reasoning procedure given in [2]. Fuzzy reasoning method for FARC-HD in [28] is summarized in four steps. In our approach, fuzzy ID3 reasoning method is combined with T-operators. T-operators were developed from the triangular inequalities [29, 30]. The combination of fuzzy set theory and T-operators are used to intersect and reunite two fuzzy sets [31, 32]. There are different types of T-operators, which are also called T-norms and T-conorms in literature [33]. These operators are used in different types of problems [33]. T-operators are two placed functions from  $[0, 1] \times [0, 1]$  to  $[0, 1]$  that are monotonic, commutative, and associative [33].

T-norm is used to find the intersection of two fuzzy sets A and B. The intersection of two fuzzy sets A and B is a fuzzy set C, written as  $C = A \text{ and } B$ , whose MF is related to those of A and B by

$$\mu_C(x) = (\mu_A(x) \wedge \mu_B(x)) \tag{10}$$

On the other hand, T-conorm is performed to achieve the union of two fuzzy sets A and B is a fuzzy set C, written as  $C = A \text{ or } B$ , whose membership function (MF) is related to those of A and B by

$$\mu_C(x) = (\mu_A(x) \vee \mu_B(x)) \tag{11}$$

T-Operators used in fuzzy reasoning method are given in **Table 1** [27].

**Nonparametric operators [27]**

Ref	T-norm operators	T-conorm operators
Zadeh [14]	$T_1(x, y) = \min(x, y)$	$T_1^* = \max(x, y)$
Product Sum [41, 42]	$T_2(x, y) = x.y$	$T_2^* = x + y - x.y$
Nonparametric Hamacher [43] ( $\lambda = 0$ )	$T_3(x, y) = \frac{x.y}{(x+y-x.y)}$	$T_3^*(x, y) = \frac{x+y-2.x.y}{1-x.y}$

**Parametric operators [27]**

Ref	T-norm operators	T-conorm operators	Parametric Range
Hamacher [43]	$T_4(x, y) = \frac{x.y}{\lambda+(1-\lambda)(x+y-x.y)}$	$T_4^*(x, y) = \frac{x+y-(2-\lambda).x.y}{\lambda+(1-\lambda)(1-x.y)}$	$\lambda \geq 0$
Yager [44]	$T_5(x, y) = \max(1 - ((1-x)^p + (1-y)^p)^{1/p}, 0)$	$T_5^*(x, y) = \min((x^p + y^p)^{1/p}, 1)$	$p > 0$
Dombi [45]	$T_6(x, y) = \frac{1}{1 + \left(\left(\frac{1}{x}-1\right)^\lambda + \left(\frac{1}{y}-1\right)^\lambda\right)^{1/\lambda}}$	$T_6^*(x, y) = \frac{1}{1 + \left(\left(\frac{1}{x}-1\right)^{-\lambda} + \left(\frac{1}{y}-1\right)^{-\lambda}\right)^{-1/\lambda}}$	$\lambda > 0$
Dubois and Prade [46]	$T_7(x, y) = \frac{x.y}{\max(x,y,\lambda)}$	$T_7^*(x, y) = \frac{(1-x).(1-y)}{\max(1-x,1-y,\lambda)}$	$\lambda = [0, 1]$
Weber [41]	$T_8(x, y) = \max\left(\frac{x+y-1+\lambda.x.y}{1+\lambda}, 0\right)$	$T_8^*(x, y) = \min(x + y + \lambda.x.y, 1)$	$\lambda > -1$

**Table 1.** T-Operators used in fuzzy reasoning method.

### 3.4.2. Fuzzy rule evaluation measures in data mining

There are two measures called as confidence and support in the field data mining to evaluate rules. Assume that fuzzy rule  $R_j$  is defined as  $A_q \implies C_q$  where  $A_q = (A_{q1}, \dots, A_{qn})$ . In [34–37], fuzzy versions of two rule evaluation measures were explained as below:

Let us assume that  $m$  labeled patterns,

$$x_p = (x_{p1}, \dots, x_{pn}), p = 1, \dots, m \quad (12)$$

are given from  $M$  classes for an  $n$ -dimensional pattern classification problem.

In literature [38–40], the compatibility grade of each training pattern  $x_p$  with the antecedent  $A_q$  is defined by the product operation as  $\mu_{A_q}(x_p) = \mu_{A_{q1}}(x_{p1}) \times \dots \times \mu_{A_{qn}}(x_{pn})$ , where  $\mu_{A_{qi}}(\cdot)$  is the membership function of the antecedent fuzzy set  $A_{qi}$ .

The confidence of the fuzzy rule  $A_q \implies C_q$  is written as follows [39, 40]:

$$c(A_q \implies C_q) = \frac{\sum_{x_p \in \text{Class } C_q} \mu_{A_q}(x_p)}{\sum_{p=1}^m \mu_{A_q}(x_p)} \quad (13)$$

The confidence is a numerical approximation of the conditional probability. On the other hand, the support of  $A_q \implies C_q$  is written as follows [39–46]:

$$s(A_q \implies C_q) = \frac{\sum_{x_p \in \text{Class } C_q} \mu_{A_q}(x_p)}{m} \quad (14)$$

The support measures the coverage of the training patterns by  $A_q \implies C_q$ .

### 3.4.3. Heuristic methods for rule weight specification

While the determination of the consequent class, there are many ways to give weights to the rules [38–40]. In general, the consequent  $C_q$  of the fuzzy rule  $A_q \implies C_q$  in [38] is settled with the class who has the maximum confidence for the antecedent  $A_q$ .

$$c(A_q \implies C_q) = \max\{c(A_q \implies \text{Class } h) | h = 1, 2, \dots, M\} \quad (15)$$

The confidence  $c(A_q \implies C_q)$  can be used as the rule weight  $RW_q$  of the fuzzy rule  $A_q \implies C_q$ .

While a set of antecedent fuzzy sets is given for each attribute, the antecedent part of each fuzzy rule (i.e.  $A_q$ ) is defined with the combination of antecedent fuzzy sets for  $n$  attributes. In [36], it is seen that the confidence is directly used for each class for the fuzzy rule with multiple consequent classes [23].

$$RW_{qh} = c(A_q \implies \text{Class } h), h = 1, 2, 3, \dots, M. \quad (16)$$

The adaptation of generalized model with weighted fuzzy reasoning based on T-operators.

The steps are given below combined with FID3 reasoning based on T-operators:

**Step 1: Antecedent degree of a rule:** In this step, the strength of activation of the if-part for all rules handled from each path of the fuzzy decision tree in the RB with the pattern  $x_p$  is computed

$$\mu_{A_j}(x_p) = T\left(\mu_{A_{j1}}(x_{p1}), \dots, \mu_{A_{jn_j}}(x_{pn_j})\right) \quad (17)$$

where  $\mu_{A_j}(x_{pi})$  is the matching degree of the example with  $i$ th antecedent of the rule  $R_j$ , which is handled from a leaf node at the end of each path. T is a T-norm (listed in Table 1) and  $n_j$  is the number of antecedents of the rule.

**Step 2: Consequent degree for a class:** The consequent degree favor of class  $l$  by the rule  $R_j$  for the pattern  $x_p$  is computed as follows where  $RW_{jl}$  the weight is computed according to the multiple consequent classes (Eq. (16))

$$b_j^l(x_p) = T\left(\mu_{A_j}(x_p), RW_{jl}\right) \quad (18)$$

**Step 3: Confidence degree for a class:** In this stage, the confidence degree for the class  $l$  according to all rules in RB is computed. To obtain the confidence degree of a class, the association degrees of the rules of that class are aggregated by using conjunction operators, where  $T^*$  is a T-conorm (listed in Table 1) [2, 27].

$$conf_l(x_p) = T^*(b_1^l(x_p), b_2^l(x_p), \dots, b_R^l(x_p)) \quad (19)$$

where  $b_j^l(x_p)$ ,  $j = 1, 2, \dots, R$ , is the association degree of the pattern  $x_p$ , to the class  $l$ , according to the  $j$ .th rule.

**Step 4: Classification:** The class is obtained with the highest confidence degree assign as the predicted one [2, 27].

$$Class = \arg \max_{l=1, 2, \dots, m} (conf_l(x_p)) \quad (20)$$

#### 4. Experimental study on fuzzy rule-based approach to geographic classification of virgin olive oil using T-operators

In this section, fuzzy rule-based approach to geographic classification of virgin olive oil problem is summarized. And, the solution is given step by step. Then, we describe the experimental study. Firstly, the description of the olive oil samples and the methodology used in chemical analyses of olive oil samples are explained in detail. Secondly, we explain performance measure and statistical tests. Fuzzy reasoning methods with nonparametric operators are examined. The behavior of fuzzy ID3 weighted fuzzy reasoning method based on different

T-operators is observed. Then, the weighted and unweighted fuzzy reasoning methods based on different T-operators are compared.

#### 4.1. Olive oil samples

Olives were collected from certain trees of the cultivars, which were determined subject matter of this work: Ayvalik, Memecik, Kilis Yaglik, and Nizip Yaglik. The samples collected in 2002–2003, 2004–2005, and 2005–2006 harvest seasons. About 101 olive oil samples [47] were used for the experimental study. These samples were collected from different regions [North Aegean (33), South Aegean (53), Mediterranean (4), and South East (11)]. The detail information about the chemical analysis of the samples was given in pioneer studies [27, 47, 48]. PCA was applied in SPSS 20.0, partition coefficients and fuzzy *c*-means algorithm were handled in MATLAB 2015. The software is designed named as OliveDeSoft in the Visual C# for the experimental study (intel i7, 2.4 GHz, 4 Gb RAM) [48]. The data fuzzification process was applied by using fuzzy *c*-means (FCM). Partition coefficient determined the number of clusters [19, 20]. The calculated partition coefficient value for each cluster is given in former study [27].

#### 4.2. Performance measure and statistical tests

In former study [27], principal component analysis is performed on this data set in order to explore the data structure. It is seen that the geographic origin of virgin olive oils on the results handled from the chemical analyses are explained clearly. Yet one region (Mediterranean) has less data than the other regions, so it is not explained. The data implementation is done in IBM SPSS 20. The chemical measurements have fuzziness. So, we prefer to use fuzzy ID3 algorithm based on fuzzy logic for the classification in our study. In classical case, ID3 algorithm works with categorical variables. It is an advantage of fuzzy ID3 algorithm. This algorithm carries out numerical variables via fuzzy variables. Each numeric variable is converted to fuzzy variable. Fuzzy *c*-means algorithm is performed for the fuzzification. This proposed approach displays eight different T-operators in the reasoning procedure. The performances of standard fuzzy ID3 represented in [2, 27] and C4.5 [49] algorithms are examined in the experimental study. Leave one out validation procedure was performed for the performances measurement of the algorithms. Accuracy rate is preferred to test different methods [13]. In experimental study, threshold value for fuzzy decision tree is set to  $\theta_r = 0.75$ . Parameters of parametric operators are fixed as Yager  $p = 2$ , Hamacher  $p = 0.25$ , Dombi = 1, Dubois = 0.25, and Weber = 15 for fuzzy reasoning procedure. The comparison of the performances of unweighted and weighted fuzzy reasoning approaches is performed.

*Studying fuzzy reasoning method with nonparametric operators:* C4.5 algorithm also uses entropy as splitting criteria. It is the improved version of ID3 algorithm. It was presented by Quinlan in 1994 to work on the numerical data [27]. The performance of it is 86.14%. Then, it is observed that the performance of fuzzy ID3 algorithm with reasoning method in [2] is 86.14% too [27].

The performance results of nonparametric approaches given in **Table 2** shows that the result handled from three nonparametric operators have the same performance value with handled from C4.5 algorithm. Yet, the accuracy handled with Zadeh T-operators is smaller value with 82.18%.

Algorithms	Accuracy rate (%)
C4.5	86.14
FuzzyID3_reasoning with Weighted Product Sum_Umano	86.14
FuzzyID3_reasoning with Weighted T-Operators $T_1$ & $T_1^*$	82.18
FuzzyID3_reasoning with Weighted Product-Sum $T_2$ & $T_2^*$	86.14
FuzzyID3_reasoning with Weighted Non Parametric Hamacher ( $\lambda = 0$ ) $T_3$ & $T_3^*$	86.14

**Table 2.** The performance results of each algorithm for nonparametric operators [27].

*Study of the behavior of fuzzy ID3 weighted fuzzy reasoning method based on different T-operators:* We have made use of the Friedman aligned ranks as a nonparametric statistical procedure to discover statistical differences among a group of results for 20 threshold ( $\theta_r$ ) values in **Table 3**.

Algorithm	Rank	Friedman aligned ranks	
Zadeh	3.02		
Umano	6.40		
Product-Sum	6.80	Total N	20
Nonparametric Hamacher ( $\lambda = 0$ )	6.88	Test Statistic	76.396
Yager	3.55		
Hamacher	6.80	Degrees of Freedom	8
Dombi	2.90		
Dubois	3.22	Asymptotic Sig. (2 sided test)	0.000
Weber	5.42		

**Table 3.** Friedman aligned ranks for weighted Fuzzy ID3 reasoning based on different T-operators.

	Weber	Zadeh	Yager	Hamacher	Nonparametric Hamacher ( $\lambda = 0$ )	Product sum	Umano	Dubois
Dombi	0.128	1.000	1.000	0.000	0.000	0.000	0.002	1.000
Dubois	0.399	1.000	1.000	0.001	0.001	0.001	0.009	
Umano	1.000	0.004	0.036	1.000	1.000	1.000		
Product sum	1.000	0.000	0.006	1.000	1.000			
Non parametric Hamacher ( $\lambda = 0$ )	1.000	0.000	0.004	1.000				
Hamacher	1.000	0.000	0.006					
Yager	1.000	1.000						
Zadeh	0.201							

**Table 4.** The results of pairwise comparisons for weighted Fuzzy ID3 reasoning based on different T-operators with 20 different thresholds (range = 0.71–0.90) via adjusted significance values.

The results of pairwise comparisons for weighted fuzzy ID3 reasoning based on different T-operators [27] with 20 different thresholds (range = 0.71-0.90) via adjusted significance values are given in **Table 4**.

**Friedman aligned ranks** test shows that  $p$ -value is equal to zero. It means that there are significant differences among the results. Then, the pairwise comparisons are performed. The results are shown in **Table 4**. These nonparametric tests were performed in IBM SPSS 20.

*The comparison of the weighted and unweighted fuzzy reasoning methods based on different T-operators:* Accuracy rates handled for different thresholds within unweighted fuzzy reasoning method based on different T-operators are given in **Table 5**. It is seen that maximum value has Dombi T-operators handled for  $\theta_r = 0.85$  with 88.11%. As a result, it is observed that we can also reach better results by using different threshold values.

On the other hand, accuracy rates handled for different thresholds within weighted fuzzy reasoning method based on different T-operators are given in **Table 6**. It is seen that Umano

$\theta_r$	Zadeh	Umano	Product-sum	Nonparametric Hamacher ( $\lambda = 0$ )	Yager ( $p = 2$ )	Hamacher ( $p = 0.25$ )	Dombi (1)	Dubois (0.25)	Weber (15)
0.71	85.15	85.15	85.15	84.16	<b>85.15</b>	<b>85.15</b>	<b>85.15</b>	82.18	51.48
0.72	85.15	85.15	85.15	84.16	85.15	85.15	85.15	82.18	51.48
0.73	85.15	85.15	85.15	84.16	85.15	85.15	85.15	82.18	85.15
0.74	85.15	85.15	85.15	84.16	85.15	85.15	85.15	82.18	85.15
0.75	86.14	86.14	86.14	85.15	86.14	86.14	86.14	83.16	86.14
0.76	86.14	86.14	86.14	85.15	86.14	86.14	86.14	83.16	86.14
0.77	84.16	84.16	84.16	83.17	84.16	84.16	84.16	82.18	84.16
0.78	82.18	82.18	82.18	81.19	82.18	82.18	82.18	82.18	82.18
0.79	86.14	84.16	84.16	85.15	84.16	86.14	84.16	84.16	84.16
0.80	86.14	84.16	84.16	85.15	84.16	86.14	84.16	84.16	84.16
0.81	86.14	84.16	84.16	85.15	84.16	86.14	84.16	84.16	84.16
0.82	86.14	84.16	84.16	85.15	84.16	86.14	84.16	84.16	84.16
0.83	86.14	84.16	84.16	85.15	84.16	86.14	84.16	84.16	84.16
0.84	<b>87.13</b>	<b>87.13</b>	<b>87.13</b>	<b>86.14</b>	<b>87.13</b>	<b>87.13</b>	<b>87.13</b>	87.13	<b>87.13</b>
0.85	<b>87.13</b>	86.14	86.14	<b>86.14</b>	86.14	<b>87.13</b>	86.14	<b>88.11</b>	86.14
0.86	<b>87.13</b>	86.14	86.14	<b>86.14</b>	86.14	<b>87.13</b>	86.14	86.14	86.14
0.87	86.14	83.17	83.17	85.15	83.17	86.14	83.17	86.14	83.17
0.88	85.15	36.63	36.63	84.16	36.63	85.15	36.63	36.63	36.63
0.89	84.16	37.62	37.62	83.17	37.62	86.14	37.62	35.64	37.62
0.90	84.16	42.57	42.57	83.17	42.57	83.17	42.57	40.59	42.57
Ave.	85.54	77.97	77.97	84.46	77.97	<b>85.59</b>	77.97	77.03	74.60

Maximum values are given as bold.

**Table 5.** Accuracy rates handled for different thresholds (%) unweighted fuzzy reasoning based on different T-operators.

$\theta_r$	Zadeh	Umano	Product-sum	Nonparametric Hamacher ( $\lambda = 0$ )	Yager ( $p = 2$ )	Hamacher ( $\lambda = 0.25$ )	Dombi (1)	Dubois (0.25)	Weber (15)
0.71	81.19	85.15	85.15	85.15	84.16	85.15	79.21	83.17	85.15
0.72	<b>82.18</b>	85.15	85.15	85.15	84.16	85.15	78.22	83.17	85.15
0.73	<b>82.18</b>	85.15	85.15	85.15	84.16	85.15	78.22	83.17	85.15
0.74	<b>82.18</b>	85.15	85.15	85.15	84.16	85.15	78.22	83.17	85.15
0.75	<b>82.18</b>	86.14	86.14	86.14	<b>85.15</b>	86.14	78.22	<b>84.16</b>	<b>86.14</b>
0.76	<b>82.18</b>	86.14	86.14	86.14	<b>85.15</b>	86.14	78.22	<b>84.16</b>	<b>86.14</b>
0.77	80.20	84.16	84.16	84.16	83.17	84.16	81.19	83.17	84.16
0.78	79.20	82.18	83.17	83.17	79.21	83.17	81.19	80.20	80.20
0.79	81.18	84.16	85.15	85.15	79.21	85.15	81.19	81.19	80.20
0.80	81.18	84.16	85.15	85.15	79.21	85.15	81.19	81.19	80.20
0.81	80.20	84.16	85.15	85.15	79.21	85.15	81.19	81.19	80.20
0.82	80.20	85.15	85.15	85.15	79.21	85.15	81.19	81.19	80.20
0.83	80.20	85.15	85.15	85.15	79.21	85.15	81.19	81.19	80.20
0.84	80.20	<b>88.12</b>	<b>88.12</b>	<b>88.12</b>	80.20	<b>88.12</b>	<b>87.13</b>	83.17	81.19
0.85	80.20	87.13	87.13	87.13	80.20	87.13	86.14	82.18	81.19
0.86	73.27	85.15	85.15	85.15	78.22	85.15	82.18	82.18	81.19
0.87	72.28	36.64	36.64	66.34	76.24	36.63	36.63	35.64	76.24
0.88	75.25	36.64	36.64	36.64	74.26	36.67	36.63	35.64	76.24
0.89	75.27	35.64	35.64	35.64	76.24	35.64	34.65	32.67	78.22
0.90	74.26	41.58	41.58	41.58	77.23	41.58	38.61	38.61	78.22
Ave.	79.56	75.76	75.94	77.36	80.65	75.95	72.27	73.10	<b>81.74</b>

Maximum values are given as bold.

**Table 6.** Accuracy rates handled for different thresholds (%) weighted fuzzy reasoning based on different T-operators.

T-operators, Product-Sum T-operators, nonparametric Hamacher ( $\lambda = 0$ ), and Hamacher ( $\lambda = 0.25$ ) reached maximum accuracy rate for  $\theta_r = 0.84$  with 88.12%. While unweighted fuzzy reasoning based on Dombi T-operators ( $\lambda = 1$ ) was handled maximum accuracy rate for  $\theta_r = 0.84$  with 88.11%, weighted fuzzy reasoning based on Dombi T-operators ( $\lambda = 1$ ) reached 87.13% for  $\theta_r = 0.84$ .

The comparison of the performances between weighted and unweighted fuzzy reasoning based on different t-operators is done for each T-operator with Wilcoxon Signed Rank Test. It is seen that the performances of unweighted and weighted fuzzy reasoning based on Zadeh T-operators ( $p < 0.001$ ), Yager T-operators ( $p < 0.001$ ), Dombi T-operators ( $p < 0.001$ ), Dubois T-operators ( $p < 0.05$ ), and Weber T-operators ( $p < 0.001$ ) are significantly different.

If the average is taken for the performances of the T-operators with 20 different thresholds (range = 0.71–0.90), Hamacher ( $\lambda = 0.25$ ) has the maximum value with 85.59% for unweighted

fuzzy reasoning approach and Weber ( $\lambda = 15$ ) has the maximum value with 81.74% for weighted fuzzy reasoning approach.

## 5. Conclusion

Geographical classification of olive oil is an important topic. This topic has crucial manner for the human health from past to present. In addition, this topic is the main topic for the traceability of designation of origin olive oil. In pioneer study, we were interested in geographic classification system of olive oil. In accordance of this paper, chemical measurements were used for the experimental study. Chemical measurements contain imprecise information. In order to deal with imprecise information, fuzzy ID3 classifier was selected for the classification of olive oil samples. In addition, fuzzy ID3 reasoning method based on T-operators has been suggested. We made the experiments for the performances of proposed fuzzy reasoning method in order to solve geographic classification problem. In this paper, we propose weighted fuzzy reasoning approach based T-operators. Three nonparametric operators [Product-Sum\_Umano, Product-Sum, and Nonparametric Hamacher ( $\lambda = 0$ )] have the same performance value with handled from C4.5 algorithm. Yet, the accuracy handled with Zadeh T-operators is smaller value with 82.18%. Then, we have checked the performance of parametric operators. Statistical procedure was performed in order to detect statistical differences among a group of results for 20 threshold ( $\theta_r$ ) values. It is observed that there are significant differences among the results between unweighted and weighted fuzzy reasoning based approaches. It is seen that weighted fuzzy reasoning approach based on Umano T-operators, Product-Sum T-operators, Nonparametric Hamacher ( $\lambda = 0$ ), and Hamacher ( $\lambda = 0.25$ ) reached maximum accuracy rate for  $\theta_r = 0.84$  with 88.12%. So, we claim that by using different parameters and weights for each rule, we can handle better reasoning performances.

