Sonia Malik *Editor*

Essential Oil Research

Trends in Biosynthesis, Analytics, Industrial Applications and Biotechnological Production

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Dedicated To my grandparents

Preface

Essential oils obtained from plants are gaining tremendous attention due to their several biological properties and use in food, cosmetics, and pharmaceutical industries. Essential oils are extracted from various plant parts by using different techniques. The chemical composition and quality of essential oils vary depending on several genetic and environmental factors. By employing diverse biotechnological methods, it is possible to improve the production of essential oils from plants. All these issues have been addressed in this book. Fourteen chapters are written by globally renowned researchers working in the area of essential oils and natural products.

Chapter 1 by Hanif et al. provides the general overview of essential oils, their chemistry, extraction methods, analyses, biological activities, applications, risks, and dangers. The chemical composition of essential oils is influenced by biotic, abiotic, and genetic factors, which are discussed in Chap. 2 by Boaro et al. Chapter 3 by Sakhanokho and Rajasekaran describes the composition and uses of essential oils from different species of *Hedychium*, while the essential oils of family Burseraceae are presented by DeCarlo et al. in Chap. 4. Chapter 5 by Guha and Nandi highlights the potential of essential oil of betel leaf in the world food sector, and Chap. 6 by Desrosiers et al. is focused on essential oils from two different species of *Artemisia.* Activity of essential oils against human oral pathogens has been detailed in Chap. 7 by Marinković et al. Chapter 8 by Blank et al. is devoted on chemical diversity and biological activities of essential oils of plants from Northeast Brazil. Satyal and Setzer discuss about adulteration in essential oils and its analysis in Chap. 9. Applications of essential oils from pines are presented in Chapter 10 by Kumar et al. Segura et al. in Chap. 11 address various biotechnological approaches to improve the yield and quality of essential oil in aromatic plants. The phytochemical composition, pharmacological activities, and biotechnological production of essential oils from geranium are summarized in Chap. 12 by Narnoliya et al. Chap. 13 by Banerjee and Roychoudhury presents the potential applications of metabolic engineering for the enhanced production of aromatic oils in plants. The role of biotechnology in obtaining essential oils from non-herbaceous plants is documented in Chap. 14 by Gounaris. Lastly, Chap. 15 by Semenova et al. aims to describe *Eremothecium* strains as essential oil producers.

Through this multi-authored book, efforts have been made to provide recent developments and techniques for extracting essential oils and various applications of biotechnological methods for their improved production in plants. This book will be a valuable reference for biotechnologists, pharmacists, food technologists, and researchers working in the area of natural plant products and medical and healthcare industries.

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Acknowledgments

Since the beginning of my task in selecting the title of this book and bringing it into the present form, I have always experienced a special source of inspiration, guidance, and shower of blessings from my grandparents and parents. They have left no stone unturned in shaping my academic career in an exceptional way. I would also like to acknowledge my brothers and their families for their love and affection.

A heartfelt appreciation goes to my husband, Dr. Surender Kumar Sharma, for his valuable advice. I would like to extend my special regards to my mother-in-law and father-in-law for their kind gesture and encouragement.

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Part I
Essential Oils Composition and Why Plants Produce Them

Chapter 1
Essential Oils

Muhammad Asif Hanif, Shafaq Nisar, Ghufrana Samin Khan, Zahid Mushtaq, and Muhammad Zubair

1.1 Introduction

The attraction of aromatic and medicinal plants grows continuously due to the increasing demand as well as interest of consumers in these plants for medicinal, culinary, and other anthropogenic applications. As consumers are increasingly informed about health, food, and nutrition issues, they are also realizing the potential and benefits of aromatic and medicinal plants and their metabolites. There are many secondary metabolites which are produced by these plants; essential oils (EOs) are among them. Composition of essential oils is very complex. Individual components present in essential oils have valuable applications in various fields like agriculture, environment, and human health. Essential oils are found as effective complements to synthetic compounds which are used in the chemical industry. The term essential oil dates back to the sixteenth century and derives from the drug Quinta Essentia, named by Paracelsus von Hohenheim of Switzerland (Brenner 1993). Essential oils (EOs) get their name because of their flammable characteristics. According to French Agency for Normalization: Agence Française

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de Normalisation (AFNOR), essential oils can be defined as (NF T 75-006): "The essential oil is the product obtained from a vegetable raw material, either by steam distillation or by mechanical processes from the epicarp of Citrus, or 'dry' distillation."

EOs are insoluble in inorganic solvents (water) while soluble in organic solvents (ether, alcohol, fixed oils). They are volatile liquids, having a characteristic odor and density less than unity, except vetiver, sassafras, and cinnamon. They are extensively used in perfumery, aromatherapy, and cosmetics industry. Aromatherapy is a therapeutic technique which includes inhalations, massage, or baths by using essential oils (volatile oils). Essential oils (EOs) also serve as chemical signals that allow the plant to control and regulate its environment (ecological role): repel predators, attract insects for pollination, inhibit seed germination, and communicate between different plants. Furthermore, EOs also possesses insecticidal, deterrent, and antifungal activities. Essential oils are present in different parts of aromatic plants such as in flowers (pink, orange, lavender, flower bud in case of clove and bracts in case of ylang-ylang), leaves (in case of mint, eucalyptus, bay leaf, thyme, sage, savory, pine needles), rhizomes (sweet flag and ginger), roots (vetiver), seeds (coriander and carvi), fruits (anise, fennel, and citrus epicarps), and wood and bark (in sandalwood, cinnamon, and rosewood).

1.2 History of Essential Oils

It is challenging to find when first essential oil was extracted; actually ancient writings which tell about the medicinal distilled waters don't exactly describe the procedure used. The very first document describes the distillation process dating back to the ninth century when the Arabs brought essential oils (EOs) into Europe. In the sixteenth century, the concept of essential oils and fatty oils, as well as methods for the separation of essences from the aromatic waters, became well known. At that time, EOs were commercialized with industrial, therapeutic, and cosmetic objectives. By the end of the nineteenth century, chemists managed to isolate, separate, and reproduce the active molecules of essential oils in perfumery, therapy, and other industries.

1.3 Sources of Essential Oils

Leaves		**Peel**	**Flowers**		**Seeds**
Basil	Oregano	Bergamot	Chamomile	Lavender	Almond
Bay leaf	Patchouli	Grape fruit	Clary sage	Manuka	Anise
Cinnamon	Peppermint	Lemon	Clove	Marjoram	Celery
Eucalyptus	Pine	Lime	Geranium	Orange	Cumin
Lemon grass	Rosemary	Orange	Hyssop	Rose	Nutmeg oil

(continued)

Melaleuca	Spearmint	Tangerine	Jasmine	Ylang-ylang	
Wintergreen	Tea tree				
Thyme					
Wood		**Bark**	**Berries**	**Resins**	**Rhizome**
Camphor	Rosewood	Cassia	Allspice	Frankincense	Ginger
Cedar	Sandalwood	Cinnamon	Juniper	Myrrh	

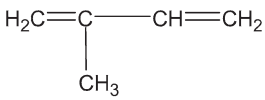
1.4 Chemistry of Essential Oils

There are more than 200 components present in the mixture of pure essential oils. Normally, these mixtures contain phenylpropanic derivatives or terpenes (have minimal structural and chemical differences) (Rao and Pandey 2007). They can be categorized into two classes:

- *Volatile fraction*: Volatile fraction has 90–95% of total oil weight. It contains monoterpenes, sesquiterpenes, and their oxygenated derivatives. Aliphatic alcohols, esters, and aldehydes may also be present in volatile fraction.
- *Nonvolatile residue*: Nonvolatile residue is 1–10% of total essential oil in weight. It contains fatty acids, hydrocarbons, sterols, waxes, flavonoids, and carotenoids.