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## Author details

Suzan Kantarcı-Savaş<sup>1\*</sup> and Efendi Nasibov<sup>2,3</sup>

\*Address all correspondence to: [suzankantarci@gmail.com](mailto:suzankantarci@gmail.com)

1 Department of Econometrics, Kırklareli University, Kırklareli, Turkey

2 Department of Computer Science, Dokuz Eylül University, İzmir, Turkey

3 Institute of Control Systems, Azerbaijan National Academy of Sciences, Baku, Azerbaijan

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# Essential Oils: Market and Legislation

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Cinzia Barbieri and Patrizia Borsotto

Additional information is available at the end of the chapter

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## Abstract

This chapter provides an overview of the market for essential oils and describes its fundamental regulation in the European Union (EU). Both processes and trends in essential oils production, trade, and consumption are analysed. Growth of the market stems from consumer interest in 'naturals' associated with health. The market is also attractive to subsistence farmers of developing countries as the raw materials (plants and plant parts), for essential oils are generally obtained from small farms. In the EU, product categories operate under specific regulations to enhance product quality and to maintain market homogeneity and consumer protection. This chapter focuses on EU legislation of general interest to the essential oils industry and regulations inherent to flavourings for food, cosmetics, and additives for animal nutrition.

**Keywords:** essential oil, regulation, production, trade, consumption, chemical, labelling, food, cosmetics, flavouring, aroma, additive, animal nutrition, EU

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## 1. Introduction

This chapter provides an overview of the essential oils market and illustrates the main regulatory aspects for their primary uses in the European Union (EU). According to the European Pharmacopoeia [1], an essential oil is an 'odorous product, usually of complex composition, obtained from a botanically defined plant raw material by steam distillation, dry distillation, or a suitable mechanical process without heating'. The norm of the International Standard Organisation (ISO) ISO 9235:2013/Cor 1:2014 defines 'Aromatic raw materials—Vocabulary' [2] as a 'product obtained from a natural raw material of plant origin, by steam distillation, hydrodistillation, dry distillation, mechanical process...'. Both sources indicate that essential oils are distilled products, as opposed to extracts [3], liquid products obtained from plant parts (leaves, stems, bark, seeds, fruits, roots, and plant exudates), or, at times, may be produced by other

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processes, including solvent extraction. They are generally of complex composition and contain alcohols, aldehydes, ketones, phenols, esters, ethers, and terpenes in varying proportions. The oils are also called 'volatile or ethereal oils' for several reasons: they evaporate when exposed to heat in contrast to fixed oils; they are odorous and volatile compounds found only in 10% of the plant kingdom; and they are stored by plants in special brittle secretory structures (glands, secretory hairs, secretory ducts, secretory cavities, or resin ducts) [4–11]. Distillation is the most common method to isolate essential oils, but other processes—including *enfleurage* (extraction using fat), maceration, solvent extraction, and mechanical pressing—are used for specific products. The most suitable extraction method depends mainly on the raw material from which the oil is being extracted, for example, expression by mechanical or cold press is commonly used to extract essential oils from citrus peels. Younger plants produce more essential oil than older ones, but old plants are richer in more resinous and darker oils due to the continual evaporation of lighter oil fractions. The function of essential oil in a plant is not well understood. Flower odours are thought to aid natural selection by acting as attractants for particular insects, while leaf-, wood-, and root-derived oils may protect against plant parasites or depredations by animals. Oleo-resinous exudations that appear with tree trunk injury prevent sap loss and create a protective sealant against parasites and disease organisms. Few essential oils are involved in plant metabolism; in fact, some investigators maintain that many of these materials are simply waste products of plant biosynthesis [12]. Of the estimated 3,000 essential oils known, approximately 150 are of commercial importance currently and traded on the world markets [13–18]. While they are called oils, they should not be confused with 'fixed' or fatty oils that actually are lipid-based and may or may not be volatile. Indeed, essential oils differ chemically and physically from fixed oils. The essential oils produced predominantly for industrial purposes are those from orange, corn mint, eucalyptus, citronella, peppermint, and lemon [18]. The oils commonly used for domestic purposes include lavender, chamomile, peppermint, tea tree oil, eucalyptus, geranium, jasmine, rose, lemon, orange, rosemary, frankincense, and sandalwood. Essential oils constitute a major group of agro-based industrial products and have applications in various industries, such as food products, beverages, perfumes, pharmaceuticals, and cosmetics [19–25]. While aromatherapy represents a recent and growth niche for the market, their use is well established in the food, flavouring, and fragrance industries. The soft drink industry is the largest user of essential oils, especially those originating from *Citrus* oil. In fact, no 'cola' is produced without lemon or lime essential oils. Other prime users include alcoholic beverage makers, manufacturers of sweet, dairy, confectionary, and dessert products, as well as fast and processed foods, all in the food sector. Essential oils also have applications in other industries: pharmaceuticals, cosmetics, supplements, herbals, and feeds. This chapter describes EU legislation for essential oils used in flavourings, cosmetics, and additives in animal nutrition. From ancient times to today, essential oils have been recognised for their powerful natural plant products and medicinal value. They have been used as perfumes and food and beverage flavourings, even to heal the body and the mind for thousands of years [26–29]. The natural chemicals of essential oils operate via multiple modes of action as follows: antifeedant and repellent activities, moulting and respiration inhibition, growth and fecundity reduction, and cuticle disruption. They can function as contact, fumigant, repellent, antifeedant, and

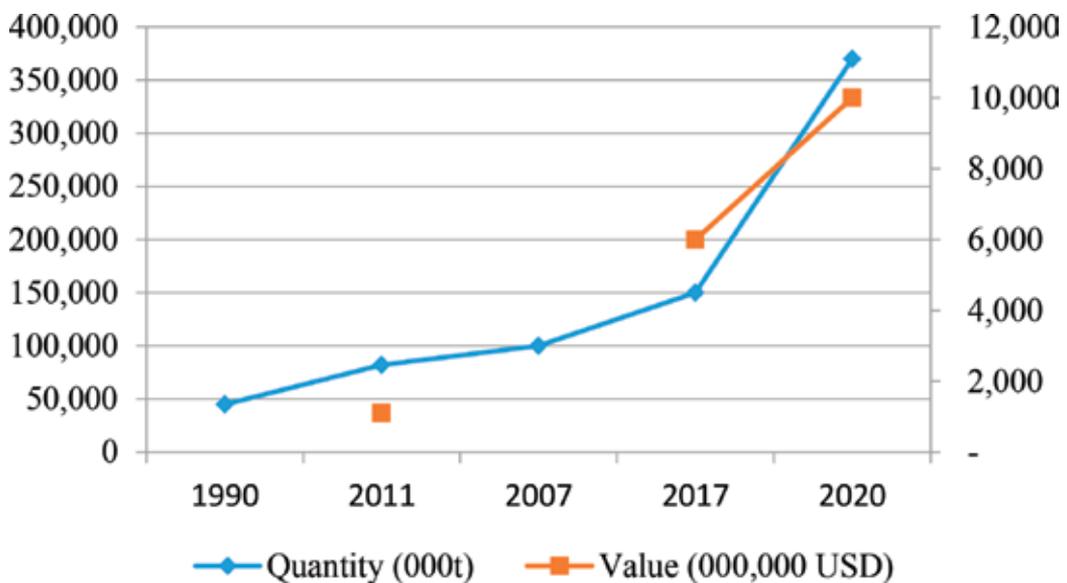
oviposition inhibition toxicants. Furthermore, essential oils have been widely used as anti-parasites, bactericides, fungicides, antivirals, and insecticides.

## 2. Markets for essential oils

The following section presents statistical data on essential oil production, trade, and consumption. Essential oils are industrial products. Available data, generally, are not specific to essential oils but are part of aggregated product data.

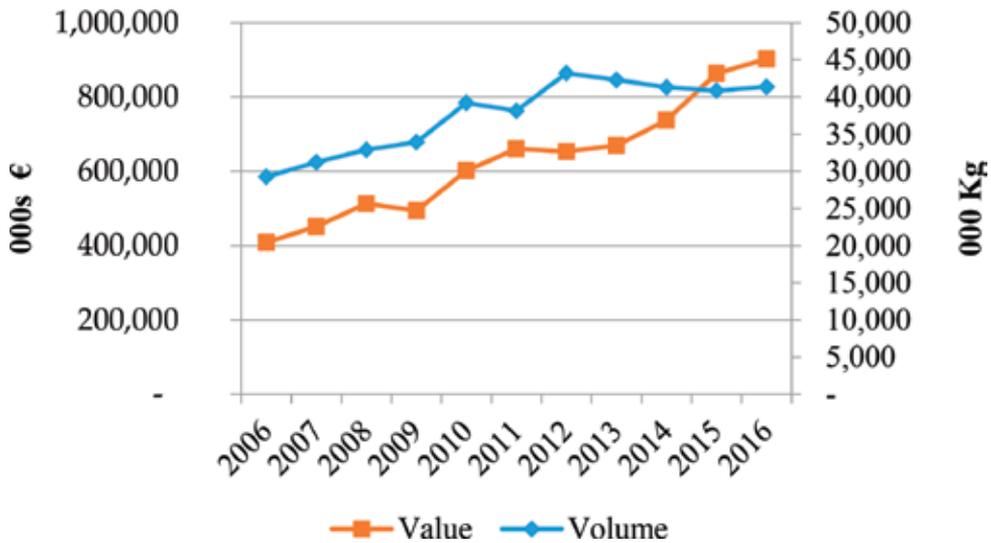
### 2.1. Production of essential oils

Estimation of essential oil world production and trade is fraught with difficulties [30]. In many countries, neither domestic production nor export statistics are recorded for some of the highest volume oils, while the rest are often buried in codes encompassing a range of products. Therefore, global production publications must be treated with caution and scepticism, as the information generally ignores domestic consumption and is frequently based on data from only a few countries. In reality, a wide range of countries from every continent are involved as producers. Worldwide essential oils production was estimated in 2017 (**Figure 1**) as more than 150,000 tonnes valued at about \$6B USD [31], which represents a tripling in volume since 1990 (45,000 tonnes), 50% of which has occurred since 2007. According to several economic analyses, growth will continue and by the 2020s is expected to reach 370,000 tonnes annually and be valued at more than \$10B USD (current dollars) [32, 33]. Essential oils are generally of



**Figure 1.** World production of essential oils (000 t; 000,000 USD). Source: EFEO, ISMEA.

complex composition and contain alcohols, aldehydes, ketones, phenols, esters, ethers, and terpenes in varying proportions. The major producers of essential oils across the world are China and India, followed by Indonesia, Sri Lanka, and Vietnam. Major essential oil-producing countries in Africa include Morocco, Tunisia, Egypt, and Algeria; the Ivory Coast, South Africa, Ghana, Kenya, Tanzania, Uganda, and Ethiopia play minor roles [34]. The North American continent is also a major essential oil producer. The United States (US), Canada, and Mexico all possess major natural aromatic plant materials, while Argentina, Paraguay, Uruguay, Guatemala, and the island of Haiti also make sizeable contributions to sector productions. Apart from the above mentioned major oil-producing countries, there are many others of less importance, such as France, Germany, Taiwan, Japan, Jamaica, and the Philippines. Many producers also come from developing country low-cost peasant-type operations and economies (65% of world production). The examples are numerous: orange derivatives produced in Brazil and China, corn mint in India and China, lemon in Argentina and Spain, Eucalyptus in China and India, peppermint in the US and India, citronella in China and Indonesia, sassafras in China, lime in Mexico, lavandin in France and Spain, and Patchouli in Indonesia and China. Key market drivers include rising consumer preference for natural products, growing popularity of relaxation therapies (spa, aromatherapy, flavourings, and fragrances), increasing disposable income from higher living standards, and growing awareness of chemical substance side effects. The excellent antibacterial and antiseptic properties and aromatic flavourings of essential oils have also boosted their overall popularity. Also of help to the market in general is the expectation that raw material prices, on which the essential oils market is highly dependent, are expected to be stable in the near future. Data from the European Federation of Essential Oils (E.F.E.O.) [3] indicates that world essential oils production covers about 600,000 ha of the 1.6 billion ha in agricultural production. Approximately 1 million farms are producers in the sector, which represents 0.06% of total farms in the world (1,600 million). The top three essential oil crops (orange, mint, and lemon), about 100,000 tonnes, represent more than two-thirds of the total essential oil crop production. A number of essential oils are produced on small farms or collected from forests: Patchouli, Litsea, Citronella, *Eucalyptus globulus*, Clove leaf (production range: 1,000–10,000 tonnes), as well as Vetiver, Geranium, Ylang Ylang, Nutmeg, Lavender (production range: 50–400 tonnes). Small farmers continue to dominate essential oils production and therefore make an important contribution to the local incomes of relatively poor rural populations in developing countries. Aside from the socio-economic importance to the producing communities, these crops play important environmental roles. Many are short- or long-term perennial crops that provide stable environments; cultivation of many relies on long-established and traditional varieties that act to balance the surrounding flora. In addition, wild crafted crops support the maintenance of natural vegetation and its flora and fauna complexes. The European market for essential oils continues to grow at a healthy rate, as demonstrated by the development of leading flavouring manufacturers. Most are based in Europe, where they produce flavourings for food and beverage manufacturers, both inside and outside Europe. According to Eurostat data, over the last decade, the EU has increased production of essential oils by 41% and more than doubled its value (**Figure 2**) [35]. As for the value trend, it is always rising, except for a 2009 drop. On the other hand, production volume has decreased since 2011, although as of 2016, a counter-trend has emerged and shown a slight rise. In fact, during 2006–2016, Germany increased its production more than 50%; similar trends occurred in Spain, Greece, and the UK. In 2016, total EU essential oils production was valued at €902 M and yielded about 41 million kg.



**Figure 2.** Essential oils production in the EU-28 (2006–2016; 000s €; 000 kg). Source: Eurostat.

Among EU member states overall, Germany consistently ranks as the top volume producer and in 2016 represented 46% of the essential oils produced (about 19 million kg) and 23% of their value. During this same decade, France made the largest gains in value by growing its sales by 90%. Over the same decade, Germany, Spain, and the US recorded even larger increases. In 2016, France was the top value producer and accounted for 34% of total EU-28 value; the country ranked second in quantity at 7 million kg. Other important European producers include Spain, the UK, and Italy (Table 1). For France, Spain and the UK, resinoides (€ 72M) deliver sizeable portions of both value and quantity production of these states, and concentrates do the same for France, Bulgaria, the UK, and Portugal.

## 2.2. World trade of essential oils

In 2016, the world exported \$4.38B USD in essential oils and import is \$4.54B USD (Figure 3) [36]. In rank order, these were the top exporters: the US (\$47B USD), Germany (\$28B USD), the UK (\$26B USD), and France (\$22B USD) [37], and the top importers were France (\$65B USD), the US (\$47B USD), Germany (\$41B USD), and Ireland (\$35B USD). As a group, the EU is the world’s biggest importer of essential oils, with France, Germany, and the UK being the major countries. Based on geography, Europe dominates the essential oils market, accounting for about 40% of world exports in 2016. The region is expected to maintain its pre-eminence throughout the analysis period due to growing demand for natural cosmetics, rising awareness, and increasing adoption of innovative essential oils in various sectors.

### 2.2.1. European imports

Over the five-year period from 2012–2016, the EU essential oils import values grew considerably to a peak (Figure 4), while import volumes remained relatively constant, which reflects a rise in unit price. Imports reached nearly 60,000 tonnes in 2016 to a value of €1.2B. Developing

Country	Essential oils		Resinoids		Concentrates of essential oils in fats... aqueous distillates, etc.	
	Value (000s €)	Volume (Kg)	Value (000s €)	Volume (Kg)	Value (000s €)	Volume (Kg)
Belgium	:C	:C	-	-	:C	:C
Bulgaria	34.413	158	:C	:C	7.412	1.022
Czech Republic	2.378	54	-	-	:C	:C
Denmark	523	18	-	-	1.052	165
Germany	206.437	18.907	:C	:C	:C	1.718
Estonia	-	-	-	-	-	-
Ireland	:C	:C	-	-	-	-
Greece	1.201	216	-	-	-	-
Spain	115.170	5.760	16.022	4.767	3.560	758
France	340.602	7.005	27.685	634	14.932	2.484
Italy	53.662	3.638	5.968	96	1.109	102
Cyprus	-	-	-	-	-	-
Latvia	-	-	-	-	-	-
Lithuania	2	0	-	-	13	34
Luxembourg	-	-	-	-	-	-
Hungary	2.961	88	-	-	-	-
Malta	-	-	-	-	-	-
The Netherlands	:C	:C	:C	:C	:C	:C
Austria	26.027	594	-	-	:C	:C
Poland	3.699	:C	:C	:C	:C	:C
Portugal	502	56	-	-	4.256	344
Romania	:C	:C	-	-	-	-
Slovenia	:C	:C	-	-	-	-
Slovakia	:C	:C	-	-	:C	:C
Finland	-	-	-	-	-	-
Sweden	-	-	-	-	-	-
The United Kingdom	98.815	3.620	10.962	CE	4.809	:C
Croatia	1.836	1	-	-	282	22
<b>EU28</b>	<b>902.439</b>	<b>41.359</b>	<b>72.410</b>	<b>6.034</b>	<b>80.000</b>	<b>7.525</b>

:C The data is confidential.

**Table 1.** EU-28 production of essential oils, resinoids and concentrates of essential oils (2016; 000 Kg; 000s €). Source: Eurostat.

countries played a relatively large and growing role by supplying Europe with 45% of its total imports. Imports from such countries represented a 2% increase as opposed to decreases of 14% and 11% from other European and non-developing countries, respectively. Of the trend in

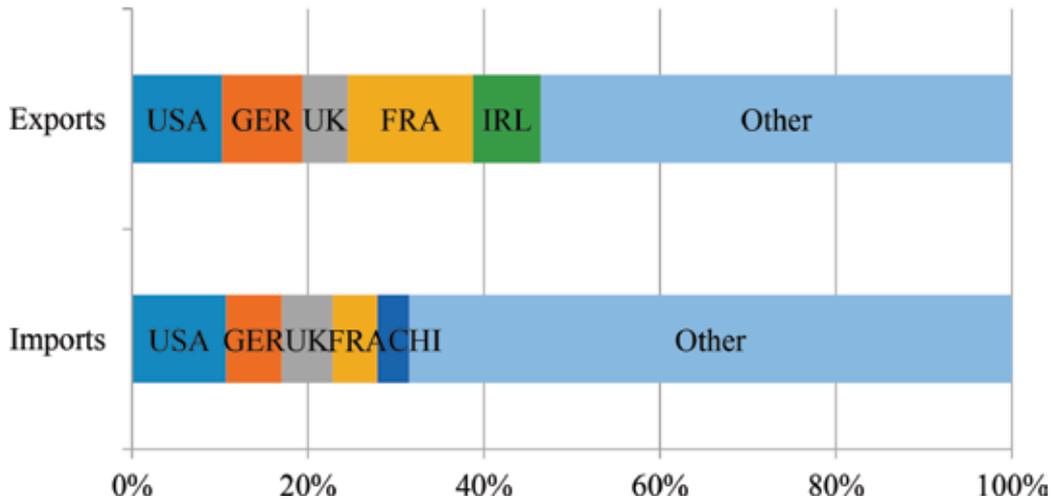


Figure 3. Import and export of essential oils in the world (2016, % value). Source: COMTRADE.

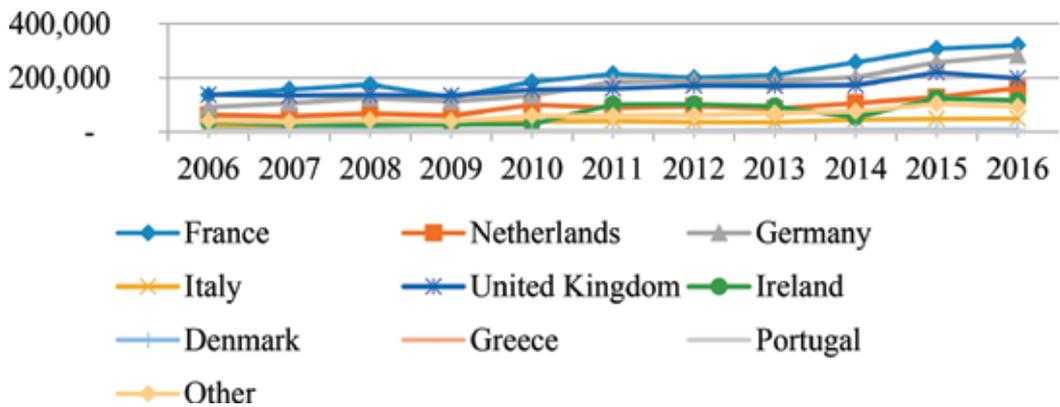


Figure 4. Import of essential oils in the EU-28 (2006-2016; 000s €). Source: Eurostat.

the EU-28, major importers (France, Germany, and the UK) all trended lower in 2012–2016, with the UK falling a remarkable 10%. The same negative trend was exhibited by Spain, Portugal, Denmark, and Ireland. The most recent year of records indicate that Greece and the Netherlands increased their import of essential oils by 27% and 35%, respectively. As mentioned earlier, for the decade ending in 2016, Germany, the Netherlands, the UK, and France were all major volume importers in Europe (Figure 5). Top ranked by volume, Germany is both a major and a stable importer of orange, peppermint, and other mint oils commonly used in the food industry. As was true for Europe generally, the value of orange oil imports by Germany grew substantially (14% annually), according to COMTRADE data. The increase resulted from price rises of orange oil after a drought in Brazil, which is the world’s principal

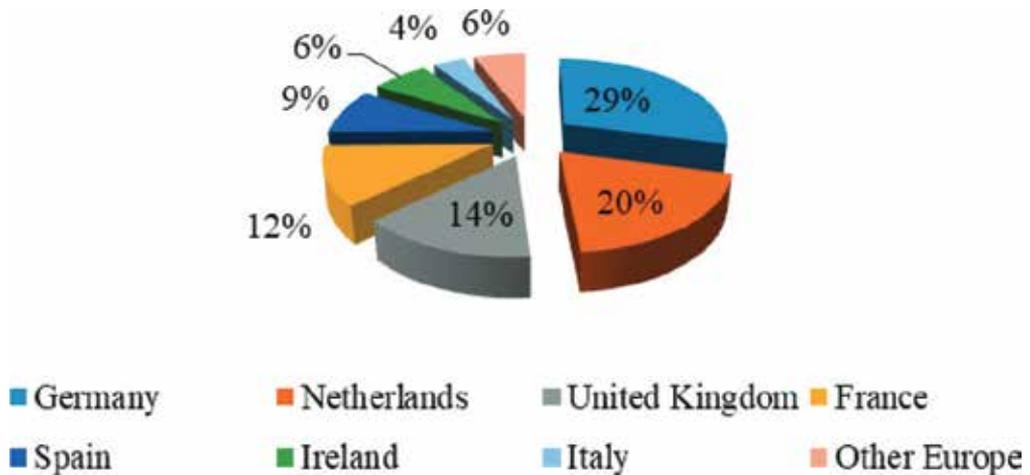


Figure 5. Leading European importers of essential oils (2016, % value). Source: Eurostat.

supplier. The Netherlands is among the leading importers of orange and citrus oils, which offers opportunities for developing countries. The UK plays a large role in the import of oils from other citrus fruits, as well as peppermint and other mints. Developing countries have benefitted most from the UK's consumption of other citrus fruit oils as its growth has been the strongest among citrus in general, and these evolving nations have satisfied half of UK demand. In 2016, France accounted for sizeable shares (12% and 24%) of total EU essential oils import volume and value, respectively. France is a major importer of oils that fall under the HS code 'not elsewhere specified' (nes), which are higher-value oils used in cosmetics. This product group includes a wide range of oils, such as rosemary, *Litsea cubeba*, tea tree, ylang-ylang, and chamomile. The French market is particularly appealing for the specialty oils used in the cosmetics sector and acts as a hub for the industry across Europe. On the contrary, some European countries mainly act as re-exporters and can be useful for getting a producer's essential oils into the European market.