1.4.1 Hydrocarbon

Essential oils contain chemical compounds that have carbon and hydrogen as their building blocks. Isoprene is the major basic hydrocarbon unit found in essential oils. Chemical structure of isoprene is as given below:



1.4.2 Terpenes

Terpenes are antiseptic, anti-inflammatory, bactericidal, and antiviral in nature. Terpenes can be classified as sesquiterpenes, monoterpenes, and diterpenes. Two, three, and four isoprene units are joined head to tail and form monoterpenes, sesquiterpene, and diterpenes, respectively. Here are some examples of general monoterpenes: pinene, limonene, camphene, piperine, etc.

1.4.3 Alcohols

Alcohols are antiseptic, antiviral, bactericidal, and germicidal in nature. Naturally, alcohols may present in free form or in combined form with other terpenes or esters. Terpenes along with hydroxyl group are called alcohols. Monoterpene combined with hydroxyl group is called termed as monoterpenol. In the body or skin, alcohols are safe to use as they show very low or completely no toxic reactions. Examples of some common alcohols present in essential oils are as follows: linalool in lavender and ylang-ylang, nerol in neroli, and geraniol in rose and geranium.

1.4.4 Aldehydes

Aldehydes are anti-inflammatory, antifungal, antiseptic, bactericidal, antiviral, sedative, and disinfectant. The presence of aldehydes in essential oils has great medicinal importance as they are effective in the treatment of candida and in many other fungal infections. Examples of some common aldehydes present in essential oils are citral in lemon, citronellal in lemon balm, citrus eucalyptus, and lemongrass.

1.4.5 Acids

Acids are anti-inflammatory in nature. In essential oils, organic acids are present in very small quantity in free form. Plant acids act as components or buffer systems to control acidity. For example, benzoic and cinnamic acids are present in benzoin.

1.4.6 Esters

Esters present in essential oil have soothing and balancing effects. Esters are effective antimicrobial agents due to the presence of alcohol in their structure. In medical field, esters are characterized as sedative and antifungal, with balancing action on nervous system. Some common esters present in essential oils are linalyl acetate in the lavender and bergamot and geranyl formate in the geranium.

1.4.7 Ketones

Ketones are cell proliferant, anti-catarrhal, vulnerary, and expectorant in nature. Essential oils (EOs) have ketones and are considered to be beneficial for promoting wound healing and also for encouraging scar tissue formation. Ketones are

generally (not always) toxic in nature. The most toxic ketone is thujone that is found in sage, mugwort, tansy wormwood, and thuja oils. Other toxic ketones found in EOs are pinocamphone in hyssops and pulegone in pennyroyal. Some nontoxic ketones are fenchone in fennel essential oil, jasmone in jasmine essential oil, menthone in peppermint oil, and carvone in spearmint.

1.4.8 Lactones

Lactones are antiphlogistic, anti-inflammatory, febrifuge, and expectorant in nature. Lactones are particularly effective due to their anti-inflammatory action. Lactones have the ability to reduce prostaglandin synthesis and show expectorant actions stronger than that of ketones (Rao and Pandey 2007).

1.5 Methods of Extracting Essential Oils

1.5.1 Maceration

Maceration in fact produces more of "infused oil" rather than that of "essential oil." In this technique, plant material is soaked in the vegetable oil and then heated and strained at a point on which produced product can be used for the massage purpose.

1.5.2 Cold Pressing

Cold pressing is a technique used for the extraction of essential oils from the citrus rinds like lemon, orange, bergamot, and grapefruit. This method encompasses the simple rind pressing followed by the separation of rinds from the fruit, chopping, and then pressing. As a result, a watery mixture is produced that contains both essential oil and liquid present in the source material. These are separated from each other by using appropriate method. It is significant to note that essential oils produced from this method have short shelf life as compared to other methods.

1.5.3 Solvent Extraction

In solvent extraction, essential oil is extracted from plant material using a suitable solvent. Generally, hydrocarbons are added as solvent into the plant material for the extraction of essential oils. After the addition of solvent into the plant material, the

produced solution is filtered and then concentrated by the process of distillation. Oil is extracted from the concentrate by the addition of pure alcohol which is then evaporated, and oil is left behind. The main drawback of using this method is that solvent residue left behind may cause allergies and also affect the immune system.

1.5.4 Enfleurage

Enfleurage is the traditional and intensive method for the extraction of essential oils from the flowers. In this process, fat is layered over the flower petal for the extraction purpose. After the absorbance of essential oils by fat from the flower petals, alcohol is used for the separation and extraction of essential oils from fat. At the end of the process, pure essential oil is collected by evaporating the alcohol.

1.5.5 Hydrodistillation

Hydrodistillation has become obsolete for the essential oil extraction process. The use of hydrodistillation in the developed countries is limited due to the production of essential oils with burnt smell. As in this process, material is overheated which causes the burning of aromatic compounds that result in the production of desired product (essential oils) with burnt smell. This process seems to be effective for powders such as groundwood, spice powders, etc. and for tough materials such as nuts, wood, or roots.

1.5.6 CO_2 and Supercritical CO_2 Extraction

This method of extraction is involved in the most modern technologies. Carbon dioxide (CO_2) and supercritical CO_2 extraction processes use CO_2 as "solvent" that carries essential oils away from the desired plant materials. In CO_2 extraction process, CO_2 is used at very high pressure. First of all CO_2 is chilled between temperatures of 35 and 55 °F and then pumped at pressure of 1000 psi through plant material. The carbon dioxide in this condition is condensed to a liquid. In supercritical CO_2 extraction (SCO_2) process, CO_2 is heated at temperature of 87 °F and at pressure of 8000 psi and pumped through plant materials. At these conditions, CO_2 is compared to dense fog or vapor. Pressure of the reaction media is released that results in the removal of carbon dioxide in gaseous form by leaving the essential oil behind. Hence essential oils get separated from the CO_2. Essential oils obtained through this process contain an essence closer to the essence of the original plant material (Reverchon 1997).

1.5.7 Turbo Distillation Extraction

Turbo distillation process is appropriate for the extraction of coarse and hard plant material like roots, seeds, and bark. In this process, plant material is soaked into the water, and then steam is circulated through the plant material and mixture of water. Throughout the process, same water is recycled through the plant material. This method allows essential oil at a faster rate from the hard-to-extract plant materials.

1.5.8 Steam Distillation

Most commonly used technique for the extraction of the essential oil from the plant material is called distillation. In this type of distillation, flowers or plants are placed on screen, and steam passed through the material. Later steam is condensed to produce water and essential oil. At the end, this mixture of essential oil and water is separated (Cassel et al. 2009).

1.6 Analysis of Essential Oils

Qualification and quantification of produced EOs are necessary to ensure its good quality. Different classical as well as modern analytical techniques are used for the analysis of produced EOs.

1.6.1 Classical Analytical Techniques

The earliest analytical techniques used for the examination of essential oils (EOs) were generally focused on the quality aspects that concern only two main properties, i.e., purity and identity (Marques et al. 2009). Titrimetry and gravimetry are classical analytical techniques that are used for the analysis of essential oils (Marques et al. 2009; Guenther 2013). Specific gravity (SG) method is frequently used for the investigation of physicochemical properties of EOs. Furthermore, classical methodologies have been also widely used for the analysis of chemical properties of essential oils (Guenther 2013).

1.6.2 Modern Analytical Techniques

Most of the analytical methods applied for the analysis of EOs are based on the chromatographic procedures that help in the component identification as well as its separation. However, other methods are also required for the confirmation to get

reliable identification and avoid equivocated characterization. In the past, researchers were devoted to develop an appropriate method in order to get deeper knowledge regarding the profiles of volatile constituents present in essential oils. However, the complexity of essential oils' structure made this analytical task troublesome. The number of known components present in essential oils has drastically increased with the improvement in instrumental analytical chemistry. In gas chromatographic (GC) analysis, the sample constituents are vaporized and eluted with the help of gas mobile phase while in case of liquid chromatographic (LC) analysis, the constituents of the sample are eluted by liquid mobile phase. In general, the GC is used for the analysis of volatile constituents present in the essential oils, and LC is used for the analysis of nonvolatile constituents present in the essential oils. Chromatography gives both qualitative and quantitative information regarding the analyzed sample (Zellner et al. 2010).

1.7 Biological Activities of Essential Oils

1.7.1 Antibacterial Activity

Essential oils show remarkable antimicrobial properties. Main feature of EOs is their hydrophobicity that allows EOs to partition into lipids of bacterial cell membrane due to which bacterial structure is disrupted and made more permeable (Sikkema et al. 1994). Hence, different ions and many other cellular molecules from the bacterial cell are leaked (Gustafson et al. 1998; Cox et al. 2000; Carson and Riley 1995; Ultee et al. 2002). However, certain amounts of ions and other cellular molecules from the bacterial cells can be endured without any loss of viability, but greater loss of cellular contents and ions can lead to bacterial cell death (Denyer 1991). Commonly, phenolic compounds present in the essential oils like eugenol, thymol, and carvacrol are responsible for the antibacterial activities of essential oils (Dorman and Deans 2000; Knobloch et al. 1986). These compounds can cause coagulation of cell contents and disruption of cytoplasmic membrane/electron flow/driving force of the proton/active transport (Denyer 1991; Pauli 2001).

1.7.2 Antioxidant Activity

Essential oils exhibit excellent antioxidant properties. The antioxidant potential of essential oils depends on the composition of essential oils. Phenolic compounds and other secondary metabolites present in essential oils (containing conjugated double bonds) generally show significant antioxidant properties (Koh et al. 2002). The essential oils obtained from nutmeg, thyme, cinnamon, mint, basil, clove, oregano,

and parsley are characterized by most vital antioxidant properties (Aruoma 1998). Most active compounds which show antioxidant properties are carvacrol and thymol. Activity of these compounds is related to their phenolic structure. Due to the redox properties of the phenolic compounds, they play a vital role in neutralization of free radicals and also in decomposition of peroxides (Burt 2004). The antioxidant activity of EOs is also due to other compounds present in essential oils like alcohols, ketones, aldehydes, ethers, and monoterpenes. Common examples of these compounds are linalool, geranial/neral, 1,8-cineole, isomenthone, menthone, citronellal, α-terpinolene, α-terpinene, and β-terpinene (Aruoma 1998).

1.7.3 Anti-Inflammatory Activity

Inflammation is an ordinary protective response which is induced by the infection or any tissue injury and functions to fight with invaders like microorganisms or nonself cells present within the body and to remove damaged or dead host cells. As a result, oxidative burst, release of cytokines, increase in permeability of endothelial lining cells, and incursions of blood leukocytes into interstitium occur. Furthermore, inflammation also stimulates the metabolism of arachidonic acid and the activity of various enzymes (nitric oxide synthases, oxygenases, peroxidases). Essential oils are used as anti-inflammation agents for the treatment of inflammatory diseases like arthritis, allergies, or rheumatism (Maruyama et al. 2005). The active anti-inflammation compounds present in essential oils act as inhibitors for the release of the histamine or reducer for the production of any inflammation mediators. For example, 1,8-cineole—important constituent of many essential oils—acts as an inhibitor for leukotrienes (LTB4) and prostaglandin (PGE2) (Yoon et al. 2000). Anti-inflammatory activities of EOs are not only due to the antioxidant activities of essential oils but also due to the interactions between EOs and signaling cascades (including regulatory transcription factors and cytokines) and due to the expression of the pro-inflammatory genes.

1.7.4 Cancer Chemoprotective Activity

Essential oils show potential activity for the treatment of cancer. Essential oils contain anticancer natural products (Edris 2007) which play a vital role in the prevention and recovery from cancer. There are certain foods like turmeric and garlic which are considered to be good sources of the anticancer agents (Edris 2007). Essential oil obtained from garlic has sulfur compounds like diallyl trisulfide, diallyl sulfide, and diallyl disulfide which show preventive effect against cancer (Milner 2001, 2006).

1.7.5 Cytotoxicity

There are no specific cellular ligands found in essential oils due to their complex chemical composition (Carson and Riley 1995). As lipophilic mixtures, EOs have an ability to degrade cell membrane layers of phospholipids, fatty acids, and polysaccharides. Furthermore, EOs may coagulate cytoplasm (Lambert et al. 2001) and also damage proteins and lipids present in cytoplasm (Ultee et al. 2002; Burt 2004). Damage to the wall and the cell membrane can lead to the leakage of macromolecules and lysis (Turina et al. 2006). Increase in the membrane permeability leads to the death of the cell by the process of necrosis and apoptosis (Oussalah et al. 2006; Novgorodov and Gudz 1996).

1.7.6 Allelopathic Activity

According to the International Allelopathy Society (IAS), allelopathy is defined as "The science that studies any process involving secondary metabolites produced by plants, algae, bacteria and fungi that influences the growth and development of agricultural and biological systems." Allelopathic interactions are derived from the secondary metabolite production by plants and many other microorganisms. The main function of secondary metabolites is to establish a wide range of defense system for plant and microorganisms. The secondary metabolites that show allelopathic activities are termed as allelochemicals (Moon et al. 2006). Bioactive terpenoids are found to have a significant part in defensive mechanisms and also in the agricultural field (Rim and Jee 2006).