### 2.2.2. European exports

EU exports reached 41,000 tonnes in 2016, with an average annual 22% increase since 2012. Export value also rose, on average 12% annually, during the same period to approximately €1B in 2016. **Figure 6** indicates the export value of essential oils (both terpeneless and not) from Europe to various regions between 2006 and 2016; **Figure 7** highlights essential oils export volumes from Europe in 2016 alone. The largest European exporters in 2016 were France at 29% by value and the following countries by volume: 26% for the Netherlands, 13% for the UK, 12% for Germany, and 10% for Italy. Together, these countries accounted for 74% of the 2016 European export volume. The 5-year data revealed different European exporters of essential oils (both terpeneless and not) exhibited different trends. While many nations began to trend up in 2009, the Netherlands did not begin to ascend until 2012. On the contrary, Spain and the UK actually decreased in the fifth year. These trends can be explained by the fact that some of the

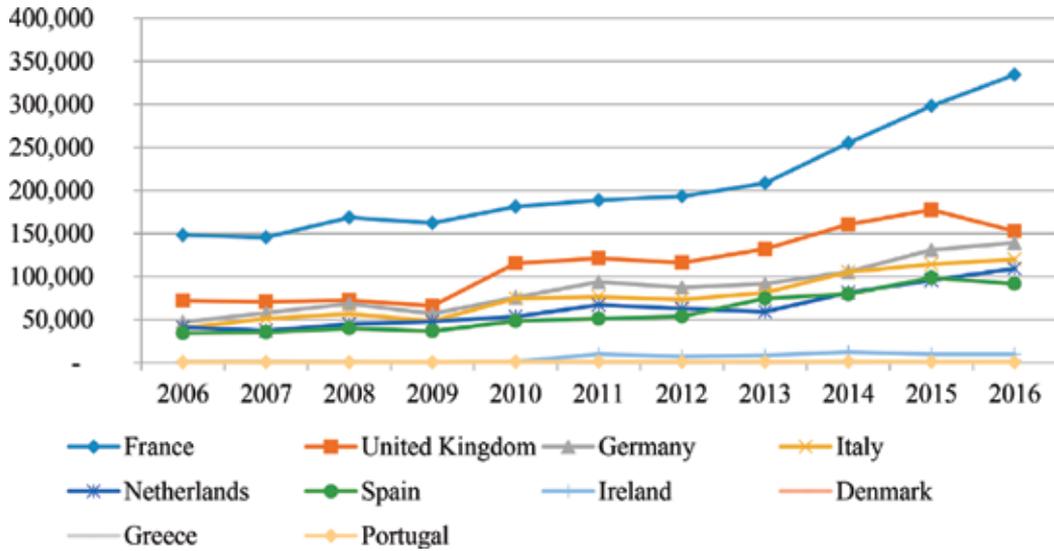


Figure 6. Export of essential oils of the EU-28 (2006-2016; 000s €). Source: EUROSTAT.

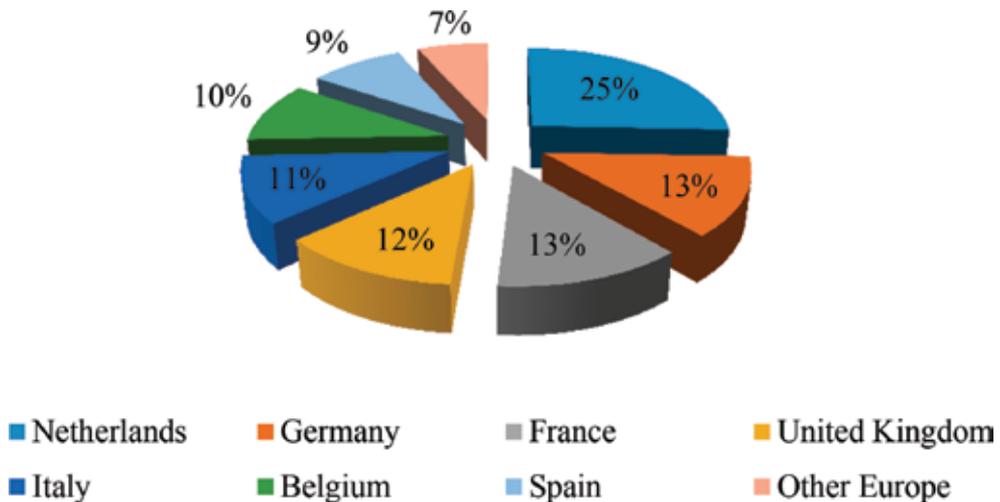


Figure 7. Leading European exporters of essential oils (2016, % value). Source: Eurostat.

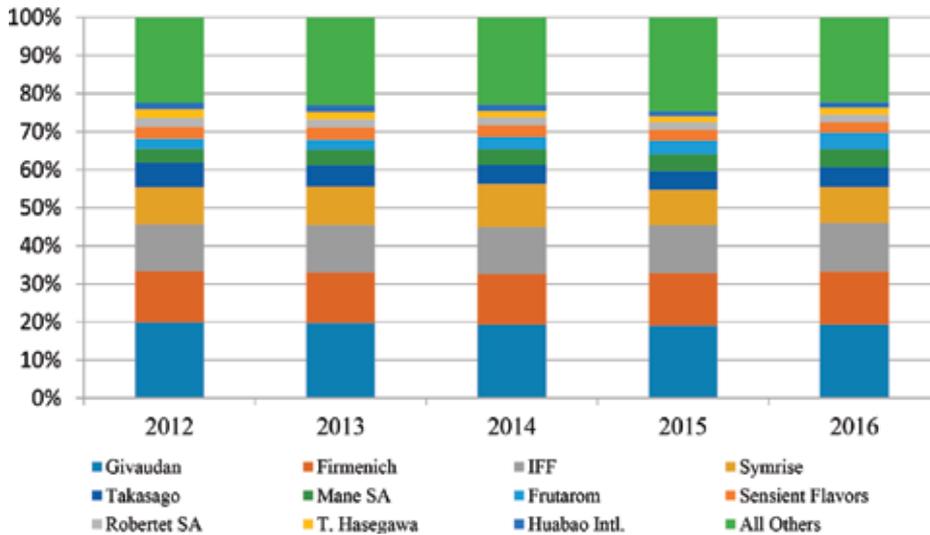
countries act mainly as re-exporters (Germany and the Netherlands) who get essential oils to European markets, while others are producers (France and Italy). Of the suppliers, France is the leading supplier of both domestically produced and re-exported high-value essential oils. In recent years, developing countries have played the largest role in essential oils that have limited production in Europe. These include orange (especially, large volume suppliers), 'other mint oils', and 'nes' essential oils. Competition from European sources may be limited for these products, but European producers have many other products for which they can otherwise compete.

### 2.3. Demand for essential oils

There is a lack of data and information about market demand for essential oils. Demand comes principally from the following markets: food and beverage (35%), fragrances, cosmetics and aromatherapy (29%), household (16%), and pharmaceutical (15%) [3]. Food and beverage is the largest segment in terms of market share, in part due to the recognition that essential oils contain health benefits as the natural ingredients that impart the essence of the source from which they have been derived. Various essential oils used by manufacturers include oils from orange, lemon, and lime. Orange oil is mainly used in foods and beverages to impart a citrus flavour and freshness to the final product. As consumers have become increasingly aware of the health benefits of essential oils, preferences for food and beverage products containing these oils as additives have grown. The global essential oils market has also been driven by the growth in demand for natural and organic hygiene products due to expanding attention to health problems among consumers. Natural flavour and fragrance demand in cosmetics, perfumes, and thermal and relaxation applications are expected to fuel demand for essential oils. Essential oils and oleoresins are not only used in the food processing and industrial seasoning sector in particular but also are important in the flavouring and perfume industry. Globally, operating flavours and fragrance manufacturers are among the main buyers of essential oils. Their sales provide an indication of developments in their market and subsequent demand for essential oils. Between 2012 and 2016, global sales of flavour and fragrance manufacturers increased by 7% to €25B. The growing demand for natural cosmetics and natural flavourings is an important driver of this sales growth. The top 11 companies represent 78% of total sales; the three major flavour and fragrance manufacturers are Givaudan, Firmenich, IFF, which as a threesome account for 46% of all sales (**Figure 8**). In 2016, Frutarom and Mane, SA, continued to accelerate their growth through various acquisitions [38].

### 2.4. Consumption of essential oils

Consumers are becoming more attentive to their health. Therefore, food and beverage manufacturers are looking to replace unhealthy ingredients that improve the texture of food with natural thickeners. Growing consumer preference for natural products has led to the development of innovative applications in personal care and beauty products as well. Rapid industrialisation and increasing disposable consumer incomes are the other major factors driving essential oil production in the developing countries of China, India, Vietnam, and Thailand. The majority of consumers by country, according to the data provided by Directorate Marketing of the USDA, are in the US (40%), Western Europe (30%), and Japan (7%) [39]. Sales of essential oils are closely tied to consumer education—the more consumers understand how and why to use essential oils, the greater the demand and sales growth. Aromatherapy is a prime example of how market sales are influenced by sector operations. The aromatherapy market relies on both professional (aromatherapists) and personal (individual consumers) utilisation for its retail purchases. In a 2013 survey of trends in the US aromatherapy market, over 62% of professional therapists developed their own products for re-sale in addition to their professional use. Almost all aromatherapists are self-employed, running their own small practices, but many also play roles in the educational (40%) and retail/wholesale (26%) ends of



**Figure 8.** Development of the world’s leading flavour and fragrance manufactures. Source: Leffingwell & Associates.

the industry, which fragments the market into one comprising many small operators. This is reflected in typical purchase volumes, where significant percentages (43%) of oil purchases are in quantities from 0.5 to 1 L/year, as opposed to purchases of 50 L or drums by industry giants. Food flavouring manufacturers are increasingly interested in self-producing flavourings from natural ingredients to meet the demand. Essential oils are some of their most important natural ingredients, and the variety of available essential oils provides many opportunities to formulate new or improved natural flavourings for use in their products. The challenge for such manufacturers is to produce consistent natural flavourings. Compared to synthetic ingredients, essential oils vary more often in composition, which can affect processing characteristics and final product performance.

### 3. European Union legislation for essential oils

The relevant legislative rules on essential oils are referred to as ‘general regulations’ (known as REACH and CLP) and ‘specific regulations’. Analysed below are general aspects of the principle regulations pertaining to essential oils and those related to individual product categories considered in this chapter (flavourings for food, cosmetics, and additives for animal nutrition).

#### 3.1. General regulations for essential oils

REACH Regulation (Regulation (EC) No 1907/2006 of 18 December 2006) [40–44] concerns the registration, evaluation, authorisation, and restriction of chemicals (in short, ‘substances’). It explicitly references the ‘Sustainable Development Implementation Plan’ (Johannesburg World Summit), which foresees that by 2020 the production and use of chemicals will be such

as to minimise the harmful effects on human and environmental health, as required by the Strategic Approach to the International Management of Chemical Substances (SAICM), Dubai, 6 February 2006. REACH applies to all individual chemicals in preparations, contained in products manufactured or imported in annual quantities above a tonne. Of course, many compounds are excluded from the regulation: radioactive substances, waste, substances subject to customs control, as well as substances used in human or veterinary medicine, in animal foodstuffs, additives in foodstuffs for human consumption, flavourings for human consumption, additives for animal food, and additives for final preparations for end users of medicines, cosmetics products, and substances for which the available data indicate minimal risk exists. A broad category excluded from REACH are substances subject to customs control (in transit or re-export) transported by rail, road, ship, or air. Creation of the Regulation arose from multiple objectives: (1) to protect human and environmental health from the use of chemical products; (2) to encourage or guarantee replacement of dangerous substances with less risky substances or technologies, to incentivise transition to more technically or economically viable alternatives; (3) to instil manufacturers and importers with the responsibility of understanding and managing the risks associated with the use of these substances; (4) to allow free circulation of substances on the EU market through similar requirements in EU countries; (5) to improve competitiveness and foster innovation in the EU chemical industry; (6) to promote the use of alternative methods to assess the substance hazards and limit animal testing. To collect the data and then make it available and useful to interested parties, the Regulation established the 'European Chemicals Agency' (ECHA) to collect, manage, and control manufacturer and importer substance registration. Collected substance and use information may be accessed to assist with activities related to implementation EU rules on voluntary labelling or to guide the EU Commission when they are revising rules concerning such substances, voluntary instruments, or community trademarks. The registration process starts with an assignment of a presentation number (identifier comprising a number and date), plus payment of a tariff. It finishes when the registrant receives notification of registration for the same identifier. The Agency is obliged to notify the EU member state in which the manufacturing site (or registered office of the manufacturer) is located that the following are available in the Agency database: registration dossier, registration date, completeness check results, and other manufacturer-provided information. The manufacturer or importer must update the registration database as needed (e.g. changes in legal status, substance composition, labelling, etc.). The ECHA website [44] hosts all registered substances statistical data under three groupings 'Full Registration', 'Transported Isolated Intermediates' and 'On-site Isolated Intermediate', for the European Economic Area (EEA) that includes the EU member states, plus Iceland, Norway and Liechtenstein. The number of registrations from 1 June 2008 and forward ===== categorized as 'Detailed Registration Statics' - is 14,640, of which 75% are 'Full registrations' that represent 6,763 'Substances'. The difference between Registrations and Substances is due to "In one registration dossier any combination of one up to three different types can be covered". The bulk of registrations is made by large enterprises (85%), as compared to small and medium enterprises (SME). In terms of registrant, 43% of registrations are done by importers, 28% by a representative of a non-EU manufacturers, 24% by manufacturers, and 5% by manufacturers/importers. Essential oils are not among the substances most registered by EEA countries; rather, these lists contain the likes of ethanol, propane-1,2-diol, silicon dioxide, and so on. The

second 'general regulation' of interest for essential oils is known as Regulation Classification, Labelling, Packaging (CLP), Regulation (CE) N. 1272/2008 16 December 2008 on classification, labelling, and packaging of substances and mixtures [45]. The law became effective on 1 June 2015 and had several objectives as follows: (1) to determine if a substance or mixture has properties that classify it as dangerous; (2) to ensure high levels of human and environmental health protection; (3) to harmonise classification, labelling, and packaging criteria; (4) to facilitate free circulation of substances, mixtures, and articles; (5) to create a single set of rules to oversee the safe transport of these substances; (6) to produce a catalogue of substances from the classification and labelling of chemicals based on the classification of chemicals used by the United Nations. The regulation requires manufacturers, importers, and downstream users to classify, label, and package dangerous substances or mixtures before market placement, based on a 'self-classification' by manufacturers, importers, and other related parties. Comparison of information on the hazard(s) of a given substance/mixture to information on the dangers established by the criteria of the CLP Regulation results in a class and category danger assignment that correlates with the related physical, human, and/or environmental health hazard(s). Hazards must be communicated and maintained via appropriate labelling throughout the supply and production chain to market for substances classified as dangerous, mixtures containing one or more substances classified as dangerous beyond a specified threshold and for articles having explosive properties. Label elements—pictographs, warnings, and standard declarations of danger, prevention, reaction, storage, and disposal—are precisely defined for each hazard class and category. In addition, the name, address, and telephone number of the supplier are provided. Packaging must be such so as to prevent escape of the contents, not attract the attention or curiosity of children, and look similar to that typically used for food, animal feed, or cosmetics. Classification and labelling of certain hazardous substances are standardised across the EU (CLH) and are found in Annex 6 of regulation classifications. The following situations allow any manufacturer, importer, downstream user, or member state to propose standard classification and labelling: (1) when a substance is carcinogenic, mutagenic, or toxic to reproduction or respiration; (2) when classification of a substance is necessary at the European level for other hazardous classes; (3) when it is necessary to add one or more hazardous classes to an existing item. Manufacturers and importers are fully responsible to keep current any classification and labelling information for substances they place on the market in a specific C&L inventory maintained by ECHA. The regulation is also the basis for legislation that manages chemical risks.

### **3.2. Regulations inherent to some uses of essential oils**

The following paragraphs describe the principal contents of regulations inherent to flavourings, cosmetics, and additives for animal nutrition. In general, regulations aim to protect consumer health and to improve the free circulation of stuffs in the EU market through common rules.

#### *3.2.1. Flavourings for food*

Regulation (EC) No 1334/2008 of 16 December 2008 is the centrepiece legislation 'on flavourings and certain food ingredients with flavouring properties for use in and on foods' [46].

It aims to protect human health and to allow free movement of food on the EU internal market. For regulation purposes, 'flavouring' is a product that is not consumed as such, but is added to food to modify its taste or aroma and is manufactured or derived from substances of plant, animal, or microbiological origin. 'Natural flavourings' are substances normally present in nature; they fall outside the scope of the legislation in the following cases: raw foods, substances with sweet, sour, or salty tastes, and mixtures like spices or herbal teas. Instead, the regulation targets flavourings used in and on foods, flavouring food ingredients, foods containing flavourings, and the basic materials for their preparation. According to Article 4 of the regulation, "flavourings/flavouring ingredients may be used only if they do not present a risk to the health of the consumer on the basis of the available scientific data and if their use does not mislead the consumer". The safety requirement demands that a risk assessment be made prior to the use as envisaged; a complementary section of the regulation, Article 8, indicates the flavouring aromas and food ingredients excluded from risk assessment and authorisation. Risks are assessed under a Commission of the Food Safety Authority (EFSA). Aromas and base material flavourings that are evaluated and authorised by the Commission are added to the EU list that allows their market entry as such or for food use. Articles 14, 15, 16, and 17 specify label standards for intermediate users and end-users sales. For market placement for intermediate users, the package or container must display aroma, food, name, company name, and address of the producer, while flavourings for final consumer use can be placed on the market only if 'for food' is present on the label, easily visible, legible, and indelible. When the product is 'natural', additional provisions apply. Finally, Article 20 of the regulation expects member states to create monitoring systems for flavouring consumption use and information collection to the Authority and Commission.

### 3.2.2. *Cosmetics*

The foundational legislation for the cosmetics sector is Regulation (EC) No 1223/2009 of 30 November 2009 [47, 48]. Essential oils are contained in many cosmetics (e.g. creams or body care products). Rules regulating the use of essential oils in cosmetics aim to balance EU market placement while considering the technological innovation in the sector. Another objective is to guarantee high quality to protect the health and safety of consumers. The regulation became effective on 1 July 2013. It contains many essential aspects: 'Basic definition set' (cosmetics, substances, manufacturers) and of the Responsible Person; specifications for labelling and advertising claims; indications for centralised notification; rules concerning animal testing; manufacturing standards (GMP); responsibilities of member states for market surveillance; indications relating to carcinogenic, mutagenic and toxic for reproduction (CMR) substances and to nano-materials. The Responsible Person (natural or legal person) is obligated to meet the regulatory requirements for each cosmetic product on the market. The individual is held to both technical and health responsibilities and must submit all security documentation from Information Documentation to Safety Reports (Annex I of the Regulation) to Safety Assessments. In addition, organised 'surveillance of cosmetics' and health authority reporting must be established. There must be a guarantee of traceability to identify the links within the supply and distribution chain for three years following the date of distribution availability. If a

cosmetic presents 'non-conformities', the Responsible Person must make the cosmetic compliant, withdraw it from the market, or recall it from all member states where it is available. The label must be void of words or other means that suggests characteristics or functions the product does not possess. The label must include the company name and address of the Responsible Person; country of origin of imported products; nominal content for packaging by weight or volume; expiration date for products properly stored; precautions for use; the manufacturing lot number or reference allowing identification of the product; ingredient list, substances, or mixtures used intentionally during manufacture. In terms of product claims, the regulation indicates that the Commission should define an 'Action Plan' to standardise the declarations applied to cosmetics and that it should set common criteria for the use of those declarations. A centralised information source provides distributor names, label dispatch, and product photographs and creates a master registration list for all EU member states. Animal product testing is under replacement in the EU with alternative methods. Animal testing is already prohibited for finished products, ingredients, and combinations of ingredients. Consequently, EU cosmetics market placement of products with formulation or ingredients or combination of ingredients is not permitted. Member states are responsible for verifying the application of manufacturing principles through ISO 22716 'Guidelines on Good Manufacturing Practices' (GMP). Member states must also monitor the market with information provided by sector operators, random testing, and ingredient lists aimed at allergic sensitivities, in particular.

### *3.2.3. Additives for animal nutrition*

There are two regulations governing additives for animal nutrition: one is Regulation (EC) No 1831/2003 of 22 September 2003 on additives for use in animal nutrition and the other is Regulation (EC) No 429/2008 of 25 April 2008 that details the rules for the implementation of the former regulation (Regulation (EC) 1831/2008) [49–51]. Article 2 of the regulation defines feed additives as "substances, micro-organisms or preparations, other than feed and pre-mixtures which are intentionally added to animal feed or water in order to perform, in particular, one or more of the functions". Five different categories of additives are identified: technological, organoleptic, nutritional, zoo-technical, coccidiostatic, and histomonostatic. The first three categories include 'functional groups' as highlighted in Annex I of the regulation itself. It clearly states that as of 1 January 2006, antibiotics are prohibited as feed additives and that only additives authorised according to Regulation 1831/2003 and its specific Authorisation Regulation can be placed on the market, used, and transformed. The regulation generally limits market readiness or use of pre-mixes, defined as "mixtures of feed additives or mixtures of one or more feed additives with feed materials or water, used as a carrier, not intended for direct feeding to animals" in the same way as described above. Additives mixtures do not need authorisation under the following circumstances: the operator complies with the requirements of Regulation (EC) No 183/2005, the additive mixture is produced only by authorised additives, and the conditions of use foreseen for each single additive are respected; the chemical, physical and biological compatibility between mixture components is ensured relative to the expected effects. The regulation also provides indications regarding labelling (Article 16). Producers and/or those who place additives and pre-mixtures of additives on the market must be

registered or recognised per Regulation (EC) 183/2005 guidelines. Finally, it should be noted that the authorisation for a new additive (Article 4) or for a new use requires submission of an application to the European Commission—Directorate General for Health and Consumer Protection (DG SANTE), that demonstrates compliance with paragraph 2 of Article 5 and at least one of the characteristics named in paragraph 3 of Article 5. Regulation (EC) No 429/2008 contains preparation and presentation rules for additive application, evaluation, and authorisation. Completed applications are forwarded to the Commission, which sends it to EFSA and to the VII-DGSAF Office of the Ministry of Health for evaluation by the member state Ministry of Health and Community Laboratory. At the end of the evaluation, EFSA issues an opinion (Article 8), which is sent to the EU Commission for preparation of an *ad hoc* regulation. Each authorised additive is entered in a community register with authorisation date, expiration date, and the relative Authorisation Regulation.

## 4. Conclusions

The first section of this chapter describes, within the limitations of the data, the fundamental aspects of the market for essential oils. *Production* is estimated to reach more than 150,000 tonnes or about \$6B USD in 2017 across the world. The main producers, by continent are China and India (Asia), Morocco and Tunisia (Africa), the USA, Canada, Mexico (Americas), and France and Germany (Europe). As a consequence of growth in consumer interest in natural and healthy living, of a rise in income in developing nations of the world, and of an increase in knowledge and use of essential oils (aromatherapy, spa therapy, and more), demand is expected to expand by 2020. Production often occurs on rural area farms (about 1.6 million farms, 0.6% of the world in total). *World trade* data values exports in excess of \$4B USD and imports above \$4.5B USD in 2016. Europe accounts for about 40% of world exports and it is expected to maintain this market share in the coming years due to the demand for essential oils in various sectors. At the country level, the US, Germany, the UK, and France are the main exporters. The latter three countries are also the main world importers. *Demand* for essential oils worldwide is driven first by companies producing flavourings and fragrances. Growing sales of flavourings and fragrances indicate future demand increases for raw materials (essential oils). The three main companies in the flavouring and fragrance sector are Givaudan, Firmenich, and IFF; combined, they represent 46% of total sales. Consumption is driven by the same factors described above that drive production. The following countries consume the most essential oils in the world: the US (40%), Western Europe (30%), and Japan (7%). The second section of the chapter examines the principal EU regulations governing essential oils. General regulations, known as REACH and CLP, refer to chemical products, which also affect essential oils. EU legislation for specific product uses (flavourings for food, cosmetics, additives for use in animal feed) also relates to essential oils. REACH and CLP concern the registration, evaluation, authorisation, and restriction of chemicals (essential oils included). They have many goals: to protect human and environmental health from the use of chemical products; to instil manufacturers and importers with the responsibility of understanding and managing the risks associated with the use of these substances; to allow free circulation of

substances in the EU market through similar requirements in EU countries; to improve competitiveness and foster innovation in the EU chemical industry; to promote the use of alternative methods to assess the substance hazards and limit animal testing. All substances subject to regulation (waste excluded) must be registered and authorised, as described by these regulations. The European Chemical Agency, established by REACH, oversees application and authorisation for registry admission and ultimate entry into the marketplace. CLP regulates substance and mixture classification, labelling, and packaging. It determines if the properties of a substance or mixture are dangerous. It also ensures high levels of human and environmental health protection, a standard classification schema, labelling and packaging criteria for EU member states, and a catalogue of substances consistent with chemical classification used by the United Nations. Pre-market entry, dangerous substances, and mixtures must be 'self-classified' by manufacturers, importers, and other related parties (downstream users) into a class and category of danger correlated with the related physical, human, and/or environmental health hazard(s). This regulation also details precise information and pictograms for substance/mixture label placement. Specific regulations for flavourings/flavouring ingredients are limited for those not of risk to consumer health on the basis of available scientific data and as long as their use does not mislead the consumer. Risks are assessed under a commission of the Food Safety Authority (EFSA) and enter into an EU list that allows their market entry as such or for use in food. These specific regulations also lay out label standards for intermediate and end-user sales. *Cosmetics-related* legislation aims to guarantee high quality and the health and safety of the consumer. It establishes the concept of a Responsible Person to submit all security documentation, safety reports, and safety assessments. Moreover, the Responsible Person must guarantee product traceability and market surveillance. The regulation also specifies label and advertising claims, rules on animal testing, manufacturing standards (GMP), indications relating to carcinogenic, mutagenic and toxic for reproduction (CMR) substances, and nano-materials. *Additives for animal nutrition* are the most important contents of the related regulation that applies 'only if the additive is authorised according to Regulation (EC) No 1831/2003 and the specific Authorisation Regulation, respecting the conditions set by this rule, can it be placed on the market, used and transformed'. The EFSA is the authority who manages the authorisation under the second principal regulation of interest for the examined products, Regulation (EC) No 429/2008 on "preparation and presentation of applications, evaluation, and authorization of additives".

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## Conflicts of interest

The authors have no conflicts of interest to declare.

## Notes

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## Author details

Cinzia Barbieri<sup>1\*</sup> and Patrizia Borsotto<sup>2</sup>

\*Address all correspondence to: [cinzia.barbieri@unito.it](mailto:cinzia.barbieri@unito.it)

1 Department of Agricultural, Forest and Food Sciences, University of Torino, Torino, Italy

2 Council for Agricultural Research and Analysis of Agricultural Economics, Centre of Policies and Bio-Economy, Torino, Italy

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# Essential Oils and Factors Related to Cardiovascular Diseases

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Geun Hee Seol and You Kyoung Shin

Additional information is available at the end of the chapter

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## Abstract

Cardiovascular diseases (CVDs) are the leading cause of mortality and a major economic burden worldwide. Various drugs, including antihypertensive, antihyperlipidemic, and antiplatelet agents, are prescribed to treat CVDs, but these agents have side effects, including serious side effects such as bleeding. Therefore, efforts are being made to develop new drugs made of natural substances with relatively weak side effects. Essential oils are natural substances extracted from aromatic plants with biological effects, such as antioxidant and antiinflammatory activities. These oils have therefore long been used in traditional medicines. This chapter reviews the effects of essential oils on CVD-related factors. Essential oils have various effects, including improvements in lipid balance, liver function, and endothelial function; reductions in blood pressure, oxidative stress, thrombosis, and inflammation; promotion of vascular relaxation; and inhibition of diabetes development and angiogenesis. Therefore, essential oils and their active components may be promising therapeutic agents for CVDs. Further studies are needed to clarify their clinical effects and to elucidate their specific mechanisms of activity.

**Keywords:** essential oil, cardiovascular disease, dyslipidemia, hypertension, endothelial dysfunction

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## 1. Introduction

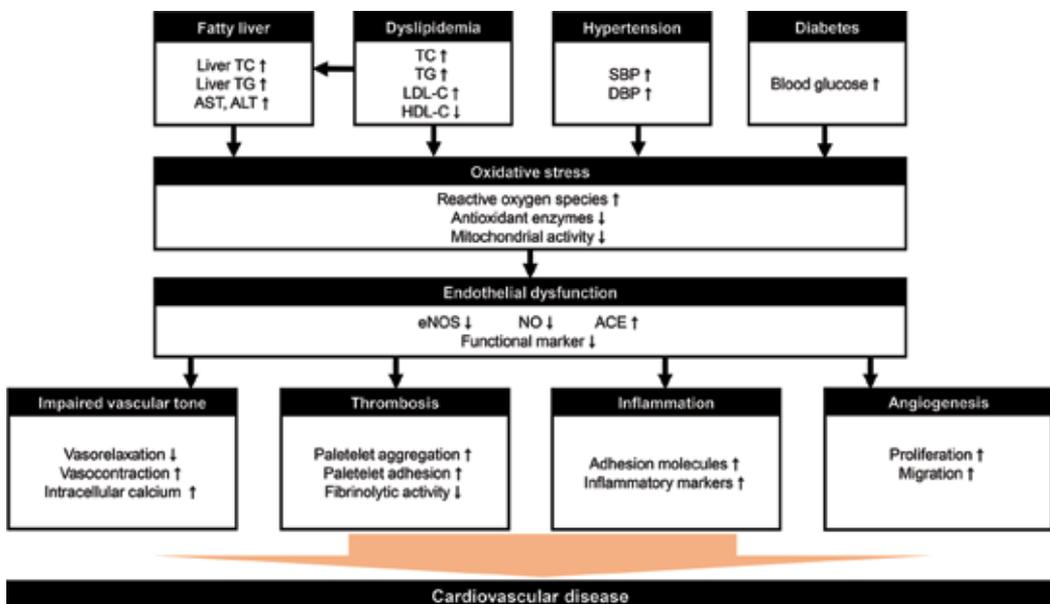
Cardiovascular diseases (CVDs) are considered the leading cause of death worldwide. CVD-related deaths accounted for 31.5% of all global deaths in 2013 [1]. In 2010, the global economic burden of CVDs was 863 billion dollars, which was estimated to increase to 1044 billion dollars by 2030 [2]. Because the incidence of CVDs increases with age [3], the aging of society is expected to increase problems caused by CVDs. Traditional risk factors for CVDs, including

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dyslipidemia, hypertension, and diabetes, cause oxidative stress. Fatty liver due to hyperlipidemia is also associated with increased lipid peroxidation [4]. Oxidative stress impairs vascular endothelial function, which is characterized by reduced nitric oxide (NO) bioavailability. Vascular dysfunction contributes to the impairment of vascular tone, characterized by a decrease in endothelium-dependent vasorelaxation [5]. Because endothelium-derived NO inhibits leukocyte adhesion, which causes inflammation [6] and inhibits platelet adhesion [7], endothelial dysfunction can lead to vascular inflammation and thrombosis. Activated platelets have been shown to increase inflammatory responses and the migration of vascular smooth muscle cells (SMCs) [8] (**Figure 1**).

Various drugs are prescribed to prevent and treat CVDs. For example, aspirin, clopidogrel, statins, beta-blockers and angiotensin converting enzyme (ACE) inhibitors are recommended for vascular protection in patients with stable angina [9]. Moreover, aspirin and statins have been reported to reduce the risks of atherosclerotic CVDs by 10 and 15%, respectively [10]. These drugs, however, have side effects [9]. For example, aspirin has been found to increase the risk of bleeding by 54% [10]. New drugs made of natural products with fewer side effects are therefore needed.

Essential oils are natural substances extracted from various organs of aromatic plants. Because these oils have pharmacological effects, they have been widely used in traditional medicines since the Middle Ages [11]. Studies have shown that essential oils and their main components have various biological properties in relation to CVDs. For example, neroli essential oil showed vasorelaxant activity, mediated by the NO-soluble guanylyl cyclase pathway and by ryanodine receptors, in mouse aortic rings [12]. Bergamot essential oil also induced



**Figure 1.** Flow chart showing the mechanisms of CVDs. Various risk factors increase levels of oxidative stress, thereby increasing endothelial dysfunction. Endothelial dysfunction, in turn, promotes abnormalities, such as impaired vascular tone, thrombosis, inflammation, and angiogenesis, which lead to CVDs.

vasorelaxation by inhibiting  $\text{Ca}^{2+}$  influx into mouse aortic rings [13]. The compound 1,8-cineole, a main component of eucalyptus essential oil, showed antioxidative and antihypertensive effects in chronic nicotine-induced hypertensive rats [14]. These findings have led to efforts to determine the efficacy and specific mechanisms of action of essential oils on CVDs. This review therefore describes the results of studies assessing the effects of essential oils on CVDs and provides new perspectives on future drug development using essential oils.

## 2. Effects of essential oils

The effects of essential oils on CVDs are summarized in **Table 1**.

### 2.1. Dyslipidemia

Dyslipidemia is a major risk factor for CVDs. Reductions in low density lipoprotein cholesterol (LDL-C) levels have been associated with decreased risks of major vascular events [53], whereas elevated triglyceride (TG) level and total cholesterol (TC)/high density lipoprotein cholesterol (HDL-C) ratios have been associated with increased risks of coronary heart disease, regardless of LDL-C levels [54]. Essential oils have been shown to improve blood lipid levels. For example, *Allium cepa* and *A. sativum* essential oils were found to reduce serum cholesterol and serum TG levels and  $\beta/\alpha$  lipoprotein ratios in cholesterol-fed rabbits, suggesting that these oils have anti-atherosclerotic properties [16]. *Dendropanax moribiferus* essential oil also showed antiatherogenic activity by reducing plasma TC, TG, and LDL-C levels and by increasing plasma HDL-C levels in high-cholesterol fed rats [26]. Similarly, *Syzygium aromaticum* essential oil significantly improved dyslipidemia by reducing plasma TC, TG, and LDL-C levels and by increasing plasma HDL-C levels in rats with metabolic syndrome induced by a high fructose diet [4]. Supplementation of a hyperlipemic diet with *Oenothera biennis* essential oil reduced plasma TG and TC levels and increased plasma HDL-C levels [43, 44], and intake of *Linum usitatissimum* L. essential oil reduced blood TC, TG, and LDL-C levels, in patients diagnosed with metabolic syndrome [34].

Efforts have been made to determine the specific mechanisms by which essential oils improve lipid metabolism. *Curcuma longa* L. essential oil improved dyslipidemia in hyperlipidemic rats by modulating the expression of peroxisome proliferator-activated receptor- $\alpha$ , liver X receptor- $\alpha$ , sterol regulatory element-binding protein (SREBP)-2, 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) and genes involved in lipid regulation in the liver [24]. Oral administration of *Nigella sativa* essential oil to hyperlipidemic rats significantly reduced plasma TG levels and increased plasma HDL-C levels by reducing liver HMGCR activity [39]. Treatment of human hepatoma (HepG2) cells with *Pinus koraiensis* essential oil suppressed the expression of lipid-related genes, such as SREBP-1c, SREBP-2, HMGCR, fatty acid synthase, and glycerol-3-phosphate acyltransferase, increased the expression of low density lipoprotein receptors and inhibited the activation of human acyl-coenzyme A: cholesterol acyltransferases (hACAT) 1 and 2 [47]. Treatment of HepG2 cells with *Lippia alba* essential oil, especially the tagetenone chemotype, decreased lipid synthesis, lipid contents, and volume of lipid droplets via the mevalonate pathway [35]. Also, incubation of HepG2 cells with *Artemisia princeps* essential oil significantly increased LDL-R expression [21].

Essential oil	Subject/route	Effects	Disease /model	Main component	Reference
<i>Aframomum melegueta</i> , <i>Aframomum danielli</i>	Pancreas and heart of rat	<ul style="list-style-type: none"> <li>• Anti-diabetes</li> <li>• Anti-oxidation</li> <li>• ACE inhibition</li> </ul>	—	<i>A. melegueta</i> : eugenol (82.2%) <i>A. danielli</i> : eugenol (51.1%)	[15]
<i>Allium cepa</i> , <i>Allium sativum</i>	Indian albino rabbit /PO	<ul style="list-style-type: none"> <li>• Fibrinolysis</li> <li>• Lipid improvement</li> </ul>	Athero sclerosis	<i>Allium cepa</i> : dimethyl-trisulfide (16.6%) <i>Allium ativum</i> : diallyl-trisulfide (33.6%)	[16]
<i>Allium sativum</i>	Human/PO	<ul style="list-style-type: none"> <li>• Fibrinolysis</li> </ul>	Myocardial infarction	Diallyl-trisulfide (33.6%)	[17]
<i>Alpinia zerumbet</i>	Wistar rat/IV	<ul style="list-style-type: none"> <li>• Blood pressure reduction</li> </ul>	Hyper tension	Terpinen-4-ol (28.1%)	[18]
<i>Alpinia zerumbet</i>	Wistar rat/PO, thoracic aorta of Wistar rat	<ul style="list-style-type: none"> <li>• Blood pressure reduction</li> <li>• Vasorelaxation</li> </ul>	Hyper tension	Terpinen-4-ol (57.4%)	[19]
<i>Aniba rosaeodora</i>	Wistar rat/IV, thoracic aorta of Wistar rat	<ul style="list-style-type: none"> <li>• Blood pressure reduction</li> <li>• Vasorelaxation</li> </ul>	—	Linalool (87.7%)	[20]
<i>Artemisia princeps</i>	HepG2 cells, isolated human LDL	<ul style="list-style-type: none"> <li>• Anti-oxidation</li> <li>• Lipid improvement</li> </ul>	—	1,8-cineole (20.1%)	[21]
<i>Citrus bergamia Risso</i>	MOVAS cells, EA.hy926 cells	<ul style="list-style-type: none"> <li>• Intracellular calcium influx inhibition</li> </ul>	—	D-Limonene (43.5%)	[22]
<i>Citrus bergamia Risso</i>	Wistar rat/IP	<ul style="list-style-type: none"> <li>• Anti-oxidation</li> <li>• Angiogenesis inhibition</li> </ul>	Vascular injury	D-Limonene (43.5%)	[23]
<i>Curcuma longa L.</i>	Golden Syrian hamster/PO	<ul style="list-style-type: none"> <li>• eNOS expression</li> <li>• Anti-platelet</li> <li>• Lipid improvement</li> <li>• Liver function improvement</li> <li>• Vasorelaxation</li> </ul>	Hyper lipidemia	Ar-turmerone (20.5%)	[24]
<i>Curcuma longa L.</i>	Wistar rat/PO, EA.hy926 cells	<ul style="list-style-type: none"> <li>• Anti-inflammation</li> </ul>	Myocardial ischemia/reperfusion injury	Ar-turmerone (20.5%)	[25]
<i>Dendropanax moribiferus</i>	Wistar rat/PO	<ul style="list-style-type: none"> <li>• Lipid improvement</li> </ul>	Hyper lipidemia	$\gamma$ -Elemene (18.6%)	[26]
<i>Euphorbiaceae</i>	Wistar rat/IV, thoracic aorta of Wistar rat	<ul style="list-style-type: none"> <li>• Blood pressure reduction</li> <li>• Vasorelaxation</li> </ul>	Hyper tension	Hexadecanoic acid, ethyl ester (46.1%)	[27]
<i>Foeniculum vulgare</i>	Swiss mouse/SC, thoracic aorta of Wistar rat, Guinea pig plasma	<ul style="list-style-type: none"> <li>• Anti-platelet</li> <li>• Anti-thrombosis</li> <li>• Vasorelaxation</li> </ul>	Pulmonary thrombo embolism	Anethole (75.8%)	[28]

Essential oil	Subject/route	Effects	Disease /model	Main component	Reference
<i>Fructus Alpiniae zerumbet</i>	HUVECs	<ul style="list-style-type: none"> <li>• Anti-inflammation</li> </ul>	High glucose induced injury	$\beta$ -Phellandrene (16.4%)	[29]
<i>Fructus Alpiniae zerumbet</i>	HUVECs	<ul style="list-style-type: none"> <li>• Anti-oxidation</li> </ul>	oxLDL induced injury	$\beta$ -Phellandrene (16.4%)	[30]
<i>Hyptis fruticosa Salzm</i>	Wistar rat/IV, superior mesenteric artery of Wistar rat	<ul style="list-style-type: none"> <li>• Blood pressure reduction</li> <li>• Vasorelaxation</li> </ul>	—	1,8-Cineole (16.9%)	[31]
<i>Lavandula hybrida</i>	Human/Inhalation	<ul style="list-style-type: none"> <li>• Endothelial function improvement</li> </ul>	—	Linalyl acetate (36.2%)	[32]
<i>Lavandula hybrida</i>	Swiss mouse/PO, Guinea pig plasma	<ul style="list-style-type: none"> <li>• Anti-platelet</li> <li>• Anti-thrombosis</li> </ul>	Pulmonary thrombo embolism	Linalyl acetate (36.2%)	[33]
<i>Linum usitatissimum L.</i>	Human/PO	<ul style="list-style-type: none"> <li>• Blood pressure reduction</li> <li>• Lipid improvement</li> </ul>	Metabolic syndrome	$\alpha$ -Linolenic acid (41.0% of total fatty acid)	[34]
<i>Lippia alba</i>	HepG2 cells	<ul style="list-style-type: none"> <li>• Lipid improvement</li> </ul>	—	<i>L. alba</i> tagetenone: myrcenone (30.4%)	[35]
<i>Mentha x villosa</i>	Wistar rat/IV	<ul style="list-style-type: none"> <li>• Blood pressure reduction</li> </ul>	Hyper tension	Piperitenone oxide (95.9%)	[36]
<i>Mentha x villosa</i>	Wistar rat/IV, thoracic aorta of Wistar rat	<ul style="list-style-type: none"> <li>• Blood pressure reduction</li> <li>• Vasorelaxation</li> </ul>	—	Piperitenone oxide (95.9%)	[37]
<i>Nardostachys jatamasi</i>	Thoracic aorta of Sprague-Dawley rat, HUVECs	<ul style="list-style-type: none"> <li>• Vasorelaxation</li> <li>• NO production increase</li> </ul>	—	Calarene (38%)	[38]
<i>Nigella sativa</i>	Wistar albino rat/PO	<ul style="list-style-type: none"> <li>• Lipid improvement</li> <li>• Anti-oxidation</li> </ul>	Hyper lipidemia	Thymol (32.0%)	[39]
<i>Ocimum gratissimum</i>	Wistar rat/IV, thoracic aorta of Wistar rat	<ul style="list-style-type: none"> <li>• Blood pressure reduction</li> <li>• Vasorelaxation</li> </ul>	Hyper tension	Eugenol (43.7%)	[40]
<i>Ocimum gratissimum</i>	Wistar rat/IV	<ul style="list-style-type: none"> <li>• Blood pressure reduction</li> </ul>	Hyper tension	Eugenol (43.7%)	[41]
<i>Ocotea quixos</i>	Swiss mouse/SC, thoracic aorta of Wistar rat, Guinea pig plasma	<ul style="list-style-type: none"> <li>• Anti-platelet</li> <li>• Anti-thrombosis</li> <li>• Vasorelaxation</li> </ul>	Pulmonary thrombo embolism	Trans-cinnamaldehyde (27.8%)	[42]
<i>Oenothera biennis</i>	Rabbit/PO	<ul style="list-style-type: none"> <li>• Anti-platelet</li> <li>• Angiogenesis inhibition</li> <li>• Lipid improvement</li> </ul>	Hyper lipidemia	Linoleic acid (71% of total fatty acid)	[43]

Essential oil	Subject/route	Effects	Disease /model	Main component	Reference
<i>Oenothera biennis</i>	New Zealand rabbit/PO	<ul style="list-style-type: none"> <li>• Anti-oxidation</li> <li>• Anti-platelet</li> <li>• Anti-thrombosis</li> <li>• Lipid improvement</li> </ul>	Athero sclerosis	Linoleic acid (71% of total fatty acid)	[44]
<i>Oenothera biennis</i> , <i>Ribes nigrum</i> , <i>Borago officinalis</i>	Spontaneously hyper tensive rat/ PO	<ul style="list-style-type: none"> <li>• Blood pressure reduction</li> </ul>	Hyper tension	<i>Oenothera biennis</i> : linoleic acid (71.0% of total fatty acid) <i>Ribes nigrum</i> : linoleic acid (45.0% of total fatty acid) <i>Borago officinalis</i> : linoleic acid (36.0% of total fatty acid)	[45]
<i>Olea</i>	Human/PO	<ul style="list-style-type: none"> <li>• Blood pressure reduction</li> <li>• Anti-oxidation</li> <li>• Endothelial function improvement</li> </ul>	High-normal BP, stage 1 essential HTN	oleic acid (55–83% of total fatty acid)*	[46]
<i>Pinus koraiensis</i>	HepG2 cells	<ul style="list-style-type: none"> <li>• Anti-oxidation</li> <li>• Lipid improvement</li> </ul>	—	Camphene (21.1%)	[47]
<i>Radix Angelica sinensis</i>	HUVECs	<ul style="list-style-type: none"> <li>• Angiogenesis inhibition</li> </ul>	—	3-Carene (32.1%)	[48]
<i>Rosa indica L.</i>	Thoracic aorta of rabbit	<ul style="list-style-type: none"> <li>• Vasorelaxation</li> </ul>	—	Acetic acid (percentage was not available)	[49]
<i>Schisandra chinensis</i>	HASMCs	<ul style="list-style-type: none"> <li>• Anti-oxidation</li> <li>• Anti-inflammation</li> <li>• Angiogenesis inhibition</li> </ul>	TNF- $\alpha$ induced injury	Borneol (43.6%)	[50]
<i>Syringa pinnatifolia</i> Hems1. var. <i>alashanensis</i>	Wistar rat/IG Kun ming mouse/IP Primary cultured rat myocyte	<ul style="list-style-type: none"> <li>• Anti-oxidation</li> <li>• Anti-platelet</li> </ul>	Myocardial infarction, hypoxia damage	$\alpha$ -Cadinol (19.9%)	[51]
<i>Syzygium aromaticum</i>	Sprague-Dawley rat/PO	<ul style="list-style-type: none"> <li>• Anti-oxidation</li> <li>• Anti-inflammation</li> <li>• Lipid improvement</li> <li>• Liver function improvement</li> </ul>	Metabolic syndrome	Eugenol (75.2%)	[4]
<i>Trachyspermum ammi</i>	Thoracic aorta of Wistar albino rat	<ul style="list-style-type: none"> <li>• Vasorelaxation</li> </ul>	—	Thymol (38.1%)	[52]

\*Polyphenol-rich olive oil was used in this study.