1.7.7 Repellent and Insecticidal Activity

Essential oils have various structurally diverse chemical compounds with a variety of repellent and insecticidal mechanisms. There are several factors that affect the commercialization of essential oils. These include biological activity, intellectual property value, product quality, regulatory requirements, and product performance (Ahmed and Eapen 1986). The EOs have toxic effect for both granary insects and flying insects. Eucalyptus (Myrtaceae) and Gaultheria (Ericaceae) oils showed very high toxic effect to kill insects (Mateeva and Karov 1983). Generally, EOs can be ingested, inhaled, or absorbed by the skin of insects. EOs also show fumigant toxicity (Regnault-Roger and Hamraoui 1995). For example, *Anopheles funestus* (Culicidae: Diptera), *Pediculus capitis* (Pediculidae: Anoplura), *Periplaneta orientalis* (Dictyoptera: Blattidae), and *Cimex lectularius* (Cimicidae: Hemiptera) are killed by the use of essential oils obtained from *Eucalyptus saligna* (Myrtaceae) within 2–30 min.

1.8 Applications of Essential Oils

1.8.1 Pharmacology and Medicinal Uses

Essential oils have an important part in the medical field due to their extraordinary medicinal properties. Several EOs show fungicidal, antidepressant, antibacterial, stimulating, and relaxant effect and can be used as an effective therapeutic agent. As essential oils exhibited remarkable therapeutic properties, that is why these oils are used effectively for the treatment of several infections caused by either pathogenic or nonpathogenic diseases. Pathogenic diseases caused by virus, fungi, and bacteria can be treated with the use of respective essential oils. Nonpathogenic diseases are also treated with the appropriate use of essential oils. For example, essential oil obtained from garlic significantly showed lowering in serum cholesterol and triglycerides (TGs) by raising the level of lipoproteins (high density) in patients with coronary heart diseases (Bordia 1981). Some EOs possess hypotensive activity and are used for the treatment of hypertension. EOs and their individual aroma constituents showed anti-cancerous properties and are used in the treatment of breast cancer, tumors, leukemia, glioma, and many others. Sesquiterpene hydrocarbon elements present in EOs in very small amounts are effective for the treatment of glioma (malignant human tumors) (DeAngelis 2001). Antiangiogenic therapy is considered to be one of the most promising methodologies to control cancer.

1.8.2 Uses in Veterinary Medicine

There are various EOs like citronella oil which are used as insecticides or as insect repellents and in veterinary applications. After the ban on the usage of antibiotics in the feed of animals, EOs have emerged as a potential alternative to antibiotics used in the feed of animals. EOs used in veterinary field are categorized into the following classes:

1. Essential oils which attract animals
2. Essential oils which repel animals
3. Antiparasitic, pest repellent, and insecticidal essential oils
4. Essential oils used in the feed of animals
5. Essential oils used for the treatment of animal disease/s

Essential oils are used in the feed of animals as an enhancer for pancreatic and gastric juice production, stimulant for the production of saliva, appetite stimulant, and antioxidant and antimicrobial for the improvement of broiler performance. EOs due to their effective nature should be used in minute quantities in animal nutrition. Otherwise, they can cause reduction in feed intake, accumulation in the animal tissues, and disturbance in gastrointestinal microflora. Taste and odor of EOs may contribute to the refusal of feed by the animals, but encapsulation of EOs is the

solution of this problem (Baser and Franz 2010). Generally, essential oils used in the treatment of the human diseases are also recommended for the treatment of animal diseases.

1.8.3 Aromatherapy

For many, the word "aromatherapy" originally became related to the idea of the holistic use of EOs for promoting the health and well-being. With the passage of time, the psychophysiological effects of EOs have been explored continuously. The use of EOs to aid sedation and to reduce anxiety is also discussed in aromatherapy. More significantly, practice of the aromatherapy is firmly related with inhalation of EOs in small doses and their applications to the skin in highly diluted form as a part of aromatherapy massage. Aromatherapy is among the complementary therapies which are used for the treatment of many diseases with the use of EOs as major therapeutic agents. Inhalation, baths, and local applications are the major approaches used in "aromatherapy" that utilize EOs to penetrate into the surface of the human skin with the marked aura. After the entrance of EOs in system, they re-modulated themselves and work in a friendly manner at affected area or at malfunction site. Aromatherapy uses several combinations and permutation to get relief from several ailments like indigestion, depression, insomnia, headache, respiratory problems, muscular pain, urine-associated complications, swollen joints, skin ailments, etc. The use of EOs is found to be more favorable when other facets of life and diet are made due consideration.

1.8.4 Agricultural Uses

Essential oils have a number of applications in sustainable agriculture due to their antibacterial activity against food-spoiling bacteria and food-borne pathogens. EOs are stated to have insecticidal properties basically as larvicidal, ovicidal, antifeedant, repellence, and growth inhibitor (Isman et al. 1990; Regnault-Roger 1997; Dale and Saradamma 1981).

1.8.5 Industrial Uses

The use of essential oils (EOs) at industrial level is a very promising area for the development of any country. The quick development of flavor and fragrance industry in the nineteenth century was largely based on the EOs and related other natural products. In 1876, Haarman and Reimer started to synthesize vanillin (synthetic aroma chemicals) and then anisaldehyde, coumarin, terpineol, and heliotropin.

Even though aroma chemicals made revolution in flavors and fragrances with top discoveries in the twentieth century, for several decades both fragrances and flavors were synthesized with elements of natural origin, nearly all of which were EOs.

1.9 Essential Oil and Health Fitness

Essential oils have the ability to promote wellness when they are used as a part of healthy lifestyle. Independently, EOs have various benefits for human body. When the use of EOs is combined with the physical activities and proper eating manner, they helped the user to feel better overall. The beauty of EOs is that they may be tailored to any type of workout by the alternation in the application methods and EO types to fit the preference and needs of the users. During routine exercise (heavy lifting, dusty hiking trail, intense cardio and recreational sports), EOs can be used to keep the body at peak performance. Essential oils are also a healthy part of weight loss program when their use is combined with the healthy eating and consistent exercise.

1.10 Risks and Dangers of Essential Oils

Essential oils (EOs) have very concentrated properties of the plant or herb from which they are derived. A very small amount of the EOs often have the qualities of several cups of herbal tea from the same plant. As an example, one drop of peppermint EO is comparable to 26–28 cups of the peppermint tea. This is not to say EOs shouldn't be used, but these oils should be utilized with great care and in safe amounts. However, there are several essential oils which are not safe to use internally, and others should really be used with great caution. As EOs are the equivalent to 10–50 cups of herbal tea (depends on the used herb) or 20× the suggested dose of herbal tincture of the exact same herb, they need to only be taken internally in circumstances where they are completely needed and with great care. However, there are many warnings about the safe utilization of EOs. EOs are excellent natural remedies when used in a proper way.

References

Ahmed S, Eapen M (1986) Vapour toxicity and repellency of some essential oils to insect pests. Indian Perfumer 30:273–278

Aruoma OI (1998) Free radicals, oxidative stress, and antioxidants in human health and disease. J Am Oil Chem Soc 75:199–212

Baser KHC, Franz C (2010) Essential oils used in veterinary medicine. In: Baser KHC (ed) Handbook of essential oils. CRC Press, Boca Raton, pp 881–894

Bordia A (1981) Effect of garlic on blood lipids in patients with coronary heart disease. Am J Clin Nutr 34:2100–2103
Brenner DM (1993) Perilla: botany, uses and genetic resources. In: Janick J, Simon JE (eds) New crops. Wiley, New York, pp 322–328
Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol 94:223–253
Carson C, Riley T (1995) Antimicrobial activity of the major components of the essential oil of Melaleuca alternifolia. J Appl Bacteriol 78:264–269
Cassel E, Vargas R, Martinez N, Lorenzo D, Dellacassa E (2009) Steam distillation modeling for essential oil extraction process. Ind Crop Prod 29:171–176
Cox S, Mann C, Markham J, Bell H, Gustafson J, Warmington J, Wyllie S (2000) The mode of antimicrobial action of the essential oil of Melaleuca alternifolia (tea tree oil). J Appl Microbiol 88:170–175
Dale D, Saradamma K (1981) Insect antifeedant of some essential oils. Pesticides 15:21–22
DeAngelis LM (2001) Brain tumors. N Engl J Med 344:114–123
Denyer S (1991) Biocide-induced damage to the bacterial cytoplasmic membrane. In: Mechanisms of action of chemical biocides. Blackwell Scientific Publications, Oxford/Boston, pp 171–188
Dorman H, Deans SG (2000) Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J Appl Microbiol 88:308–316
Edris AE (2007) Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. Phytother Res 21:308–323
Guenther E (2013) The essential oils-vol 1: history-origin in plants-production-analysis. Read Books Ltd, New York
Gustafson J, Liew YC, Chew S, Markham J, Bell HC, Wyllie SG, Warmington J (1998) Effects of tea tree oil on Escherichia coli. Lett Appl Microbiol 26:194–198
Isman MB, Koul O, Luczynski A, Kaminski J (1990) Insecticidal and antifeedant bioactivities of neem oils and their relationship to azadirachtin content. J Agric Food Chem 38:1406–1411
Knobloch K, Weigand H, Weis N, Schwarm H, Vigenschow H (1986) Action of terpenoids on energy metabolism. Walter de Gruyter, Berlin, Germany
Koh K, Pearce A, Marshman G, Finlay-Jones J, Hart P (2002) Tea tree oil reduces histamine-induced skin inflammation. Br J Dermatol 147:1212–1217
Lambert R, Skandamis PN, Coote PJ, Nychas GJ (2001) A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J Appl Microbiol 91:453–462
Marques CA, Leitão GG, Bizzo HR, Peixoto AL, Vieira RC (2009) Anatomy and essential oil analysis of the leaves from Hennecartia omphalandra J. Poisson (Monimiaceae). Rev Bras 19:95–105
Maruyama N, Sekimoto Y, Ishibashi H, Inouye S, Oshima H, Yamaguchi H, Abe S (2005) Suppression of neutrophil accumulation in mice by cutaneous application of geranium essential oil. J Inflamm 2:1
Mateeva A, Karov S (1983) Studies on the insecticidal effect of some essential oils. Nauchni Trudove-Vissh Selskost Inst Vasil Kolarov 28:129–139
Milner JA (2001) A historical perspective on garlic and cancer. J Nutr 131:1027S–1031S
Milner JA (2006) Preclinical perspectives on garlic and cancer. J Nutr 136:827S–831S
Moon T, Wilkinson JM, Cavanagh HM (2006) Antiparasitic activity of two Lavandula essential oils against Giardia duodenalis, Trichomonas vaginalis and Hexamita inflata. Parasitol Res 99:722–728
Novgorodov SA, Gudz TI (1996) Permeability transition pore of the inner mitochondrial membrane can operate in two open states with different selectivities. J Bioenerg Biomembr 28:139–146
Oussalah M, Caillet S, Lacroix M (2006) Mechanism of action of Spanish oregano, Chinese cinnamon, and savory essential oils against cell membranes and walls of Escherichia coli O157: H7 and Listeria monocytogenes. J Food Prot 69:1046–1055
Pauli A (2001) Antimicrobial properties of essential oil constituents. Int J Aromather 11:126–133

Rao VPS, Pandey D (2007). A project report on Extraction of essential oil and its applications for Bachelor of Technology (Chemical Engineering) at Department of Chemical Engineering National Institute of Technology Rourkela-769008 Orissa, India

Regnault-Roger C (1997) The potential of botanical essential oils for insect pest control. Integr Pest Manag Rev 2:25–34

Regnault-Roger C, Hamraoui A (1995) Fumigant toxic activity and reproductive inhibition induced by monoterpenes on Acanthoscelides obtectus (Say)(Coleoptera), a bruchid of kidney bean (Phaseolus vulgaris L.). J Stored Prod Res 31:291–299

Reverchon E (1997) Supercritical fluid extraction and fractionation of essential oils and related products. J Supercrit Fluids 10:1–37

Rim I-S, Jee C-H (2006) Acaricidal effects of herb essential oils against Dermatophagoides farinae and D. pteronyssinus (Acari: Pyroglyphidae) and qualitative analysis of a herb Mentha pulegium (pennyroyal). Korean J Parasitol 44:133

Sikkema J, de Bont JA, Poolman B (1994) Interactions of cyclic hydrocarbons with biological membranes. J Biol Chem 269:8022–8028

Turina ADV, Nolan M, Zygadlo J, Perillo M (2006) Natural terpenes: self-assembly and membrane partitioning. Biophys Chem 122:101–113

Ultee A, Bennik M, Moezelaar R (2002) The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen Bacillus cereus. Appl Environ Microbiol 68:1561–1568

Yoon HS, Moon SC, Kim ND, Park BS, Jeong MH, Yoo YH (2000) Genistein induces apoptosis of RPE-J cells by opening mitochondrial PTP. Biochem Biophys Res Commun 276:151–156

Zellner BDA, Dugo P, Dugo G, Mondello L (2010) Analysis of essential oils. In: Handbook of essential oils. CRC Press, Taylor and Francis Group, London, pp 151–184

Chapter 2
Factors Influencing the Production and Chemical Composition of Essential Oils in Aromatic Plants from Brazil

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2.1 Introduction

Metabolism is the set of chemical reactions occurring in plant cells. Specific enzymes provide direction to these reactions, called metabolic routes, which are primarily aimed to obtain nutrients for the cell, such as energy (ATP), reducing power (NADPH), and biosynthesis of compounds essential for their survival, including macromolecules such as carbohydrates, lipids, and proteins (Taiz and Zeiger 2010).