**Table 1.** Effects of essential oils in CVDs.

## 2.2. Hypertension

Hypertension is also a major risk factor for CVDs. In the United States, the prevalence of hypertension in adults aged over 60 years was 67.2% from 2011 to 2014 and hypertension was the third leading cause of death from CVDs [55]. Thus, aggressive blood pressure control is needed and essential oils are thought to be helpful. Intragastric administration of *Alpinia zerumbet* essential oil to N-nitro-L-arginine methyl ester (L-NAME)-induced hypertensive rats for 30 days reduced systolic arterial pressure, diastolic arterial pressure, and mean arterial pressure in a time-dependent manner. These hypotensive effects of *A. zerumbet* essential oil were due to its vasorelaxing and Ca<sup>2+</sup> antagonist effects [19]. In spontaneously hypertensive rats, *Ribes nigrum* essential oil reduced systolic blood pressure (SBP) significantly when compared with sesame oil [45]. Although *Oenothera biennis* and *Borago officinalis* essential oils also reduced SBP, these effects were not statistically significant. In addition, intravenous administration of *Ribes nigrum* essential oil reduced mean arterial pressure (MAP) in spontaneously hypertensive rats. Intravenous administration of *Alpinia zerumbet* [18], *Euphorbiaceae* [27], *Mentha x villosa* [36], and *Ocimum gratissimum* [40, 41] essential oils to deoxycorticosterone-acetate (DOCA)-salt induced hypertensive rats, reduced MAP. Moreover, *Aniba rosaeodora* [20], *Hyptis fruticosa* Salzm [31], and *Mentha x villosa* essential oils [37] reduced MAP in normotensive rats.

The antihypertensive effects of essential oils have also been demonstrated in human studies. In a randomized controlled trial, oral administration of *Linum usitatissimum* L. essential oil significantly reduced SBP and diastolic blood pressure (DBP) in patients with metabolic syndrome [34]. In addition, polyphenol-rich *Olea* essential oil reduced SBP and DBP in women diagnosed with stage 1 hypertension and those with high-normal blood pressure [46].

## 2.3. Fatty liver

Several essential oils effective in the treatment of dyslipidemia also improved liver fat contents and liver function. Oral administration of *Syzygium aromaticum* essential oil, the main component of which is eugenol, to rats fed a high fructose diet, was found to reduce total fat, TC, and TG levels in the liver. In addition, *S. aromaticum* essential oil reduced the plasma concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and direct bilirubin in these rats, thereby improving fatty liver and liver dysfunction [4]. Similarly, oral administration of *Curcuma longa* L. essential oil to hyperlipidemic hamsters not only reduced hepatic cholesterol levels but also decreased plasma ALT and AST concentrations [24].

## 2.4. Diabetes

Because chronic hyperglycemia associated with diabetes increases oxidative stress, a cause of vascular endothelial dysfunction, via several pathways such as polyol flux [56], the anti-diabetic effect of essential oils is noteworthy. *In vitro* studies showed that the essential oils of *Aframomum melegueta* and *A. danielli*, the main component of which is eugenol, had anti-diabetic properties. Although both essential oils inhibited  $\alpha$ -glucosidase and  $\alpha$ -amylase, *A. melegueta* essential oil had much higher inhibitory activities, indicating greater antidiabetic effects, than *A. danielli* oil [15].

## 2.5. Oxidative stress

Excessive production of reactive oxygen species (ROS) induces endothelial dysfunction, an early stage of atherosclerosis [57]. Patients with coronary artery disease (CAD) have higher lipid peroxidation activity but significantly lower antioxidant enzyme activities than individuals without CAD [58], indicating the importance of maintaining a balance between ROS production and antioxidant defense systems. Several essential oils have shown the ability to reduce oxidative stress. For example, pretreatment of human aortic SMCs with *Schisandra chinensis* essential oil blocked tumor necrosis factor (TNF)- $\alpha$ -induced ROS [50]. *Fructus Alpiniae zerumbet* oil attenuated oxidative stress in human umbilical vein endothelial cells (HUVECs) exposed to ox-low density lipoprotein (LDL), not only by reducing malondialdehyde (MDA) contents but also by increasing the activities of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase, and catalase [30].

Essential oils have also been shown to be active in models of acute myocardial ischemia. For example, *Syringa pinnatifolia* Hems1. var. *alashanensis* essential oil inhibited the reduction of SOD activity and increased mitochondrial activity in cardiac myocytes [51]. *Aframomum melegueta* and *A. danielli* essential oils showed radical scavenging activity, as well as dose-dependently ameliorating lipid peroxidation in rat heart and pancreas [15]. Similarly, *Artemisia princeps* essential oil displayed radical scavenging activity and inhibited the production of thiobarbituric acid-reactive substances, a marker of LDL oxidation [21]. *Pinus koraiensis* [47] and *Olea* essential oils [46] also inhibited LDL oxidation.

In addition, essential oils have also been found to reduce MDA contents. For example, *Citrus bergamia* Risso essential oil reduced MDA production in carotid arteries injured by balloon angioplasty [23] and *Nigella sativa* essential oil reduced plasma MDA formation in hyperlipidemic rats [39]. In rabbits, an atherogenic diet supplemented with *Oenothera biennis* essential oil inhibited platelet MDA production [44]. Oral administration of *Syzygium aromaticum* essential oil to rats with metabolic syndrome reduced plasma MDA concentrations [4].

## 2.6. Endothelial dysfunction

NO is a typical vasodilatory substance produced from L-arginine by the enzyme endothelial NO synthase (eNOS) in vascular endothelial cells [59]. Thus, endothelial dysfunction is associated with decreased NO bioavailability [57]. In hyperlipidemic hamsters, *Curcuma longa* L. essential oil, at a concentration of 300 mg/kg body weight, increased the expression of aortic eNOS, suggesting that *C. longa* L. essential oil protects against endothelial dysfunction [24]. Treatment of HUVECs with *Nardostachys jatamansi* essential oil increased NO production by phosphorylating eNOS, a reaction mediated by the phosphatidylinositol 3-kinase/protein kinase B signaling pathway and changes in intracellular Ca<sup>2+</sup> [38]. *Aframomum melegueta* and *A. danielli* essential oils were found to inhibit ACE activity *in vitro*, suggesting that these oils have antihypertensive activity, with *A. danielli* oil having greater activity than *A. melegueta* oil [15].

Flow mediated dilatation (FMD) is a widely used marker of vascular endothelial cell function. A study of night-shift medical workers found that FMD was significantly higher after a 30 min inhalation of *Lavandula hybrida* essential oil than before inhalation and than in a control group

[32]. A randomized crossover study in women with stage 1 hypertension or high-normal BP found that a diet containing polyphenol-rich *Olea* essential oil increased hyperemic areas after cuff-induced ischemia, another test of vascular endothelial function [46].

## 2.7. Impaired vascular tone

Many essential oils have been found to induce vascular relaxation *in vitro*. In a hyperlipidemic animal model, oral administration of *Curcuma longa* L. essential oil, at a dose of 300 mg/kg body weight for 28 days, restored acetylcholine-induced vasorelaxation, as well as increasing eNOS expression and decreasing cholesterol contents in the aorta [24]. *Ocimum gratissimum* essential oil showed partial endothelium-dependent vasorelaxing activity in aortic rings from DOCA-salt induced hypertensive rats. This vasorelaxant activity was mainly attributed to an inhibition of Ca<sup>2+</sup> influx rather than Ca<sup>2+</sup> release from the sarcoplasmic reticulum [40]. In addition, essential oils of *Aniba rosaedora* [20], *Euphorbiaceae* [27], *Foeniculum vulgare* [28], *Mentha x villosa* [37], *Nardostachys jatamasi* [38], *Rosa indica* L. [49], and *Trachyspermum ammi* [52] were found to induce vasorelaxation in rat thoracic aorta pre-contracted with KCL or phenylephrine. In particular, the vasodilatory effects of *Nardostachys jatamasi* essential oil were mediated by increased NO production [38]. *Ocotea quixos* essential oil also relaxed aortic rings pre-contracted with U46619 [42]. *Alpinia zerumbet* essential oil was also shown to relax aortic rings pre-contracted with KCL or phenylephrine. *A. zerumbet* oil also inhibited CaCl<sub>2</sub>-induced vascular contraction, an effect resulting from the inhibition of Ca<sup>2+</sup> influx through voltage-operated and receptor-operated Ca<sup>2+</sup> channels [19]. Similarly, the treatment of the rat superior mesenteric artery with *Hyptis fruticosa* Salzm essential oil resulted in vascular relaxation and inhibition of CaCl<sub>2</sub>-induced vascular contraction in a concentration-dependent manner [31]. Adequate regulation of cytosolic Ca<sup>2+</sup> is important in maintaining vascular tone. *Citrus bergamia* Risso essential oil inhibited Ca<sup>2+</sup> influx into HUVECs [22].

## 2.8. Thrombosis

Platelet aggregation and adhesion play important roles in thrombus formation. *Curcuma longa* L. essential oil has been shown to reduce hyperlipidemia-induced platelet activation by suppressing platelet aggregation and adhesion in hyperlipidemic hamsters [24]. Other essential oils were found to inhibit platelet aggregation or adhesion. For example, essential oils of *Foeniculum vulgare* [28], *Lavandula hybrida* [33], and *Ocotea quixos* [42] not only inhibited agonist-induced platelet aggregation *in vitro* but also inhibited thrombin-induced clot retraction in guinea pig plasma. In addition, these three essential oils prevented paralysis in an animal model of acute pulmonary thromboembolism, indicating that these oils had antithrombotic activity. *Syringa pinnatifolia* Hems1. var. *Alashanesis* essential oil was found to inhibit agonist-induced platelet aggregation in rat whole blood. This result, together with its antioxidant effects, suggests that *Syringa pinnatifolia* Hems1. var. *Alashanesis* oil has cardioprotective activity [51]. In addition, an atherogenic diet enriched with *Oenothera biennis* essential oil reduced agonist-induced platelet aggregation in whole blood and platelet thromboxane B2 production, thereby inhibiting platelet activation [44]. Both *Allium cepa* and *A. sativum* essential oils have been shown to increase the fibrinolytic activity of garlic and onions in atherosclerotic rabbits [16]. In addition, *A. sativum* essential oil was found to significantly increase the fibrinolytic activity in patients with chronic myocardial infarction and in patients after acute myocardial infarction [17].

## 2.9. Inflammation

The levels of expression of intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 in endothelial cells increase during inflammation [60], with the expression of these adhesion molecules being regulated by inflammatory cytokines such as interleukin (IL)-1 and TNF- $\alpha$  [61]. Several essential oils have been shown effective in inhibiting this process. For example, the treatment of HUVECs exposed to high glucose with *Fructus Alpiniae zerumbet* essential oil was shown to reduce the secretion of IL-8, TNF- $\alpha$ , ICAM-1, and VCAM-1 by inhibiting nuclear factor kappa B (NF- $\kappa$ B) signaling, suggesting that this essential oil has endothelial protective effects [29]. Similarly, the treatment of human aortic SMCs with *Schisandra chinensis* essential oil decreased TNF- $\alpha$ -induced matrix metalloproteinase-9 (MMP-9) activation, inducible NO synthase and cyclooxygenase-2 (COX-2) expression by inhibiting NF- $\kappa$ B signaling [50]. In an animal study, oral administration of *Curcuma longa* L. essential oil to rats exposed to myocardial ischemia/reperfusion injury reduced endothelial cell-induced inflammation by decreasing the expression of E-selectin and ICAM-1 [25]. Similarly, *Syzygium aromaticum* essential oil reduced plasma TNF- $\alpha$  concentration in rats fed a high-fructose diet [4].

## 2.10. Angiogenesis

The proliferation and migration of vascular SMCs play essential roles in the pathophysiological changes of cardiovascular systems. *Radix angelica* essential oil was found to dose-dependently inhibit HUVEC proliferation and migration, and, at concentrations above 20  $\mu$ g/ml, to reduce endothelial tube formation, indicating that *R. angelica* essential oil has antiangiogenic effects [48]. Similarly, *Schisandra chinensis* essential oil effectively decreased the TNF- $\alpha$ -induced migration of human aortic SMCs. These findings, together with the antiinflammatory and antioxidant effects of this oil, suggested that *Schisandra chinensis* oil has antiatherosclerotic activity [50].

Animal studies have also assessed the effects of essential oils on angiogenesis. For example, supplementation of a hyperlipidemic diet with *Oenothera biennis* essential oil for 6 weeks reduced endothelial lesions of the aorta and neointimal proliferation of the arterial wall in rabbits [43]. *Citrus bergamia* Risso essential oil reduced the neointima/media ratio and the cross-sectional area of the carotid artery in rats that underwent balloon-induced vascular injury, with these effects accompanied by decreased expression of lectin-like receptor for oxidized LDL [23].

## 3. Conclusions

Essential oils are natural substances extracted from aromatic plants with biological properties, including antioxidant and antiinflammatory activities. This chapter reviewed the effects of essential oils on CVD-related factors. Evidence has shown that essential oils have multiple effects, improving lipid balance, liver function, and endothelial function; reducing blood pressure, diabetes induction, and oxidative stress; enhancing vascular relaxation; and inhibiting thrombosis, inflammation, and angiogenesis. Essential oils and their active components may therefore be promising therapeutic agents for CVDs. Studies are needed to clarify the effects of these oils on patients and to elucidate their specific mechanisms of action.

## Acknowledgements

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## Conflict of interest

None.

## Author details

Geun Hee Seol\* and You Kyoung Shin

\*Address all correspondence to: [ghseol@korea.ac.kr](mailto:ghseol@korea.ac.kr)

Department of Basic Nursing Science, School of Nursing, Korea University, Seoul, Republic of Korea

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# Antifungal Effect of Essential Oils

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María Paz Arraiza, Azucena González-Coloma,  
Maria Fe Andres, Marta Berrocal-Lobo,  
José Alfonso Domínguez-Núñez,  
Avanor Cidral Da Costa Jr,  
Juliana Navarro-Rocha and  
Carlos Calderón-Guerrero

Additional information is available at the end of the chapter

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## Abstract

Essential oils are employed in agriculture, medicine and food industries among others, due to their antimicrobial, antiviral, insecticidal and antifungal properties. In this chapter, we will focus on the control of fungal plant pathogens with essential oils. Fungal diseases in agricultural crops and forestry alter the physiology of plants, disrupting their normal functioning, reducing their yield and sometimes causing their death. Recent studies show antifungal effects of many essential oils against plant pathogenic fungi, which make them candidates for the development of new fungicidal agents. This chapter presents a review of the most recent advances in this area, as well as the future trends in this field.

**Keywords:** antifungal, plant pathogens, active compounds, essential oils, biotechnology

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## 1. Introduction

Diseases caused by plant pathogens significantly contribute to annual loss in crop yield worldwide [1], being fungi the major pathogens with the greatest impact regarding diseases and crop production losses [2]. Application of chemical fungicides is the most prevalent and effective control method of these plant diseases, posing a serious threat to the environment and public health besides causing resistance in the pathogens [3]. Therefore, in recent years, there has been a clear tendency toward finding safer alternative methods for fungal disease control in agriculture [4]. In January 2009, The European Parliament agreed the text

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of a Regulation on Plant Protection Products (91/414/EC) [5]. Integrated Plant Management (IPM) is the effort to control plant diseases with alternative methods to chemical fungicide, eliminating or controlling their use and implementing the application of alternative control methods such as natural fungicidal substances. Therefore, the industrial research aimed at the discovery and optimization of botanical fungicides needs to address the following aspects: (a) the product must overcome resistance problems to the established commercial products, (b) the product must have lower toxicity to nontarget species and acceptable levels of persistence in the environment, and (c) the product must have market and technical advantages for the agrochemical companies [6].

Essential oils (EOs) represent a new class of crop protectants due to their effects, short shelf-life and low toxicity to the environment [7]. In addition, the probabilities of creating new resistant strains by using essential oils as fungicidal agents are low since their constituents can act as synergists [8]. Usually, mono- and sesquiterpenes such as phenols, alcohols, ethers, carbohydrates, aldehydes and ketones are the major constituents of essential oils, which are responsible for the biological activity as well as for their fragrance [9]. In fact in recent years, researchers have reported many mono- and sesquiterpene hydrocarbons as inhibitors of microbial pathogens [10]. Compounds such as carvacrol, thymol, linalool, cymene, pinene are known to exhibit antimicrobial activity [11–14]. These are the major components of essential oils with promising antifungal applications. Many essential oils have been reported as active against animal pathogenic fungi with no side effects [15–17]. Currently, there are some reviews on antifungal activity of plant extracts, generally structured according to the botanical family of plant species source of the active EOs [16] or to the active compounds of plant extracts [18]. The present review is up to date and focused on plant essential oils with antifungal activity against plant pathogenic fungi.

## 2. Main forest pathogenic fungi

Forest pathology deals with the diseases of forest trees, which are mainly caused by fungal and oomycete pathogens, in both their fundamental and applied aspects. The development and dissemination of effective control measures is vital to the protection of forest health. An evolution has been observed over the past few decades in terms of techniques and attitudes toward pest control. In the early 1960s, a variety of methods were used to control forest insect pests and diseases including mechanical, silvicultural, chemical and biological methods, with chemical control the most commonly used. By the 1970s, environmental concerns were being increasingly raised about the use of chemicals. As a result, research into the use of biological control agents in conjunction with silvicultural methods or pheromones began in earnest. In the last decade, integrated pest management involving a combination of control measures began to be considered the most effective way to deal with forest pests. Applications of biological control agents and microbial insecticides have become major components of pest management programs and considerable emphasis is placed on prevention and early detection as a means to avoid future pest problems [19]. There is a growing trend toward adopting more sustainable forest management strategies to contain forest pests, particularly in developed countries [20]. This movement is related to changes in the perception and role of forests, which are increasingly valued not just for economic reasons but also for their ecological and

social functions. Forest insect pests, diseases and other pests are having significant impacts on forests worldwide. While the devastating impacts of indigenous forest pests are already recognized, those of introduced species are increasingly being recognized as well. Rapid transports, ease of travel, and free trade have facilitated the spread of pests [19]. A review of forest pests in both naturally regenerated forests and planted forests [19] was carried out from 2005 to 2008 in 25 countries, including a number of major forest countries (Brazil, China, Indonesia), in Africa, Asia and the Pacific, Europe, Latin America and the Caribbean, and the Near East. In this global review, the frequency of disease-causing pathogens was reported: ascomycota 59%, bacteria 3%, basidiomycota 33%, oomycota 4%, and phytoplasma 1%. In the Global Forest Resources Assessment 2010 [21], countries were also invited to list and rank up to 10 major outbreaks of insects and diseases that have occurred since 1990; the most prevalent fungal pathogens reported (in order of importance) are as follows: *Armillaria* spp., (Armillaria root disease), *Cryphonectria parasitica* (chestnut blight), *Heterobasidion* spp. (annosum root rot), *Melampsora larici-populina* (poplar rust), *Mycosphaerella pini*, (red band needle blight), *Sphaeropsis sapinea* (diplodia tip blight), *Chalara fraxinea* (ash dieback), *Gremmeniella* sp., and *Melampsora allii-populina* (poplar rust).

## 2.1. Emerging forest fungal diseases

In the last 15 years, two major changes affecting forest pathology—the world movement of species with trade and the rise of plantation forestry to meet growing needs of an increasing human population—have led to an increasing number of emerging diseases [21–22]. Ghelardini et al. [23] showed seven pathways driving the emergence of diseases threatening natural and planted forest ecosystems around the world: invasions by alien pathogens, climate change, emergence of new virulent and aggressive strains or species, rise of hybrid fungal species, latent and cryptic pathogens, establishment of new associations between vectors and pathogens, and the introduction of new crops and cultivation practices.

Native forests of Europe, Asia and North America have particularly suffered from invasive alien pathogens, which in the last century have caused the decline of key tree species. Among the most striking historical examples are the destruction of chestnuts by *Cryphonectria parasitica*, the alien ascomycete responsible for chestnut blight; the devastating epidemics of Dutch elm disease (DED) caused by *Ophiostoma ulmi* and *O. novo-ulmi*, two alien and highly aggressive fungi previously unknown to science; the huge damage inflicted to white pines by *Cronartium ribicola*, the invasive agent of white pine blister rust (WPBR); and the devastation of plane trees, especially obtrusive in Southern Europe, caused by the introduction of *Ceratocystis platani*, the agent of plane canker stain. In the last years, the number of described *Phytophthora* species has dramatically increased and it is now clear that forest soils host numerous and diverse resident communities of *Phytophthora* species [24]. Recently, the introduction of *Fusarium circinatum* in Spain [25] or, as a late and worrisome case, the fast-spreading epidemics of European ash dieback caused by *Hymenoscyphus fraxineus* [26–28], an anamorphic fungal pathogen with putative origin in eastern Asia [29] should be added to the list. In relation to climate change, *Phytophthora cinnamomi* is forecast to benefit from warmer winters, possibly expanding its geographic range by kilometers and reaching unaffected host populations or new host species [30]. Otherwise, [31] found that in the last 15 years, the emerging pine shoot pathogen *Diplodia sapinea* spread in France probably because of a

climate shift to milder winters and wetter summers. Dutch elm disease (DED) is frequently mentioned in forest pathology reviews as the best example of a destructive disease of alien origin since it almost destroyed the elm populations of Europe, North America, and parts of Asia. DED reemerged in the 1970s in Europe as a devastating disease, which killed also elm genotypes that had been resistant in the first epidemic (at the beginning of the twentieth century). This new epidemic was caused by the emergence of the separate and highly virulent species *Ophiostoma novo-ulmi* [32] consisting of the subspecies *novo-ulmi* and *Americana* [33]. Also, [34] provided strong evidence that *Mycosphaerella populorum*, the Septoria canker of poplars, has adapted to infect, colonize, and cause mortality on poplar woody stems as a result of horizontal transfer of the necessary gene battery from ascomycete fungi associated with wood decay and from prokaryotes.

In fungal pathogens of woody plants, emergence of new interspecific hybrids was described in *Melampsora* [35], *Phytophthora* [36], *Ophiostoma* [37], *Cronartium* [38], and *Heterobasidion* [39]. An up-to-date case of a worrisome forest pathogen that may have a latency period in asymptomatic infected plants is *H. fraxineus*, the agent of European ash dieback, which penetrates into wood tissues from infected leaves and may not produce external necroses until the next growing season [40]. The *Botryosphaeriaceae* are a classical example of a very diverse group of fungi, which comprises well-studied endophytes and latent pathogens of woody plants that typically cause disease associated with some types of stress [41]. A key factor in the spread of *Diplodia sapinea* and *D. scrobiculata* is the latency period within host plant tissues. These fungi are able to live within the host without causing any visible symptoms but rapidly shift to a pathogenic interaction when an environmental stress factor primes the host (e.g., local or large-scale climate change) [31].