The processes essential to plants are called primary metabolism, which is characterized by large production, wide distribution, and essential functions. The specialized metabolism (Buchanan et al. 2015), on the other hand, is characterized by the biosynthesis of molecules with structural diversity and complexity, produced in a small scale with restricted distribution and specificity, having an adaptive role in the medium, defense against herbivores and microorganisms, protection against UV rays, attraction of pollinators, and seed-dispersing animals (Lima et al. 2014; Wink 2016).

The specialized metabolites often present interesting biological activities of great relevance in the pharmaceutical, food, agronomic, and perfume industries (Facanali et al. 2015; Matos-Rocha et al. 2016; Simões et al. 2017).

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The production of specialized metabolites is the result of complex interactions between biosynthesis, transport, storage, and degradation, being influenced by genetic or epigenetic factors, ontogeny (state of development), and environment. In several plant species, the biosynthesis site is restricted to one organ, while the products are accumulated in any plant or in different organs, by means of an intercellular transport system (Gobbo-Neto and Lopes 2007; Rehman and Hanif 2016; Nagegowda 2010; Chezem and Clay 2016; Chacón et al. 2013).

The specialized metabolites originate from the intermediates acetyl-CoA, shikimic acid, mevalonic acid, and methylerythritol phosphate (Dewick 2009).

Essential oils are constituted by volatile substances belonging to specialized metabolism, which may be extracted from root, stem, leaf, fruit, flower, and plant seeds (Marques et al. 2012). The main classes of metabolites in essential oils are monoterpenes, sesquiterpenes, and phenylpropanoids. The monoterpenes and sesquiterpenes are derived from the union of isoprene (C_5) units. The isoprene unit is formed from two biosynthetic routes: (i) the intermediate route from mevalonic acid (MVA), which occurs in cytosol and is responsible for the formation of sesquiterpenes (C_{15}), and (ii) the alternative route of methylerythritol-4-phosphate (MEP), also known as the independent route or deoxyxylulose-5-phosphate, which occurs in chloroplasts, leading to monoterpenes (C_{10}). Mevalonic acid (MVA) is formed by the union of three molecules of acetyl-coenzyme A and methylerythritol-4-phosphate (MEP) by intermediates of the glycolytic route, pyruvic acid, and glyceraldehyde-3-phosphate (Dewick 2009; Taiz and Zeiger 2010). Phenylpropanoids are produced from the shikimate pathway and derived from cinnamic acid. The deamination of the phenylalanine by the enzyme phenylalanine ammonia lyase (PAL) originates cinnamic acid, which is hydroxylated by the enzyme cinnamate 4-hydroxylase to form *p*-coumaric acid. *Trans*-cinnamic and *p*-coumaric acids and their derivatives are called phenylpropanoids (Dewick 2009; Taiz and Zeiger 2010).

Essential oils are biosynthesized and accumulated in specialized structures, such as osmophores, glandular trichomes, idioblasts, ducts, and cavities. These structures have a variety of shapes, sizes, and compositions and may be found in vegetative and reproductive organs of plants (Langenheim 2003; Antunes et al. 2004; Marin et al. 2006; Dickison 2000; Schilmiller et al. 2008).

The biological activities of essential oils and/or their isolated substances have shown promising results, with the possibility of being a source of raw material for pharmaceutical, cosmetic, and food industries (Amdouni et al. 2016; Ferraz et al. 2013; Silva et al. 2015; Singh and Sharma 2015). However, for the conservation and sustainable exploitation of these genetic resources, studies are needed on the chemical and genetic diversity of species, aiming to obtain information in elaborating strategies for conservation and sustainable use, contributing to the quality of commercial products (Allendorf et al. 2010; De Queiroz et al. 2017; Oliveira et al. 2012; Zucchi 2009).

In plants, essential oils play important roles in the adaptation to the environment (Kroymann 2011; Meldau et al. 2012), defense against pathogens (Goggin 2007), herbivores (De Vries et al. 2017), interactions with pollinators and mycorrhizal fungi, signaling among plants, and protection against abiotic stresses,

such as light, water, temperature, nutrients, types of soils, pH, and altitude, among others (Amdouni et al. 2016; Morshedloo et al. 2017; Verpoorte 2000).

Some plants when attack by herbivores, release mixtures of volatile compounds, which attract organisms that feed on the parasites, thereby reducing the damage and benefiting the plant (Degenhardt et al. 2009; Duarte et al. 2010; Jamieson et al. 2017).

Biotic factors, related to plant species, and abiotic factors, related to the environment, influence the production and chemical composition of essential oils (Aslam et al. 2017; Shao et al. 2007; Silvas et al. 2013).

Among the many species distributed in several families with medicinal and aromatic potentials, we will highlight in this chapter some belonging to the families Lamiaceae (*Mentha x piperita* L., *Ocimum selloi* Benth, reclassified as *Ocimum carnosum* (Spreng.) Link & Otto ex Benth, *Ocimum basilicum* L., *Origanum vulgare* L., and *Thymus vulgaris* L.), Asteraceae (*Lychnophora ericoides* Mart., *Lychnophora pinaster* Mart., and *Baccharis dracunculifolia* DC), and Boraginaceae *Varronia curassavica* Jacq (synonym: *Cordia verbenaceae* DC) (Bueno 2004; Scavroni et al. 2005; Valmorbida et al. 2006; De Fazio 2007; David 2007; Vasques 2007; De Fazio 2011; Carboni 2013; Facanali et al. 2015; Búfalo 2015; Bolina 2015; Búfalo et al. 2016; Haber 2008; Isobe 2012; Silva 2013; Vieira et al. 2014; Silva 2016; Belini 2015).

2.2 Medicinal and Aromatic Plants

Medicinal and aromatic plants are plant species that have one or a group of substances that have biological activities, such as insecticidal, larvicidal, anticancer, antifungal, anti-inflammatory, analgesic, antiemetic, antimalarial, carminative, stimulant, antispasmodic, antiulcer, antimicrobial and antirheumatic, among others (Park et al. 2009; Abdelwahab et al. 2010; Rana et al. 2011; Stojkovic et al. 2011; Millezi et al. 2012; Millezi et al. 2013; Oliveira et al. 2012; Oliveira et al. 2013; Aznar et al. 2015). Many species that exhibit intense odor due to the release of volatile substances are classified as aromatic plants and may not be medicinal.

Since ancient times, medicinal and aromatic plants have been used as culinary spices to enhancing the organoleptic properties of foods (Bozin et al. 2006); as natural medicines, especially due to their antimicrobial properties (Dorman and Deans 2000); and as an alternative to the use of synthetic chemicals in agriculture (Antunes and Cavaco 2010).

The family Lamiaceae has approximately 245 genera and 7886 species. Among these, 46 genera and 525 species have been identified in Brazil (Harley et al. 2015). Many of these genera were brought by the colonizers and acclimated easily, being grown in gardens and vegetable gardens, such as *Mentha* (mint), *Ocimum* (alfavaca), *Origanum* (oregano), *Rosmarinus* (rosemary), and *Salvia* (salvia) (Joly 1993).