An example of new association between vectors and pathogens is the spread of *C. parasitica* on chestnuts by *Dryocosmus kuriphilus*, the oriental chestnut gall wasp, in Europe [42]. *D. kuriphilus* is an invasive insect of Asian origin. Also, a new association was recently reported between *D. sapinea* and *Leptoglossus occidentalis* [43], the so-called western conifer seed bug (WCSB), an invasive coreid, accidentally introduced to Italy from the US in 1999 [44], and nowadays present in several parts of Europe [45]. This association might be beneficial for both partners: the insect enables the fungus to reach a higher number and variety of host trees, either pines or other conifers, while the fungus stimulates the tree's production of monoterpenes, signaling the status of weakness of the tree and attracting more insects [43]. Regarding the new silvicultural practices, commercial plantations of poplars may be severely damaged by emerging plant pathogens worldwide [46]. In northeastern and north-central North America, one of the most harmful poplar diseases is *Mycosphaerella populorum* (Peck). Also, the epidemics of *Phytophthora ramorum* on *Larix kaempferi* (Lamb.) Carr. in UK might have been driven by the intrinsic fragility of clonal monocultures on great areas due to ecosystem simplification, extreme mechanization, and reduced genetic diversity [47, 23]. Looking ahead, authors of [48] propose an evolutionary ecology perspective that could provide new directions for forest research or disease management: (1) fungal evolutionary diversity (species diversity of forest pathogens and their ecological niches), (2) pathogen evolution (how forest pathogens become adapted to their hosts), (3) forest resistance to disease, especially in relation to tree breeding (trade-offs, tolerance, emerging properties in populations), and (4) the role of hyperparasites

and tree microbiota in the regulation of pathogen populations and disease. In this ecosystem perspective, pathogens are no longer “enemies” but are key actors of the evolution and ecology of local communities, and more generally of the ecosystem health (e.g., [49–50]).

### 3. Biotechnological approach: genomics-proteomics-metabolomics

Plants in nature are constantly challenged by several harmful phytopathogens including bacteria, fungi, nematodes, or virus, producing a high and negative impact on crop productivity worldwide [51]. An uncontrolled amount of synthetic and chemical pesticides used during past decades makes necessary to adopt new strategies allowing a sustainable plant protection in crops and forest systems. The use of natural compounds as plant biostimulators of growth or biotic and abiotic stress responses in plants is getting importance in the last decade because of legal restrictions on the use of phytosanitary products on crops [52, 53]. European Union policy works upon a significant reduction in pesticide use in the short future [54]. One alternative are natural origin compounds with priming capacities, such as the essential oils (EOs) [55]. This section describes examples of recent molecular approaches studying EOs and discusses the use of EOs as an alternative of nonpollutant primers to induce plant resistance for environmental-friendly plant protection.

#### 3.1. The “priming” process

Priming is “the physiological state that enables cells to respond to very low levels of a stimulus in a more rapid and robust manner than non-primed cells. In plants, priming plays a role in defense and development” [56, 57]. A classical priming defense strategy consists in the use of very well-conserved molecules into the phytopathogen structure called damage/pathogen/microbe-associated molecular patterns (DAMPs/PAMPs/MAMPs), such as the lipopolysaccharides (LPS), peptidoglycan (PGN), bacterial flagellin, fungal chitin, bacterial Ax21, or elongation factor Tu (EF-Tu). MAMPs are recognized by plasma-membrane receptors in plants called pattern recognition receptors (PRRs). PAMPs recognition activates a pattern-triggered immunity (PTI) associated with the increase in intracellular calcium, phosphorylation processes mediated by MAPKinase cascades, production of reactive oxygen species (ROS), plant protective compounds, induction of defense-related transcription factors, and corresponding plant pathogenesis-related proteins (PRs) such as glucanases and chitinases, as well as proteins and compounds involved in plant cell wall fortification, such as callose or lignin. PTI might be suppressed by host-adapted phytopathogens, producing an effector-triggered susceptibility (ETS), and adapted plants might block those effectors, activating a robust effector-triggered immunity (ETI) [53, 58–60]. In parallel to the PAMP response, each pathogen specifically triggers a cascade of signaling pathways mediated by phytohormone receptor and recognition of salicylic acid (SA), jasmonate acid (JA), or ethylene (ET). Commonly, it is well accepted that SA is induced by biotrophic and hemibiotrophic phytopathogens, while ET and JA are activated by necrotrophic ones and also by some hemibiotrophs. Those pathways are also interconnected, in order that the activation of one of them currently down-regulate the other one or vice versa [56]. A new mechanism called EMPIS (ETI-Mediating and PTI-inhibited

sector) inhibits unnecessary immune responses in plants, limiting the fitness cost of the robust ETI, when PTI is enough effective [61]. Additionally to MAMPs, hormone-mimic-related compounds have been used as classical biostimulators of priming on plants; some examples are the synthetic chemical compounds such as: benzo (1,2,3)-thiadiazole-7-carbothiolic acid (BTH), a SA analog which activates systemic acquired resistance (SAR) in crops [62], and the  $\beta$ -aminobutyric acid (BABA), a nonprotein amino acid priming compound with a direct fungitoxic effect [63] or the nonprotein amino acid pipercolic acid [64]. The recent advances in metabolic profiling have led to the discovering of certain new plant secondary metabolites that play significant roles as priming molecules at nature, during biotic and abiotic plant stress responses and in the plant-to-plant communication; at this point, EOs might play an important role in future biotechnological approaches [65, 66].

### 3.2. Metabolic engineering improving EO yield

A line of research on EO biotechnology consists in improving EO yield in plants using metabolic engineering. One of the plant species in which biotechnology approaches has been applied because its commercial interest is peppermint, and [67] transformed peppermint with various gene constructs by overexpressing genes involved in the supply of precursors through the 2C-methyl-D-erythritol 4-phosphate (MEP) pathway. The overexpression of the MEP pathway gene 1-deoxy-D-xylulose 5-phosphate reductoisomerase increased up to 78% of the oil yield over wild-type controls in a multiyear field trials. Current genetic manipulation on EO synthesis pathway was also useful improving the EO production in the same species [68]. The inhibition of the mevalonate pathway also enhanced the carvacrol biosynthesis and DXR gene expression in shoot cultures of *Satureja khuzistanica* Jamzad. *S. khuzistanica* shoots were treated with fosmidomycin (an inhibitor of the nonmevalonate pathway) and mevinolin (an inhibitor of the mevalonate pathway). The last one induced the gene expression of DXR, measured by heterologous QRT-PCR, increasing the DXR enzyme activity and allowing higher levels in carvacrol biosynthesis on plants compared to controls [69].

### 3.3. Molecular mechanism of EOs in fungi

Recent studies have been made in order to elucidate the molecular mechanisms underlying the phytotoxic effect for some of those compounds on phytopathogenic fungi, but still are limited. The lipophilic or hydrophobic nature of many EO components allows them to interact directly with the fungal membrane, resulting in the alteration of membrane properties including the fluidity. An active transport via trans-membrane pumps has not been yet demonstrated [55]. A recent study based on RNA-Seq-transcriptomic analysis of the fungus *Fusarium oxysporum* f. sp. *niveum*, responding to thymol, shows that most of glycosphingolipid and sphingolipid metabolism-related fungal genes were downregulated upon this treatment, while genes involved in an antioxidant activity, chitin biosynthesis, and cell wall modification were up-regulated. The authors propose that the thymol acts by disrupting fungal cell wall and cell membranes through increasing the production of ROS on the fungal cell surface as well as by blocking the fungal molecular genes necessary for cell wall fortification and cell membrane synthesis [70]. Those molecular data are in line with the results obtained by [71], showing that thymol strongly inhibited conidial production and hyphal growth on *Fusarium graminearum* via inducing lipid peroxidation and disrupting ergosterol biosynthesis, which

are essential for plasma membrane structure. A similar mechanism of action was observed on carvacrol and thymol acting against vineyard and wine spoilage yeast [72].

### 3.4. Plant signaling pathways and EOs

Emerging molecular studies try to elucidate the molecular effects of EOs produced by plants to the surrounding ones [52]. This old natural process is currently known as “allelopathy” or the ability of a plant to produce biomolecules, especially secondary metabolites, to affect another plant beneficially or vice versa [73]. In 1997, [74] demonstrated that methyl salicylate (MeSA), the volatile benzenoid and secondary metabolite, which is easily metabolized on the plant to SA, activates disease resistance and the expression of defense-related genes in neighboring plants and in the healthy tissues of the infected plants. Later on, other research works have shown that MeSA mediates plant-plant communications during immune responses. MeSA, which is an important insect-attracting pollinators [75], is not induced by wounding but is induced by tobacco mosaic virus and *Pseudomonas syringae* pv. *maculicola* ES4326 and *Pst* DC3000 pv. *tomato*, where both are SA inducers [76]. The plant molecular response to MeSA has been studied into essential oil extracts from *Gaultheria procumbent* (GEO), whose metabolic profile has been characterized recently [77]. GEO induced defense response against the hemibiotrophic fungus *Colletotrichum higginsianum* and was very effective in inducing SA plant defense-related genes similarly to the synthetic MeSA and also induced some marker genes of JA pathway [78]. A recent study investigated the role of volatile organic compounds inducing systemic acquired resistance (SAR). The headspace exposure of arabidopsis to a mixture of the bicyclic monoterpenes,  $\alpha$ -pinene and  $\beta$ -pinene, induced the accumulation of ROS and the expression of SA- and SAR-related genes, including AZELAIC ACID INDUCED1 (AZI1) and three of its paralogs. Pinene-induced resistance was dependent on SA biosynthesis and signaling and on AZI1. Arabidopsis geranylgeranyl reductase1 mutants with reduced monoterpene biosynthesis were SAR-defective, but showed normal local resistance and MeSA-induced defense responses, suggesting that monoterpenes act independently of SA-mediated pathway. The volatile emissions composed by  $\alpha$ -pinene,  $\beta$ -pinene, and camphene induced plant defense in neighboring plants, activating SAR responses on them. The impaired SAR immunity lines *eds1-2* and *ggr-1-1* showed reduced emissions of  $\alpha$ -pinene,  $\beta$ -pinene, and camphene [79]. *Pseudomonas syringae* pv. *maculicola* ES4326 also induced terpenoid production of (E,E)-4,8,12 trimethyl-1,3,7,11-tridecatetraene (TMTT),  $\beta$ -ionone, and  $\alpha$ -farnesene, depending on JA signaling and independently on SA pathway in *Medicago truncatula* [80]. Copper sulfate, which activates JA biosynthesis in plant by camalexin biosynthesis, induced VOs in arabidopsis wild-type plants but not in *tps4* mutant showing that TMTT is induced by JA pathway [80]. TMTT and other VOs were also induced in lima beans by herbivory [81]. However, the significance on the *Pst* induction of TMTT in plants is still unknown.

## 4. EOs in the control of phytopathogenic fungi in agricultural crops

In agriculture, the losses caused by plant diseases reach an average of 12% per year. Among the pathogens, fungi are considered the most important. There are around 8.000 species of fungi that attack plants, distributed in more than 64 genera of fungi [82]. Added to the importance of plant diseases caused by phytopathogenic fungi, we have two other factors that must be considered.

The first concerns the constant need to produce food to feed the planet's growing population. According to the Food and Agriculture Organization of the United (FAO), global food demand in 2050 is estimated to be 60% higher than in 2006. The population living in poverty could rise from 35 to 122 million by 2030. This increase of the poor will be higher in sub-Saharan Africa, largely because of the heavy dependence of the economy of these regions on agriculture. The second factor refers to the use of pesticides. The increase in the use of pesticides is due to the increase in the cultivable area and consequently the increase in the consumption of fertilizers and pesticides. Misuse of pesticides has led to serious public and environmental health problems. The United Nations has proposed the creation of a global treaty to regulate and stop the use of pesticides in agriculture. Current patterns of production and use of pesticides are very different in each country. According to the World Health Organization (OMS), pesticides cause 200.000 deaths from poisoning each year. Almost all fatalities, or 99%, occur in developing countries. Exposure to pesticides is linked to the risk of cancer, Alzheimer's and Parkinson's disease, hormonal, developmental, and fertility problems. The rural community made up of farmers and families who live near plantations and indigenous communities is the most vulnerable. In Brazil, for example, data from the Impact of Agrochemicals on Health released in 2015 by the Brazilian Association of Collective Health (ABRASCO) show that Brazil is the largest consumer of pesticides in the world, with a 288% increase in pesticide use. The data also show that 64% of the food marketed is contaminated and that the number of poisoning by agrochemicals reaches 34.147 cases. It is believed that these statistics should be even higher due to under-reporting, i.e., subacute intoxications caused by moderate or small exposure to products of high toxicity, slow onset and subjective symptomatology, and chronic intoxications requiring months or years of exposure. Resistance of fungi to fungicides has been recorded since the 1960s. The first case of resistance was found with the use of Benomyl to control the mildew of cucurbits, caused by the fungus *Sphaerotheca fuliginea* [83] and later to control the fungus *Botrytis cinerea* in the culture of the cyclamen [84]. Since then, more than half of the known fungus species have shown some resistance to fungicides in more than 100 plant-pathogenic combinations [85]. Over the past 55 years, it has been proposed to develop agriculture under Integrated Pest Management (IPM), and this has become the main global holistic strategy for phytosanitary protection. It provides for the production of food in a sustainable agroecosystem, with the management of the soils, from the point of view of the increase in organic matter, fertility and vegetation cover, the adequate use of water for irrigation, the use of resistant varieties for different soil and climatic conditions and the use of temporal and spatial distribution of crops, the encouragement of the application of agroecology to grow food, as well as the encouragement of family agriculture, the production and preservation of creole seeds, the diversity of plant species, and reduction of pesticide use for pest and disease control as opposed to increased use of biological control. The search for biopesticides has aroused much interest from the scientific community due to the expansion of organic farming, more restrictive regulations to chemical pesticides, and the demand for healthier and safer products. Essential oils (EOs), included within the group of biopesticides of botanical origin, are complex mixtures of volatiles, mainly products of plant secondary metabolism, which comprise terpenes (mainly mono-, sesqui-, and some diterpenes) and phenolic compounds phenylpropanoids), although other groups of compounds may also occur in relevant amounts. These volatiles have aromatic components that give odor, flavor or aroma, distinct from each plant, and are part of defense mechanisms of the plant to the attack

of microorganisms. Most plant species have 1–2% EOs, but in some plant species, this value can reach 10%, as in *Ocimum basilicum* [86].

#### 4.1. Phytopathogenic fungi of agronomic interest

The antifungal properties of EOs and their constituents have been reported in several studies, most of which are due to inhibition of fungal mycelial growth in vitro. The mycelium supports all fungal activity, from the spore germination to the formation of the fruiting body, and thus represents a good indicator of fungus survival. Studies with plants of the Lamiaceae family showed positive results in the control of several phytopathogenic fungi. The EOs of oregano (*Oreganum vulgare*) and thyme (*Thymus vulgaris*) were effective against *Aspergillus niger*, *A. flavus*, *A. ochraceus*, *F. oxysporum*, *F. solani*, *Penicillium* sp., *Phytophthora infestans*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, *B. cinerea*, *Monilinia fructicola*, *Rhizopus stolonifer*, *Sclerotium rolfsii*, *Macrophomina phaseolina*, and *Pythium* sp. [87]. *R. solani*, for example, represents an important phytopathogen of agricultural crops around the world. The fungus has a host range of more than 500 species of plants, with a complex ecology and is difficult to control. Seema and Devaki [88] studied the antifungal activity of several EOs against *R. solani* and revealed that cinnamon's EO (*Cinnamomum zeylanicum* Breyne) completely inhibited the growth of the fungus at a concentration of 500 ppm. The EOs of *T. vulgaris* [89], *Salvia fruticosa* [90], *Mentha piperita* [91–94], *Monarda* spp. [95], *Calocedrus macrolepis* var. *formosana* [96], *Bunium persicum* [94] were also effective in mycelial inhibition of the fungus. In [97], it was reported that the foliar application of *Desmos chinensis* reduced the intensity of the disease caused by *R. solani* in rice. Arici and Şanlı [98] studied the EO efficiency of *Cuminum cyminum*, *Anethum graveolens*, *Salvia officinalis*, *Origanum onites*, *Rosmarinus officinalis*, and *Lavandula intermedia* against *R. solani* and *Streptomyces scabies* on potato and found that EO of *S. officinalis* reduced *R. solani* infection in 4.2%, and oregano's EO reduced the disease severity caused by *S. scabies* to 1.8%. Fusarium species are also important phytopathogens. The EO of *Artemisia absinthium* showed effectiveness against *Fusarium moniliforme*, *F. oxysporum*, *F. solani* [99]. Other positive results have also been reported in field experiments. Citral, methyl anthranilate, and nerol tested at the concentration of 5.0 ml/L reduced 78.1 and 80% of *Cercospora beticola* and *Alternaria* (*Alternaria tenuis*) in sugar beet, respectively [100]. El-Mohamedy and Abd-El-latif [101] tested the EO of *T. vulgaris* applied alone or in combination with humic acid and observed a 92.2% reduction in tomato blight caused by *P. infestans* when tested at the concentration of 6.0 ml/L. In postharvest, treatment with EOs of basil (*Ocimum basilicum* L.), fennel (*Foeniculum sativum* Mill.), lavender (*Lavandula officinalis* Chaix), marjoram (*O. majorana* L.), oregano (*O. vulgare* L.), mint (*Mentha piperita* L.), rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.), savory (*Satureja montana* L.), thyme (*T. vulgaris* L.), and wild mint (*Mentha arvensis* L.) was effective against *B. cinerea* and *Penicillium expansum* [102]. Al-Reza et al. [103] tested the EO of *Cestrum nocturnum* L. at 1000 ppm concentration and showed that EO inhibited up to 80.6% growth of *B. cinerea*, *Colletotrichum capsici*, *F. oxysporum*, *F. solani*, *P. capsici*, *R. solani*, and *S. sclerotiorum*. The EO of *C. nocturnum* also inhibited the spore germination and reduced the disease by 82–100% in pepper seedlings. Muchembled et al. [104] studied some OEs against *Venturia inaequalis* strains of apples with different sensitivities to Tebuconazole compared to the application of copper sulfate and highlighted the effectiveness of clove EOs

(*Syzygium aromaticum*), eucalyptus (*Eucalyptus citriodora*), mint (*Mentha spicata*), and savory (*Satureja montana*) with priority components such as eugenol and carvacrol. They also found that each strain of the fungus reacted differently to each treatment, indicating that each strain of the pathogen had different survival mechanisms.

## 5. Industrial applications in agronomy and agrifood industry

Essential oils are employed in agriculture, medicine, and food industries among others, due to their antimicrobial, antiviral, insecticidal, and antifungal properties. They are specially employed in agriculture, against phytopathogenic fungi such as *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizoctonia*, and other fungi, which produce many losses in agronomic crops. Also, these fungi are pathogenic to many forest species, and nowadays, we are losing many trees due to these fungi. Essential oils and their effect as antifungal agents must be approached from a biotechnological point, taking in account their genomic, proteomic, and metabolomic functioning. Finally, industrial and commercial applications are being developed, so these products can reach easily their target and have the desired effect for which they are designed. Antimicrobial volatile substances from plants, such as essential oils (EOs) present an alternative to chemical fungicides and food preservatives. Their main new uses and industrial applications of essential oils as antifungals in agronomic crops and in the agrifood industry are the pre- and postharvest treatment of vegetables such as fruits and grains in order to prevent their decay and increase their time of storage, to protect seeds against fungal attack, to prevent food spoilage due to fungal attack, and to produce active containers for vegetables and other food.

Eos are employed to avoid pre- and postharvest fungal diseases of vegetables, but their stability, solubility, and bioavailability are limited and the use of EOs as antifungal agents is limited due to the degrading ability of these volatile compounds under the action of heat, pressure, light, and oxygen. In addition, the fact that there are not water soluble limits their use in certain applications, especially when a controlled release is required [105]. Also, it must be considered that the application of these natural products may alter the characteristics of food, such as aroma or taste, so this is another factor which to be taken in account. The main ways of application of EOs as fungicidal in the agrifood industry, from crops to preservation, are emulsions, encapsulation, and vapor application. All these biotechnologies allow a good contact of the EOs with the plant, a time controlled release, and avoid the alteration of the properties of vegetables. It must be also taken in account that the antifungal effect of the EOs depends on the application method. Suhr and Nielsen [106] have studied how larger phenolic compounds such as thymol and eugenol (from thyme, cinnamon and clove) have best effect against rye bread spoilage when applied directly to the medium, whereas other smaller compounds such as allyl isothiocyanate and citral (from mustard and lemongrass) are most efficient when added as volatiles.

EOs can be prepared into **emulsions** by different techniques. **Microemulsion** of EOs is prepared with EO, Tween 20, and ethanol, and can be unlimitedly diluted with water, being stable

for long time. *Laurus nobilis* EO has been proven to be effective in cherry tomatoes applied in this way [107]. **Nanoemulsions** of thymol without carrier oil have also been studied to avoid the deployment of wheat due to *Fusarium gramineum* [108]. **Double w/o/w emulsion** type prepared lipophilic and hydrophilic emulsifiers with xanthan gum as thickener showed stability and water-dilution tolerance and retained most of the electrolytes included in the internal aqueous phase. Antifungal activity of the EOs increased, and the absence of organic solvents makes these formulations environmentally safe. Also, the property of controlled electrolyte release makes these formulations attractive [109].

The **microencapsulation** in porous materials allows direct contact between the fungus and the microparticle in the soil, which acts more efficiently against the fungus. That is, these could be put directly into the crop acting as biopesticides throughout the growth of the vegetables. Microencapsulation can be done by simple coacervation [110–111] and it has been tried already in fruits such as mango with thyme and rosemary EOs [110] and to preserve peanut seeds with *Lippia turbinata* EO [111]. Carvacrol and thymol from oregano and thyme have also been studied in microcapsules of mesoporous silica and B-cyclodextrin, together with cinnamaldehyde and eugenol from cinnamon and clove, respectively. **Nanoencapsulation** is also used to enhance antifungal activity and stability of the oils against fungi. Nanoencapsulation in chitosan nanoparticles (CSNPs) is done by an ionic gelation technique. This technique has shown a controlled and sustained release of EOs for 40 days in comparison with unmodified EOs [101]. Nanoparticle carriers of EOs, as compared to microsize carriers, show a better surface area rate, solubility, bioavailability, controlled release, and targeting of the ingredients [101]. Nanoencapsulation of EOs has been studied also for their incorporation into fruit juices to prevent fungal activity while not affecting on the quality attributes of the product [112].

**Simple vapor application** of EOs can change the sensory profile of fruits and vegetable [113–114]. EOs from cinnamon (*Cinnamomum zeylanicum* Nees.), thyme (*Thymus vulgaris* L.), oregano (*Origanum vulgare* L.), clove (*Syzygium aromaticum* L.), lemongrass (*Cymbopogon citratus* [DC] Stapf.), and ginger (*Zingiber officinale* Rosc.) have shown to inhibit the growth of *Aspergillus* spp. in oats [114]. But furthermore, there are new technologies of application of the EOs, such as the combination with **warm air flow (WAF)**, that can be used in the control of postharvest fungal pathogens of grains [115], being more effective compared to standard vapors in disc volatilization [113] with very low effect on their sensory profile.

EOs are a very good source of natural additives for **active packaging (films & coatings)**, which refers to the incorporation of additives into the packaging material, maintaining its properties without adding active agents in the food product, thus reducing the use of aggressive techniques and synthetic chemicals in food. Oregano is one of the EOs that has been positively tested in this way [116]. In that sense, chitosan **composite films** enriched with essential oils of cinnamon, thyme, clove, and lime alone or in combination have been tried against *Colletotrichum gloeosporioides* in papaya fruit. This coating can be an alternative to potentially reduce the need for cold storage during postharvest handling [117]. Edible coatings with oregano EO have been proved for the preservation of tomatoes against *Alternaria alternata* growth maintaining the sensorial acceptability of tomatoes [118].

## Author details

María Paz Arraiza<sup>1\*</sup>, Azucena González-Coloma<sup>2</sup>, Maria Fe Andres<sup>2</sup>, Marta Berrocal-Lobo<sup>1</sup>, José Alfonso Domínguez-Núñez<sup>1</sup>, Avonor Cidral Da Costa Jr<sup>3</sup>, Juliana Navarro-Rocha<sup>4</sup> and Carlos Calderón-Guerrero<sup>1</sup>

\*Address all correspondence to: paz.arraiza@upm.es

1 Department of Forestry and Environmental Engineering and Management, Technical University of Madrid, Madrid, Spain

2 Instituto de Ciencias Agrarias (ICA), Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain

3 Universidade Federal Rural de Pernambuco, Pernambuco, Brazil

4 Departamento de Ciencia, Tecnología y Universidad, Centro de Investigación y Tecnología Agroalimentaria de Aragón, Gobierno de Aragón, Zaragoza, Spain

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# Coriander and Its Phytoconstituents for the Beneficial Effects

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Alev Önder

Additional information is available at the end of the chapter

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## Abstract

Coriander (*Coriandrum sativum* L.) is a very popular medicinal plant that belongs to Apiaceae family in taxonomic classification, which is widely used as a spice and also in pharmacy and in food industries. The plant used in folk medicine, especially in Egypt, it has been known as one of the earliest spices. Coriander (Cilantro, Kışniş, Chinese parsley, *Coriandrum sativum*) is extensively recognized in almost every recipe. The genus Coriander was represented in *Flora of Turkey* by two different species called *C. sativum* L. and *C. tordylium* (Fenzl) Bornm. It is mainly cultivated for the seeds (fruits) that contain essential oil, fatty acids, coumarins, flavonoids, and polyphenols. Nowadays, the fruits exhibit internally carminative, spasmolytic, and galactagogic effects in many disorders. The use of coriander in different forms includes a wide range of fresh and dried herbs. The current section focuses on coriander oil in different forms, including the plant, its terpenoid profile, and all the miracle effects of coriander together with future prospects.

**Keywords:** Apiaceae, coriander, *Coriandrum sativum*, Kışniş, oil

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## 1. Introduction

Medicinal and aromatic plants have been very popular in all the time for the culinary, medicinal, and many other purposes. The plants and their secondary metabolites (phytochemicals) take part in increasingly in foods, in health, and in nutritive products. The essential oils are the most popular secondary metabolites of the plants, used for thousands of years (over 5000 years) regarding the variety of objectives, principally for their health benefits [1]. In the history, the term essential oil dated back to the sixteenth century and comes from the drug Quinta essentia, named by Swiss physician Paracelsus von Hohenheim of Switzerland.

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Essential oil or essence is referred to by this name due to their flammable principle. In many kinds of the literature, the essential oils have been defined [2]. Briefly, essential oils are secondary metabolites biosynthesized in different plant organs [3] obtained by mainly hydrodistillation from almost all parts of the aromatic plants growing temperate regions in the world, of the secretory special elements with volatile properties along with characteristic fragrances, as limpid and rarely colored, soluble, in organic solvents [4]. The chemical composition of the essential oils is quite complex including mostly terpenes (monoterpenes and partly sesquiterpenes formed by isoprene) and aromatic compounds derived from phenylpropane and phenolic constituents [3, 5]. The essential oils known as fragrant oils, steam volatile liquids, or semiliquids, ethereal oils are concentrated hydrophobic aromatic oil. The flavor (fragrance and flavor) of the essential oils is marvelous due to reflecting their corresponding sources as mean basically plant [3]. The physiological effects of the essential oils are not clear but can say that protect the plants against microorganisms, insects, or attract them for the pollination [5]. The conventional methods to obtain the essential oils are hydrodistillation, steam distillation, solvent extraction, Soxhlet extraction, cold pressing method, enfleurage, cohobation, and maceration. By the way, distillation is absolutely the most common method of obtaining essential oils. In addition, innovative techniques can be covered supercritical fluid extraction (SFE), microwave-assisted hydrodistillation (MAHD), ultrasound-assisted extraction (UAE), solvent-free microwave extraction (SFME) and microwave hydro diffusion and gravity (MHG) [3]. Analytical monographs have been published by European Pharmacopeia (EP), International Organization of Standardization (ISO), World Health Organization (WHO), Council of Europe (COE) to ensure good quality of essential oils. The essential oils are exhibited in many important biological activities [1–5] and discussed in many kinds of the literature but mainly antibacterial and antifungal activities are dominated [6]. Essential oils are also commercially important, especially in the pharmaceutical, agronomic, food, sanitary, and cosmetic and perfume industries. Moreover, essential oils are the main therapeutic agent in aromatherapy as it forms mixtures with vegetal oil in several forms [3, 7, 8]. Essential oils are classified as “Generally Recognized as Safe” (GRAS) by the Food and Drugs Administration (FDA); therefore, they are not counted as harmful and, due to their natural origin, are more widely endorsed by consumers than “synthetic” agents [6], if it is used reasonably and carefully. In fact, there are so many things to say regarding essential oils, but this section will be interested in coriander as aromatic plant and its essential oil. Hence, in this chapter, we discuss the essential oil of coriander, which is very important in many fields called as food, spice, cosmetic, and of course pharmaceutical. Therefore, you can find much information regarding coriander, which has great importance almost in every field. Consequently, the coriander will be handled with the latest articles in details according to an order.

## 2. Methodology

The phytochemical constituents and pharmacological activities regarding the coriander and its essential oil have been investigated with the help of PubMed, Science Direct, Scopus, and Google. Generally, the latest articles were used when writing this review in this process.

### 3. Description of coriander

*Coriandrum sativum* L. (Coriander) is a member of the Apiaceae family (previously Umbelliferae) [9, 10] and known as cilantro, cilantrillo, Arab parsley, Chinese parsley, Mexican parsley, Dhania, and Yuen sai. It is an annual herb commonly used in Middle Eastern, Mediterranean, Indian, Latin American, African, and Southeast Asian cuisine [11]. Although cilantro and coriander seem to describe the same thing, it actually carries different meanings. When the plant is freshly harvested, the green leaves of the plant is called cilantro; if the dried fruits are used, the herb is called as coriander [12].



**Figure 1.** Coriander (The leaves and flowers from nature). Photo: M. Necat IZGI.

The plant grows all over the world [13], but originally from the Mediterranean regions, cultivated mainly in the tropical areas such as Ukraine, Russia, Romania, Morocco, Mexico, India, and Argentina [14]. Especially in a cool and comparatively dry frost, the free climate at the optimum temperature of germination and early growth of coriander is 20–25 °C. It grows best in dry climates, but it can grow in any type of soil like light, well-drained, moist, loamy soil, and light to the heavy black soil [15].

The genus *Coriandrum* L. (**Figure 1**) has been represented by two species in Flora of Turkey called *C. sativum* L. and *C. tordylium* (Fenzl) Bornm. [10]. *C. sativum* L. is coriander, approximately 30–100 cm in height, with strong-smelling leaves. It is cultivated as a domestic plant. In addition, in commerce, coriander has two varieties such as *C. sativum* L. var. *microcarpum* DC, the small-fruited species called as oil-rich Russian coriander and *C. sativum* L. var. *vulgare* Alef., the larger-fruited species with low oil content called as Moroccan, Indian and some other Asiatic types [16, 17]. The coriander has been described as glabrous aromatic, herbaceous, erect annual plant with a pronounced taproot, and slender branching stems up to 20–70 cm

in height. The leaves are lanceolate, green or dark green, glabrous on both surfaces and are variable in shape and lobed. The flowers are arisen in small umbels, white or light pink, asymmetrical, with the petals pointing away from the center. The coriander fruits are almost ovate globular dry schizocarp with two mericarps as usual and multiple longitudinal ridges on the surface possessing a sweet, slightly pungent, citrus-like flavor resembling sage [10, 17].

#### 4. History of coriander

*Coriandrum sativum* L. has a long history as a culinary herb [17]. Sanskrit literature before 5000 BC and Greek Ebers Papyrus earlier 1550 BC have been mentioned from this herb. The coriander name comes from the “korannon,” which is derived from the words “koris” and “annon” in Greece. By the way, the genus name as “Coriandrum” the first used by Pliny the Elder [18, 19]. It was said that the coriander seed emerged in the Neolithic level of the Nahal Hemel Cave in Israel. On the other hand, interestingly about one-half liter of coriander seeds was found in the tomb of Tutankhamun (Ramesses II) in Egypt. In Egypt, the herb was known as “spice of the happiness” may be attributed to its aphrodisiac property. In Greece, coriander seems to have been cultivated, since at least the second millennium BC, where the plant was used in perfumes and in cooking besides used as traditional medicine by Hippocrates [12, 19]. The herb was also widely used in the Roman Empire. As an example, Apicius (a collection of Roman cookery recipes) includes some 70 recipes using coriander in his cookbook. In Germany, coriander was used about ~900 AD [12]. The first factory to obtain the essential oil of coriander by the steam distillation was built in Russia in 1885 in the Voronezh district [20]. I came across a section regarding coriander written by Dioscorides in the literature. As shown below, Dioscorides emphasized how important it is [12]:

Dioscorides\* wrote about *Coriandrum sativum*.

III. 63. κοριον The coriander: it has a cooling property, wherefore when plastered on with bread or barley groats, it cures erysipelas and shingles; with honey and raisins, it treats pustules that are most painful at night, testicular inflammations, and carbuncles; and with bruised corn, it dissolves scrofulous swellings of the glands and tumors. A small quantity of its seed drunk with grape syrup expels intestinal worms and furthers the production of semen, but if too much is taken, it dangerously disturbs the thinking process; this is why one must guard against drinking it to excess and continuously. Anointed with white lead or with litharge, and with vinegar and unguent of roses, the juice benefits surface tumors that are inflamed.

\*Source: Beck LY, transl. Pedanius Dioscorides of Anazarbus. In: The Materia.

Medica: Ancient Scientific Texts and Studies, vol. 38 [in German]. Hildesheim, Germany: Olms-Weismann.

#### 5. Phytochemical constituents of coriander

*Coriandrum sativum* L. is a medicinal plant native to the eastern Mediterranean where it may have spread like many other aromatic plants to India, China and rest of the world [17]. In this frame, the essential oil is the main secondary metabolite of coriander. However, a different group of active substances will also be mentioned in present compilation.

The fruits contain sugars, alkaloids, flavones, resins, tannins, anthraquinones, sterols, and fixed oils [21, 22]. We can say that the most important constituents of coriander fruits are the essential oil and fatty oil [23]. The fatty acids in coriander fruits are petroselinic acid (*cis*-6-octadecenoic acid, 18:1), linoleic acid (18:2), oleic acid (18:1), and palmitic acid (16:0) [16, 17]. It was reported that coriander like all other green leafy vegetables is a rich source of vitamins (high amount of vitamin A/ $\beta$ -carotene: 12 mg/100 g and vitamin C: 160 mg/100 g) besides minerals and iron [24], very low in saturated fat and cholesterol and a very good source of thiamine, zinc, and dietary fiber. Unripen coriander contains 84% water [15]. Here, the most important phytoconstituents will be mentioned in the order.

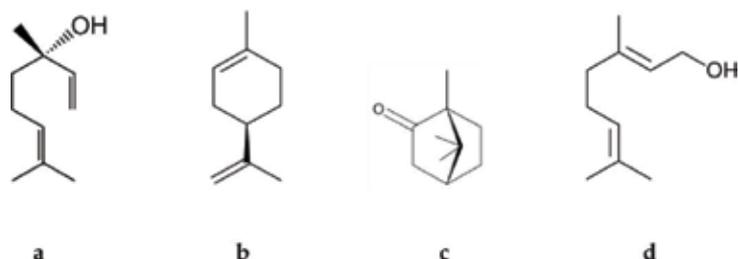
### 5.1. Essential oils

There is much work to be performed collected from different localities of coriander essential oil. Because of the fact that, the coriander is one of the most important essential oil-bearing spices in its leaves, flowers, stem, seeds, roots, and bark; however, the composition of the parts can be different. Its chemical composition undergoes changes during ontogenesis, which affects the aroma of the plant, and thus interestingly the coriander fruit (seed) aroma is completely different from the aroma of the herb. Immature fruits and leaves have an unpleasant odor called a “stink bug smell” which is due to the presence of *trans*-tridecen in the oil [17]. On the other hand, the fragrance in the mature fruits pleasantly is similar to citrus peel and sage [16, 17]. By the way, Burdock et al. have mentioned about specifications of coriander oil according to the Food Chemical Codex (FCC) as given below [19]:

Characteristics	Metrics
Angular rotation	Between +8 and +15
Appearance	Colorless or pale yellow liquid
Heavy metals (as Pb)	Passes test
Identification	Infrared absorption spectrum
Odor	Characteristic of coriander
Solubility in alcohol	Passes test. 1 ml dissolves in 3 ml of 70% alcohol
Specific gravity	Between 0.863 and 0.875
Refractive index	Between 1.462 and 1.472 at 20°C

Coriander oil specifications according to the FCC, 2003 [19].  
 FCC = Food Chemicals Codex.

Coriander fruits contain about 0.2–1.5% of essential oil and 13–20% of vegetal oil (fixed oil); however, it has been recorded that some cultivars contain essential oil up to 2.6% [15, 25]. Another literature mentioned that coriander contains up to 1% essential oil where monoterpenoid linalool is the major compound (>50%), and limonene, camphor, and geraniol (**Figure 2**) are present in significant quantity [26]. Sometimes, the essential oil of the coriander obtained from the fruits was recorded approximately as 0.5–2.5%. It seems that different cultivars and



**Figure 2.** The major constituents in the essential oil of coriander fruit: (a) linalool, (b) limonene, (c) camphor, and (d) geraniol.

regions have been present in a different ratio of the essential oil concentration. However, in the majority of studies the main component is defined as linalool (60–70%) [16, 17]; sometimes up to 87.54%. In addition,  $\alpha$ -pinene, camphor and geraniol are also known as other important components and are responsible for the character of fragrance and aroma of the plant. The investigation on two coriander varieties (*vulgare* and *microcarpum*) from Turkey has resulted in oil content like 0.15–0.25% in *vulgare* (linalool 42.1–52.7%); and 0.31–0.43% in *microcarpum* (linalool 63.5–71.0%) [9]. In Iran, the essential oil from the dried fruit of the coriander has been found in the range of 0.1–0.36% represented by 34 compounds, linalool (40.9–79.9%) as major component [27].

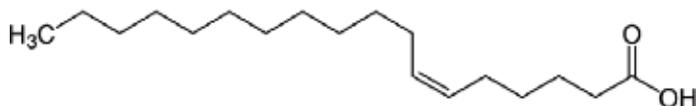
In a study, the essential oil of coriander was obtained by supercritical water extraction, hydrodistillation, and Soxhlet extraction methods. The main component found was linalool (82.916%) chosen as the key component to find the best-operating conditions [20]. In fact, the parameters exhibit different impact to reach the volatile oil; pressure and temperature control influenced the yield and composition. In an example, supercritical CO<sub>2</sub> fluid extraction to obtain the volatile oil from Italian coriander fruits was performed under different temperature conditions. A decrease in the particle size improved the volatiles' yield so as to more ducts were destroyed during the milling process. Optimum supercritical fluid extraction conditions were found to be as follows: Pressure 90 bar, temperature 40°C, 1.10 kg/h and 0.6 mm. The compositions of each supercritical fluid extraction samples were analyzed by GC and GC/MS. The major components were linalool (65–79%), the others  $\gamma$ -terpinene (4–7%), camphor (3%), geranyl acetate (2–4%),  $\alpha$ -pinene (1–3%), geraniol (1–3%) and limonene (1–2%) [28]. Linalool was determined as the highest percentage composition in the essential oil of *C. sativum* fruit (73.11%) [29], but this rate can vary during different maturation periods. Essential oil obtained by hydrodistillation increased markedly during the maturation process, and geranyl acetate (46.27%), linalool (10.96%), nerol (1.53%) and neral (1.42%) were the major compounds at the first stage of immature fruits. At the middle stage, linalool (76.33%), *cis*-dihydrocarvone (3.21%) and geranyl acetate (2.85%) were reported as the main constituents. Essential oils at the final stage of maturity (mature fruits) include mainly of linalool (87.54%) and *cis*-dihydrocarvone (2.36%) [30]. In another study, the volatile oils of coriander have been compared each other by conventional methods such as hydrodistillation and solid-liquid extraction with methylene chloride and newer methods such as supercritical fluid extraction or subcritical water extraction which are safe environmentally. The highest crude extract was obtained by Soxhlet extraction and supercritical fluid extraction on the optimization conditions of 300 bar and 40°C (14.45% and 8.88%, respectively), while

subcritical water extraction on 100°C provided the lowest yield of lipid extract (0.36%). Similarly, the major compounds from coriander oil [ $\gamma$ -terpinene, (+)-limonene, linalool, camphor, and geraniol] were obtained by Soxhlet extraction (785.05, 26.73 and 21.96 mg/100 g of coriander fruits, respectively). By the way, subcritical fluid extraction provided extraction of vegetal oil, while polyphenolics were also extracted by subcritical water extraction, increasing health value of obtained extracts, and presenting good alternatives for traditional techniques [26]. Although the examples given are reproduced, the volatile oil content and their rates are almost the same.

## 5.2. Fatty acids

The vegetal oil (fixed oil/fatty oil) of coriander fruits, especially from French origin (23% yield), contain a high amount of monounsaturated fatty acids (1.8%); particularly petroselinic acid (73%) [22, 26]. The vegetable oil of coriander has been labeled as a Novel Food Ingredient (NFI), and it can be safely consumed as a food supplement by healthy persons, in maximum 600 mg/day dosages [22]. That is why, the essential oil of *C. sativum* fruits is called as triglyceride oil, due to the presence of petroselinic acid (**Figure 3**). The plant is recognized a potential source of lipids (rich in petroselinic acid) and essential oil (high in linalool) isolated from the fruits and the aerial parts [17]. The presence of petroselinic acid makes coriander more precious and interesting.

Petroselinic acid (18,1n-12) is classified as a monounsaturated the major omega-12 fatty acid exists in coriander oil, is a positional isomer of octadecenoic acid (its double bond being in position 6 instead of 9). Petroselinic acid has been detected at a level between 68 and 83% in coriander oil [31], or between 65–70% and 80.9% [9]. In a study, changes in fatty acids during maturation of coriander fruits cultivated in the North-East of Tunisia (Charfine) were studied. At full maturity, the main fatty acids were petroselinic acid ( $80.9 \pm 5.7\%$ ), followed by linoleic ( $13.6 \pm 2.9\%$ ), palmitic ( $3.6 \pm 0.1\%$ ), and stearic ( $0.7 \pm 0.1\%$ ) acids. During maturation of coriander fruit, saturated and polyunsaturated fatty acids decrease significantly, and monounsaturated fatty acids increase. It is necessary to underline that coriander fruits at the first four stages of maturity have a healthy nutritional value and the last five stages were with important economic and industrial applications [32, 33]. Commercial production of vegetable oils from oil-rich materials is based on through two traditional processes like the mechanical expression and extraction. In this manner, the maximum yield was obtained with single screw extruder for a configuration allowing the strongest oil expression. Comparing with a mechanical press, the maximum yield was obtained by the Soxhlet extraction with 21.25%, and the oil quality was found high grade. The acid value was under 1.8 mg of KOH/g of oil and iodine values were tolerable (44 mg of iodine/100 g of oil). In the oil, nine fatty acids were identified, with petroselinic acid accounting for 74–77% of the total fatty acids, followed by linoleic (12–13%), oleic (4–6%), and palmitic acids (3%). Moreover,  $\beta$ -sitosterol was the major sterol (28%) in all oils. The next major sterols in all oils were stigmasterol (24–27% of total sterols) and  $\Delta^7$ -stigmasterol (14–18% of total sterols) [9, 34, 35].



**Figure 3.** The structure of petroselinic acid.

### 5.3. Polyphenols

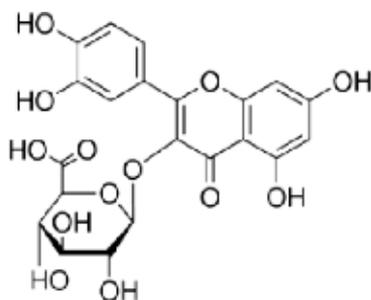
The polyphenols are very important secondary metabolites in coriander fruits and leaves because of their strong biological activities. Generally, flavones, tannins, and anthraquinones have been mentioned as the fruits phenolic constituents [21]. In coriander leaves, some phenolic compounds could be tentatively characterized using LC/MS. The polyphenol profile between leaves and fruits have been detected, and quercetin-glucuronide (**Figure 4**) was found as the major compound in plant parts, leaves and fruits [36]. Moreover, coriander leaves contain high amounts of caffeic, ferulic, gallic, and chlorogenic acids [12].

### 5.4. Carotenoids

Commercial varieties of coriander were analyzed by HPLC/MS and evaluated for carotenoids as  $\beta$ -carotene (vitamin-A precursor). In all varieties grown on similar conditions,  $\beta$ -carotene content was found higher in foliage at a mature stage, than in seedlings and seeds. For example, one of the varieties produced the highest biomass ( $6.18 \pm 0.73$  g/plant), total carotenoids ( $217.50 \pm 5.6$  mg/100 g DW) and  $\beta$ -carotene ( $73.64 \pm 0.3$  mg/100 g DW) at the pre-flowering stage. When the drying conditions are compared, microwave drying of foliage with oven drying one; the results showed that microwave drying protects pigments and trans- $\beta$ -carotene [37]. The carotenoid content such as  $\beta$ -carotene (**Figure 5**),  $\beta$ -cryptoxanthin epoxide, lutein-5,6-epoxide, violaxanthin, and neoxanthin from the ether extract of coriander, the  $\beta$ -carotene was represented 61.14% of the carotenoids detected in the extract [38].

### 5.5. Isocoumarins

There are no recent articles regarding the isocoumarins from coriander. The isocoumarins, coriandrone A and B, together with coriandrin (**Figure 6**) and dihydrocoriandrin were isolated from the aerial parts of *C. sativum* [39]. In addition, coriandrone C-E were also isolated from the methanolic extracts of the aerial parts of the coriander cultivated in the botanical garden of the Osaka University of Pharmaceutical Sciences [40]. Extracts of coriander were analyzed for photoactive constituents using HPLC and photobiological assay. Similarly, photoactive furoisocoumarins named as coriandrin and dihydrocoriandrin were also obtained, and their structures determined by  $^1\text{H}$ ,  $^{13}\text{C}$  NMR and X-ray crystallography [41].