Mentha x piperita L., known as peppermint and mint, is widely grown and contains essential oils used in the pharmaceutical, alcoholic beverage, food, and cos-

metic industries (Gupta 1991; Munsi 1992). Among the mints, *Mentha x piperita* L. presents the higher content of menthol (Loewenfeld and Back 1980), a substance with important biological activities (Table 2.1). It is an annual or perennial aromatic plant, semi-erect, and about 30 cm high. Its branches range from dark green to purplish purple, and its leaves are elliptic-acuminate, jagged, and pubescent (Goutham 1980; Simões and Spitzer 2000; Lorenzi and Matos 2008).

Table 2.1 Biological activity of essential oil or its volatile substances isolated from aromatic species

Scientific name	Popular name	Substance/ essential oil	Biological activity(ies)	Reference(s)
Mentha x piperita	Mint	Menthol	Analgesic, antifungal, antibacterial, and anticancer	Stengel et al. (2007); Edris and Farrag (2003); Trombetta et al. (2005); Monteith et al. (2007); Kim et al. (2012); Wang et al. (2012); Li et al. (2009)
		Essential oil	Antifungal, larvicidal, and antispasmodic	Carreto (2010); Kumar et al. (2011); Sousa et al. (2010)
Mentha x *villosa*	Creeping mint	Essential oil and rotundifolone	Antischistosomal	Matos-Rocha et al. (2013), (2017)
Ocimum selloi	Basil	Essential oil	Anticancer	Cola et al. (2003)
Ocimum basilicum	Sweet basil	Essential oil	Antibacterial	Valeriano et al. (2012)
Ocimum basilicum	Sweet basil	Essential oil	Antifungal	Saggiorato et al. (2012)
Origanum vulgare	Oregano	Essential oil	Antibacterial	Cattelan (2015)
Thymus vulgaris	Thyme	Thymol	Antibacterial and antifungal	Giordani et al. (2004); Klaric et al. (2007)
Lychnophora ericoides	Arnica	Essential oil and *ortho*-acetoxy-bisabolol	Anti-inflammatory and analgesic	Pavarini et al. (2013)
		Essential oil	Acaricidal	Baldin et al. (2010)
Lychnophora pinaster	Arnica	Essential oil	Antibacterial	Queiroz (2012)
Baccharis dracunculifolia	Rosemary	Essential oil	Antiviral	Sforcin et al. (2012)
Varronia curassavica	*Erva baleeira*	*trans*-Caryophyllene	Anti-inflammatory	Fernandes et al. (2007)
		α-Humulene		
Psidium guineense	Brazilian guava	Essential oil	Antioxidant, antiproliferative, and anti-inflammatory	Nascimento et al. (2017)
		Spathulenol		
Turnera subulata	*Chanana*	Essential oil	Antibacterial	Fernandes et al. (2014)

Ocimum selloi Benth, reclassified as *Ocimum carnosum* (Spreng.) Link & Otto ex Benth, a native species occurring in the southeastern and southern regions of Brazil, is herbaceous, perennial, and up to 1.20 m tall and blossoms throughout the year. The inflorescence is a terminal spike with purple flowers (Morhy 1973). It is popularly known in the states of Rio de Janeiro and Espírito Santo as paregoric elixir, in Minas Gerais as *anis and alfavaquinha,* and in São Paulo as *atroveran* (Martins et al. 1997). The species is used in popular medicine as a digestive and for treatment of gastritis, vomiting, cough, and bronchitis. Pre-clinical tests (Vanderlinde et al. 1994) refer to its antidiarrheal, antispasmodic, and anti-inflammatory properties (Table 2.1).

Ocimum basilicum L. known as basil, alfavaca, great basil, and Saint-Joseph's wort is cultivated in many countries, representing a source of raw material (extracts and essential oils) for the industries that produce cosmetics, perfumes, pesticides, pharmaceuticals, and food (Umerie et al. 1998; Keita et al. 2001; Pascual-Villalobos and Ballesta-Acosta 2003; Prasad et al. 1986; Hussain et al. 2008). In popular medicine, the species is used due to its carminative, stimulating, and antispasmodic properties (Marotti et al. 1996). Basil is an annual herb, used in culinary preparations in Mediterranean countries (Sifola and Barbieri 2006), native to India and other regions of Asia (Klimankova et al. 2008), presenting purple or white flowers. According to the aroma, basil may be classified as sweet, lemon, cinnamon, camphor, anise, and clove (Blank et al. 2004). Basil plants are also used for ornamental, medicinal, and aromatic purposes, its essential oil being valued in the international market due to the high content of the compound linalool (Blank et al. 2004). Its leaves are used dry or fresh, such as flavoring in foods, confectionery, and beverages (Kopsell et al. 2005).

Some of the essential oil components, such as eucalyptol, linalool, and camphor, are known to have biological activity (Morris et al. 1979), such as antibacterial (Elgayyar et al. 2001) and insecticidal (Bowers and Nishida 1980).

Oregano (*Origanum vulgare* L.) is native to the mountainous regions of Southern Europe and grown in Brazil as a spice used in culinary. The plant is used in home medicine, and its essential oil is used in the composition of food flavorings and perfumes (Lorenzi and Matos 2008). Studies of the subspecies *Origanum vulgare* L. ssp. *vulgare*, very widespread in Italy (Ietswaart 1980), describe essential oils germacrene D, β-ocimene, β-caryophyllene, and sabinene as essential substances (Mockute et al. 2001; Mockute et al. 2003), whose biological activities are shown in Table 2.1.

Thymus vulgaris L., a shrub that grows up to 50 cm high, has white or purple flowers and is an aromatic species, which has a slightly bitter taste and is popularly known as thyme or common thyme. Infusion of leaves and floral buds are used in popular medicine. Flowers and dried plants are used as tonic, emmenagogue, antispasmodic, antiseptic, antiparasitic, sleep inducer and relief of headache (Naghibi et al. 2005; Figueiredo et al. 2008). The major substances of its essential oil are thymol, *p*-cymene, carvacrol, 1,8-cineole, borneol, and linalool. Thymol is a phenolic monoterpene that has several pharmacological properties (Table 2.1), including antibacterial and antifungal properties (Giordani et al.

2004; Klaric et al. 2007, Braga et al. 2007; Ahmad et al. 2011; Sienkiewicz et al. 2012).

Asteraceae, the largest family of eudicotyledons, presents 1911 genera, with 3293 species (Nakajima et al. 2015). In Brazil, its species represent the equivalent of 10% of vascular plants. They are commonly found in open fields, being uncommon in humid tropical forests (Funk et al. 2005). They have high adaptability to the most diverse habitats and climatic conditions, and their sizes vary from sub-shrubs, annual, or perennial grass to shrub or liana. Inflorescence of a flower head type and fruit of cypsela type, also called achene, are its main characteristics (Funk et al. 2005; Canceli et al. 2007; Heiden et al. 2007; Souza and Lorenzi 2008).