**Figure 4.** The structure of the quercetin-glucuronide.

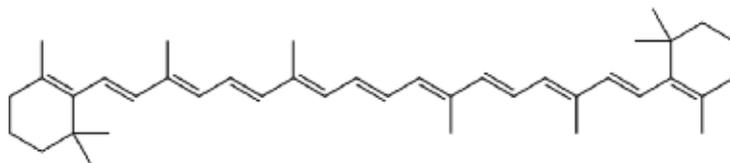


Figure 5. The structure of  $\beta$ -carotene.

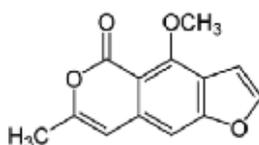


Figure 6. The structure of coriandrin.

## 6. Biological activities and usages of the coriander

In the food industry, coriander is approved in food-use by the Food and Drug Administration (FDA in the USA), the Flavor and Extract Manufacturers Association and the Council of Europe. The plant can be mainly used as a spice, medicine, and raw material in food, beverage and pharmaceutical industries [16, 17]. Microwave-drying characteristics of coriander leaves were examined in designing and modeling heat and mass transfer processes during storage and other possible operations, necessary in food and chemical industry [42]. Moreover, the encapsulation (400 nm–7  $\mu$ m) of the essential oil of coriander with chitosan obtained from the waste shells of crayfish (*Astacus leptodactylus*) has been arranged via the spray drying method led to the much higher antimicrobial activity and antioxidant activity than the oil and the pure chitosan. The created product could be beneficial in the food and pharmaceutical industries as a natural antioxidant and antimicrobial agent [43]. One of the reasons for being famous as a spice in the food industry is to use as a common flavoring substance for the pleasant aromatic odor of their stems, leaves, and fruits. That is why it is used in seasonings for sausages, and other meat products besides in baked goods, beverages, sweets, chewing gums, tobacco products, condiments, preservative, and also functions as an essential ingredient in curry powder. On the other hand, it should not be forgotten that coriander and its oil are used especially in perfumery and cosmetics. The warm and sweet notes of coriander oil mixed with other floral notes for oriental type perfumes have a different effect. It is used not only in perfumes but also in soaps, creams, lotions and in aromatherapy as much as covering the taste of some medicines [19]. The leaves are used for flavoring curries, sauces, and soups, while the whole young plants are used for preparing chutneys. All parts of *Coriandrum sativum* are edible and used as a spice in daily life [44]; however, its fresh leaves and dried fruits are the most frequently used parts. Green foliage, known to be rich in proteins, vitamins, and minerals (such as calcium, phosphorus, and iron), fibers and carbohydrates, are reportedly used as vegetables in salads. While the coriander fruits are very popular as health-supporting as the healing spices, the essential oil of coriander can be marketed as a food supplement at a maximum dosage of 600 mg per day. On the other hand, the fruits are a rich source of lipids (fatty acids) (28.4%), which may be of great

importance in the food industry. Petroselinic acid (18:1), linoleic acid (18:2), oleic acid (18:1), and palmitic acid (16:0) are valuable fatty acids of coriander fruits [16, 17]. Petroselinic acid is an important fatty acid to show substantial biological effects. It significantly increased the production of anti-inflammatory precursor and decreased pro-inflammatory precursor [13], and it has the capability to the reduction of arachidonic acid in the heart and liver of rats. When petroselinic acid was orally administered, the overproduction of arachidonic acid has been restricted [45]. Moreover, it has antimicrobial properties and can be a competitive inhibitor of topoisomerases; therefore, it is a potential agent in the treatment of cancer [46]. Topoisomerases modify the structure of DNA and play a role in the progression of several proliferative diseases [45]. In addition, the major components of the essential oil of coriander, linalool has antioxidant, anticancer, neuroprotective, anxiolytic, anticonvulsant, relieving migraine, analgesic, hypoglycemic, hypolipidemic, blood pressure lowering effects [47]. A syrup prepared by the ethanolic extract of the coriander fruits exhibited short-term considerable effects on migraine reducing duration and frequency of the migraine attacks and diminishing pain degree, in a month [48].

In short, coriander fruits and its oil have been used for many diseases [13] such as for the treatment of rheumatism, gastrointestinal upsets, insomnia, flatulence, and joint pain in humans [49]. Moreover, coriander has a positive influence on lipid profile in plasma of rats [50]. The hydroalcoholic extract of the coriander fruits has exhibited anti-inflammatory, and anti-granuloma properties are reducing TNF-R1 expression on peritoneal macrophages in an experimental model [51]. The fruits of the plant are famous for carminative, diuretic effects and used in the treatment of cold, fever, nausea, and stomach disorders [16]. The fruit extract has been found as a strong analgesic agent than dexamethasone [52]. Laribi et al. discussed with all aspects regarding the pharmacological effects of coriander in a review [9]. In this manner, the most frequent effects of coriander will be debated in an order.

### 6.1. Antioxidant effects

The antioxidant potencies of polyphenolic compounds from *Coriandrum sativum* against hydrogen peroxide-induced oxidative damage in human lymphocytes have been investigated in some articles. It was seen that the treatment with polyphenolic fractions (50 µg/mL) was increased the activities of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione-S-transferase) and glutathione content. On the other hand, it reduced the levels of TBARS significantly [53]. The aqueous coriander extracts (2.734 mg) of total phenolics (catechin equivalents) per 100 g of a dry sample exhibits considerable antioxidant activity according to the  $\beta$ -carotene/linoleic acid model. The caffeic acid (4.34 and 2.64 mg/mL), protocatechuic acid and glycitin (6.43 and 3.27 mg/mL) were found in high concentration in the aqueous extract [54]. Moreover, it seems that subcritical water extraction showed significant improvement comparing the yields of phenolics and flavonoids obtained with this technique against conventional solid-liquid extraction and modern extraction techniques, such as ultrasound-assisted and microwave-assisted extraction. When the temperature, pressure and extraction time were optimized, the highest yields and lowest  $IC_{50}$  were observed. Total phenolics and total flavonoids content were maximized, while  $IC_{50}$  value was minimized, and optimum conditions were determined using desirability function. The most efficient extraction conditions for all three responses were the temperature of 200°C, the pressure of 30 bar and extraction time

of 28.3 min [55]. In another study, the hot water extract of the coriander fruits has been evaluated for their antioxidant characters [56]. The aerial parts of the hydroalcoholic extract of *C. sativum* showed important antioxidant activity besides anticonvulsant effects [57]. The antioxidant activity of the essential oil of coriander conducted by different types of antioxidant tests have been investigated, and the total phenolic content of the sample is found the 52.3 mg GAE/L [17, 58].

The potency of the antioxidant activity of coriander is indeed attributed to its carotenoid content. Carotenoid extract of the coriander showed high antioxidant activity with an  $IC_{50}$  value of  $14.29 \pm 1.68$   $\mu$ g/mL, scavenging hydroxyl radicals and providing higher protection to DNA than by standard gallic acid ( $IC_{50} = 357.21 \pm 4.29$ ) [37]. It was seen that there was a synergistic action between the carotenoids compared to the crude extract [38]. Obviously, the aqueous or alcoholic extracts of the coriander have significant antioxidant capacity depending on the polyphenolic content. Carotenoids in coriander show higher antioxidant capacity in the extracts.

## 6.2. Antimicrobial effects

The antimicrobial activity of the coriander has been arisen from the essential oil content. The essential oil of coriander has been exhibited potent antimicrobial activity against oral pathogens, and a dental gel formulation [59]. Moreover, the aqueous extract of coriander was found to have potency against acne-inducing bacteria (MIC values are 1.7 mg/mL for *Propionibacterium acne* and 2.1 mg/mL for *Staphylococcus epidermidis*). The formulations commercially developed for the treatment of acne, showed the same activity [60]. The development of advanced anti-acne formulations, the coriander oil is the good options due to the antibacterial activity [61]. Coriander is also found as an important herbal remedy for its antioxidant, anti-inflammatory, analgesic and antimicrobial properties of diaper dermatitis which is a common dermal disorder [62]. Moreover, coriander oil exhibited powerful activity against *Bacillus cereus*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella typhi*, and *Acinetobacter baumannii* with different degrees of inhibition. *Bacillus cereus* was the most sensitive strain along with one of the multidrug-resistant clinical strains of *Acinetobacter baumannii* (MIC 50.1%, v/v), while *P. aeruginosa* was the most resistant to growth inhibition by the tested oil, showing the highest determined MIC (1.6%, v/v), with the exception of *B. cereus* and *E. faecalis*. On account of this, the use of coriander oil can be encouraged in antibacterial formulations owing to the fact that coriander oil effectively kills pathogenic bacteria related to foodborne diseases and hospital infections [63]. In many studies, coriander essential oil was investigated for the antibacterial and antifungal activity and has been found a good potency of the antimicrobial activity [17, 58, 64]. The antifungal activity of the essential oil obtained by hydrodistillation from *Coriandrum sativum* has been examined against different *Candida* species and exhibited antifungal activity against all tested species of *Candida*, except for *C. tropicalis* CBS 94. Hence, it was concluded that the oil could be used as potential antimicrobial agents to treat or prevent *Candida* yeast infections [65]. In another study, the essential oil of *C. sativum* leaves growing in Kenya showed antimicrobial activity against clinical isolates of Gram (+) (*S. aureus*, *Bacillus* spp.) and Gram (-) (*E. coli*, *Salmonella typhi*, *K. pneumoniae*, *Proteus mirabilis*, *P. aeruginosa*) bacteria but not a pathogenic fungus, *C. albicans* [66]. It was observed that the fruit oil of coriander displayed very good activity against *S. aureus*, *S. haemolyticus*, *P. aeruginosa*, *E. coli*, and *Listeria monocytogenes* [12].

In addition, the coriander essential oils which are obtained by hydrodistillation and microwave-assisted hydrodistillation have been compared for their antimicrobial potency. No distinctive activity differences were found except for energy and time savings [67].

### 6.3. Antidiabetic effects

In many articles, we can find the antidiabetic effects regarding the coriander. In fact, coriander has been confirmed as an antidiabetic remedy. The studies have confirmed the antihyperglycemic effect of coriander in streptozotocin-diabetic mice. The mechanism of action of the antihyperglycemic action of the aqueous extract of the coriander fruits is connected with stimulation of insulin secretion, enhancement of glucose uptake and metabolism by muscle. In general, the effect is generated by one or more components existed in the extract. Therefore, *C. sativum* is acceptable as a possible antihyperglycemic dietary supplement and can be accounted for a potential source of a new orally active agent for diabetes [68]. In another study, a single dose of coriander fruit-extract or glibenclamide suppressed hyperglycemia in obese-hyperglycemic-hyperlipidemic *Meriones shawi* rats. After administration, the insulin resistance significantly decreased in the rats. Interestingly, the hypoglycemic effect was lower in normal rats, its mean; the test substances reduced plasma glucose, insulin and insulin resistance, cholesterol, LDL-cholesterol, and triglyceride [69]. Moreover, it was observed that a dose of coriander fruit decrease and regulate blood sugar and dyslipidemia at typical traditional doses in the patients who have noninsulin-dependent diabetes mellitus. In a study of 40 volunteers, 20 subjects took 2.5 g of ground coriander fruit twice daily for 60 days and 20 volunteers served as controls. The treatment group had a significant declining in fasting blood-sugar levels; a significant reduction in lipid peroxidation in red blood cells; and rises in serum  $\beta$ -carotene, vitamin A, vitamin C, vitamin E, and glutathione levels [12]. In addition, the animals in the two groups showed almost similar weight gain, and the diet consumption was similar in both groups. There is a significant decrease in fasting blood glucose level and increase in the concentration of hepatic glycogen in the rats of the experimental group. Hexokinase and phosphoglucomutase activity increased significantly in the liver of rats administered coriander fruits. The glycogen synthase activity in the liver was increased, and that of glycogen phosphorylase showed a decrease in the rats of the experimental group compared to the control group. Significant reduction in glucose-6-phosphatase activity was observed in the experimental group, whereas glucose-6-phosphate dehydrogenase activity showed a significant increase [70]. In this frame, coriander, especially the fruits of the plant found in the receipts can be also acceptable for the treatment of hepatic fibrosis and chronic liver diseases [71].

### 6.4. Anxiolytic effects

The aqueous extract of coriander fruits has an anxiolytic activity and may possess sedative and muscle relaxant effects dose-dependently in mice. Its utility in clinical applications may be similar to that of diazepam. The effect of coriander at a dose of 100 mg/kg in mice was found almost equivalent to that of 0.3 mg/kg diazepam on the plus-maze test. According to the studies on animal and on human, 7.5 g dry extract of coriander fruit may be suggested as effective dose for a 75 kg adult man. This corresponds to an infusion of approximately 20 g of coriander fruit in 100 mL water, considering the yield of the extract in the range of the coriander doses, tentatively used in traditional medicine. However, the effect of the extract in a clinical application was not determined to reach the optimum therapeutic dose for a human [49].

For example, the aqueous extract of coriander fruits (200, 400, and 600 mg/kg per day), alcohol extracts (400 and 600 mg/kg per day), and essential oil (600 mg/kg per day) increased pento-barbital-induced sleeping time. In a mouse study, coriander fruits (50, 100, and 200 mg/kg) was compared with diazepam (0.5 mg/kg) in animal models of anxiety. Coriander fruits were almost recorded equivalent to diazepam as an anxiolytic at the two higher doses [12].

### 6.5. Cardioprotective effects

The hydro-methanolic extract of coriander fruits has been found cardioprotective potential. This effect should be attributable to its high polyphenol content in the fruits likewise. The preventive effect of coriander on cardiac damage has been investigated by isoproterenol induced cardiotoxicity model in male Wistar rats and found that the methanolic extract of the fruits prevent myocardial infarction by inhibiting myofibrillar damage on rats [72]. The coriander fruits caused a significant decrease in all cholesterol-associated lipids, while the extract reduced high-density lipoprotein (HDL) cholesterol; the extract also improved the cardioprotective indices. Coriander fruits also reduced dyslipidemia in rabbits. All blood-fat values improved significantly with the coriander diet. It means that the extracts have beneficial profits on cardioprotective effect [12].

### 6.6. Anthelmintic effects

The anthelmintic activities (*in vitro*) of crude aqueous and hydroalcoholic extracts of the fruits of *Coriandrum sativum* were investigated on the egg and adult nematode parasite called as *Haemonchus contortus* and the aqueous extract of coriander for *in vivo* anthelmintic activity in sheep infected with *Haemonchus contortus*. Both extract types inhibited completely leaving eggs at a concentration less than 0.5 mg/mL. ED<sub>50</sub> of aqueous extract was found 0.12 mg/mL while that of the hydroalcoholic extract was 0.18 mg/mL [73]. Moreover, all essential oil dosages showed a significant level of toxicity to the *Sitophilus granarius* (an insect) after 5 days in chickpea grains [29].

### 6.7. Antiulcer effects

Coriander is a potential herb to protect the body against absorption of heavy metals and other dietary toxins. Moreover, the herb can be able to prevent the formation of gastric ulcers and *Helicobacter pylori*. In a study, the antigastric ulcer and antisecretory activity of coriander have been confirmed and concluded that the effect might be linked to the antioxidant property of different constituents present in Coriander, involved in scavenging of the reactive oxygen species on the surface of gastric mucosa, or might also form a protective layer by hydrophobic interactions. That is why, it protects the cells from gastric injury [74]. In a recent work, the animal study showed that coriander fruits (250 mg/kg and 500 per os) protected the animals against the ulcerogenic effects of salt, sodium hydroxide, ethanol, indomethacin, and pylorus ligation dose-dependently [12].

### 6.8. Antiaging effects

The long chain fatty acids are potentially beneficial in antiaging products for local use, helping to restore barrier properties of the epidermis and prevent moisture loss. Therefore, the long chain fatty acids can be considered as potential antiaging agents. Coriander fruit oil is very

rich in these types of the fatty acids. The studies done as a topical treatment for a variety of skin conditions with coriander-fruit oil and as a component of herbal sunscreens seem very impressive [12]. The oil may contain ceramides of petroselinic acid as well. The extract also functions as an anti-irritant and helps to maintain skin texture and tone. A specially prepared extract from coriander fruits such as Umbelliferin® (INCI: *Coriandrum sativum* (coriander) extract is a trademarked product containing petroselinic acid triglycerides obtained as a nonlauric fraction from coriander fruit oil) helps in supporting skin barrier functions [45]. Preparations using coriander/oil as single form or in combination with the other plants can be developed in the future and may become famous as one of the secrets of staying young for a long time.

### 6.9. Anticancer effects

The biochemical effect of coriander fruits on lipid parameters in 1,2-dimethylhydrazine induced colon cancer has been studied in rats. The concentrations of cholesterol and cholesterol to phospholipid ratio declined while the level of phospholipid increased significantly in 1,2-dimethylhydrazine control group compared to the coriander administered group. Fecal dry weight, fecal neutral sterols, and bile acids showed a sharp increase in the coriander-fed group compared with the DMH-administered group. Thus, it seems that the coriander plays a protective role in the lipid metabolism of colon cancer [75]. Although there are not many studies on the anticancer effect of coriander, there are some studies based on antioxidant effect.

### 6.10. Diuretic effects

There is no more study on diuretic effect of coriander to mention in this compilation. In a study, the aqueous extract of coriander fruits was implemented by continuous intravenous infusion (120 min) at two doses (40 and 100 mg/kg) under anesthetize conditions. A diuretic-Furosemide (10 mg/kg) has been used as the standard drug. In the assay, water and electrolyte excretion (sodium, potassium, and chloride) were measured in urine, and glomerular filtration ratio (equal to creatinine clearance) was determined. The crude aqueous extract of coriander fruits increased diuresis, excretion of electrolytes, and glomerular filtration rate in a dose-dependent way; but furosemide was found more potent as a diuretic and saluretic. By the way, the mechanism of action of the plant extract appears to be similar to that of furosemide. In Moroccan pharmacopeia, the coriander is listed and indicated that the aqueous extract of coriander fruits has diuretic and saluretic activity verifying the use of coriander as a diuretic plant [11].

## 7. Coriander in traditional medicine

All parts of this herb are in use as a flavoring agent (culinary purposes) and/or as traditional remedies for the treatment of different ailments in the folk medicine on different civilizations [15, 76] especially in digestive disorders. The fruits of this herb are very popular as a spice in Mediterranean countries [9]. Hippocrates (460–377 BC) used coriander in ancient Greek Medicines. Decoction and tincture of powdered fruits of *C. sativum* alone or in combinations

with other herbals are recommended for dyspeptic complaints, loss of appetite, convulsion, insomnia, and anxiety. Coriander essential oil has also a long history in traditional medicine. The essential oil was found to improve blood glucose control and promise as an antihyperglycemic (antidiabetic) agent [17]. On the other hand, the aqueous extract of coriander fruits is used in traditional Moroccan medicine in the treatment of diabetes and dyslipidemia besides to treat a variety of disorders [69] including Saudi Arabia and Jordan [9]. In addition, Moroccan and Palestinian pharmacopeias have been mentioned the usages of coriander as a traditional diuretic and treat urinary infections [15]. In Iranian traditional medicine, coriander fruits have a long history of use as an anxiolytic and a sedative in insomnia. The fruits were widely used internally as a carminative, digestive, spasmolytic, and galactagogic as usual. Moreover, it is also known as an anti-inflammatory agent in Iranian traditional medicine, still in herbal formulations, might be beneficial in human inflammatory bowel diseases [77]. Coriander is highly reputed Ayurvedic medicinal plant commonly known as “Dhanya” in India [15]. Usage of coriander leaves is not clear on diabetes as suggested on Persian folklore medicine, but Ayurvedic medicine also recommends the regular use of a decoction of coriander fruits (seeds) and mentioned about effects in the treatment of arthritis and other inflammatory disorders [9, 12]. Anyway, it is the main ingredient in curry powder in Indian food; the fresh green leaf is dominated in Thai and Vietnamese foods. Moreover, the roots of coriander have been used in Asian cuisine for intense flavor [9]. Moreover, in some regions of India, the plant has been used traditionally for its “anti-inflammatory” principals; besides, the fruits are used to treat spermatorrhea, leucorrhoea, and rheumatic fever [16, 78].

In the United States, coriander has recently been studied for its cholesterol-lowering effects [16]. Moreover, in some parts of Europe, coriander has traditionally been referred to as an “antidiabetic” plant [16, 78]. In Pakistan, the whole plant part is used for the treatment of flatulence, dysentery, diarrhea, cough, stomach complaints, jaundice, and vomiting. In Turkey, it is noted that the fruit infusions are useful in indigestion and as an appetizer [9]. However, in history, it is mentioned that coriander has an aphrodisiac effect as many other spices [78].

In traditional medicine, the usual dose of fruit powder is from 1 to 5 g, three times per day. This translates to a 43–71 mg/kg dose for a 70 kg individual [12]. Most of the traditional usages of the coriander have been supported by scientific data as mentioned in the text. This point is very important that the plant has been integrated between traditional and scientific usages.

## 8. Toxicity of coriander

Coriander fruits at a dose of 750 mg/kg caused no mortality in rats, and LD<sub>50</sub> (lethal dose that kills 50% of test subjects) for the oil was found 4.13 g/kg. However, high doses of coriander fruits (500 mg/kg) inhibited implantation in female rats significantly and had a small abortifacient (but no teratogenic) effect on the rats. In the Ames test, a dried leaf extract produced a mutagenic effect [12]. By the way, coriander juice extracts were neither toxic nor mutagenic in the range of concentrations tested (50–1000 µL/coincubation flask); the chlorophyll content in whole juice extracts was 0.0325 µg/mL [79].

## 9. Conclusion

The new attraction for natural products like essential oils is important to develop a better understanding of their mode of biological action for new applications in human health, agriculture, and the environment. The essential oils could find many applications as an ingredient in different industries, like the cosmetic, the pharmaceutical, and the food industries. Updates on coriander usefulness, based upon the scientific studies, have been given in this compilation, with emphasis on its essential oils. The coriander as an aromatic plant is an edible herb, famous spice, and nontoxic to humans. The healing properties of coriander can be attributed to exceptional phytochemicals. Considering these potentials of coriander and its biomolecules can be significant along with a tremendous future. The essential oil of coriander is also rich in beneficial phytonutrients, and the fruits have a health-supporting reputation that is almost on the top of the list of the healing spices besides many other traditional health benefits mostly supported by scientific reports. The other uses of coriander are amazing, but information on their possible benefits remains ambiguous. By the way, exciting essential and fatty oil combination in a magical proportion in the fruit composition makes the plant still worthy of future investigations and utilization. Finally, it is strongly recommended that coriander is an incredibly safe herb, and it would be beneficial to increase coriander use in diet.

## Author details

Alev Önder

Address all correspondence to: [pharmacogalev@gmail.com](mailto:pharmacogalev@gmail.com)

Faculty of Pharmacy, Department of Pharmacognosy, Ankara University, Ankara, Turkey

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Essential oils have recently received much attention globally due to the increased use of essential oils as well as the positive impacts from economic backgrounds.

New compounds of essential oils have been discovered from medicinal plants and used in anti-disease treatment as well as in most houses as a source of natural flavor.

This book covers some interesting research topics for essential oils, including identification of active ingredients from wild and medicinal plants. This book will add significant value for researchers, academics, and students in the field of medicine.

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