Lychnophora ericoides Mart. and *Lychnophora pinaster* Mart. are endemic aromatic and medicinal species in Brazil, presenting near-morphological characteristics, making it difficult to distinguish between them (Semir 1991; Semir et al. 2011). Due to the polymorphism and overlays of characters, Coile and Jones (1981) synonymized *L. pinaster* and *L. ericoides*. *L. pinaster* shows polymorphism in the size, diameter of the branches, shape, length, and width of the leaves. Although *L. pinaster* usually presents smaller individuals, with sub-bush size and thinner branches, it may also present individuals with similar dimensions to *L. ericoides*, who show a generally larger size and more robust branches. The species present medicinal potential, being used in popular medicine as anti-inflammatory and analgesic (Table 2.1).

Lychnophora ericoides, popularly known as arnica, false arnica, or candle, blossoms and fruits throughout the year, with reproductive periods that may vary among populations. *L. ericoides* occurs in sites with altitudes between 950 m and 1800 m, in the states of Minas Gerais and Goiás. In the Minas Gerais it is distributed along of the Serra do Espinhaço, Planalto de Diamantina, Furnas, Serra da Canastra and in some places in southeast of state. In Goiás occurs in Cristalina city, Chapada dos Veadeiros, Serra Dourada, Serra dos Pireneus, and Serras near Brasília (Semir et al. 2011).

Lychnophora pinaster Mart., also popularly known as arnica, may be found in rocky, dirty, and clean fields (Rodrigues 1988). In addition, it also occurs in canga fields, between blocks of rocks or on top of small hills exposed to intense sunshine, and in xeric environments, where winters are humid and cold and summers are dry and warm (Semir 1991). The species is common in southeast of Serra do Espinhaço, in the state of Minas Gerais, southeast region of Brazil (Flora do Brasil 2020).

Baccharis dracunculifolia DC, also known as rosemary, field rosemary, and field broom, occurs in Brazil, Paraguay, Argentina, Uruguay, and in the high valleys of Bolivia, reaching up to 3280 m in altitude (Cassel et al. 2000). Woody shrub, measuring up to 4.0 m high, presents rapid growth and occurs in southeast, central-west, and southern regions of Brazil (Nakajima et al. 2015). The origin of one of its popular names is due to the fact that the species has been very used in the making of rustic brooms. It is also used as an ornamental plant, being important for the local population for its use in home medicine to mainly combat gastric disorders, physical fatigue, inappetence, febrile affections, and organic weakness (Carneiro and Fernandes 1996; Mors et al. 2000). The essential oil extracted from the field rosemary

leaves is valued in the fragrance industry (Molt and Trka 1983), due to the high content of *trans*-nerolidol (Table 2.1), and is also the main botanical source for the production of green propolis in southeast Brazil (Park et al. 2004). The species should be highlighted in the regeneration of natural vegetation after disturbances, such as fire (Tabarelli and Mantovani 1999; Galindez et al. 2009).

The family Boraginaceae presents 4 subfamilies, namely Ehretioideae, Cordioideae, Helitropioideae, and Boraginoideae (Miller and Gottschling 2007), 130 genera, and 2500 species (Al-Shehbaz 1991), distributed in tropical, subtropical, and temperate regions, being rare in the temperate zones of the Northern Hemisphere (Al-Shehbaz 1991). It was from this family the production of the first Brazilian commercial herbal medicine with anti-inflammatory activity (Table 2.1) from the essential oil of *Varronia curassavica* Jacq (synonymy: *Cordia verbenaceae* DC), medicinal plant native to Brazil, which occurs in coastal regions (Vaz et al. 2006; Passos et al. 2007), from Amazon region (Akisue et al. 1983) to Rio Grande do Sul (Montanari Jr 2000).

It is popularly known as *erva baleeira*, *salicina*, *catinga-de-barão*, *cordia, erva-balieira, balieira-cambará, erva-preta, maria-milagrosa, maria-preta, catinga-preta, maria-rezadeira, camarinha, camaramoneira-do-brejo,* and *pimenteira* (Montanari Jr 2000; Carvalho Júnior et al. 2004; Lorenzi and Matos 2008). Other botanical synonyms are *Cordia curassavica*, *Cordia salicina* DC., *Cordia cylindrostachya*, *Lithocardium fresenii*, *Lithocardium salicinum,* and *Lithocardium verbaceum* (Carvalho Júnior et al. 2004). The aerial part of the species has a strong and persistent odor (Passos et al. 2007). Standing bush, very branched, with the end of the hanging branches and stems covered by fibrous bark, the plant reaches between 1.5 m and 2.5 m high. It has simple, alternating, coriaceous, aromatic leaves that are 5 to 9 cm in length (Lorenzi and Matos 2008). It is commonly used in the form of alcoholic extracts, decoctions, and infusions due to its antiulcer, antimicrobial, anti-inflammatory, antirheumatic, analgesic, and tonic properties (Akisue et al. 1983; Vaz et al. 2006; Medeiros et al. 2007; Passos et al. 2007).

The medicinal property of the species is due to the presence of α-humulene in its essential oil, a sesquiterpene substance that presents anti-inflammatory action (Montanari Jr 2000).

2.3 Essential Oils: Methods of Extraction and Identification

Excluding the extraction of essential oils from citrus fruits, the main methods of extraction of essential oils are steam distillation and hydrodistillation, which consist of the vaporization of essential oil, which is drawn together with steam into a condenser, where it is cooled, returns to the liquid phase, and is stored in a separator vessel.

In the process steam distillation, the steam is generated either in a boiler separated from the vegetal material. The water vapor stream generated is passaged in matriz vegetal and the mixture of water and essential oils is condenses and collected

in a recipiente for separation of phases. In the hydrodistillation method, the vegetal material is submerged in boiling water during the entire extraction process (Guenther 1948; Lawrence 1995; Marques et al. 2013).

The analysis of essential oil ingredients is performed by gas chromatography with flame ionization detector (GC-FID) and by gas chromatography-mass spectrometry (GC/MS). The identification of the substances is performed by comparing the retention time (RT) and composite mass spectra with the mass spectra described in the literature (Adams 2017) and commercial pattern matching.

In case of substances not reported in the literature, the substances are isolated by different chromatographic techniques (column chromatography, preparative thin-layer chromatography, high-performance liquid chromatography) and identified by the hydrogen nuclear magnetic resonance (^{1}H-NMR) and carbon 13 nuclear magnetic resonance (^{13}C-NMR), one (1D) and two dimensions (2D).

2.4 Influence of Genetic, Epigenetic, Abiotic, and Biotic Factors on the Production and Chemical Composition of Essential Oils

The quantitative diversity, expressed by yield, and the qualitative diversity, related to the chemical composition of essential oils, may be influenced by genetic and epigenetic (Gupta et al. 2016; Pandotra et al. 2013; Trapp and Croteau 2001; Richards 2006; Bird 2007) and abiotic factors, including mineral nutrition, water, light, temperature, and soil types, and biotic factors, such as attacks of pathogens, pests, and herbivores. All these factors may act and increase or decrease both the yield and the chemical composition of essential oils. In addition, the factors must be considered together, characterizing the seasonality, potential causes of stresses that may influence the primary metabolism, and, consequently, the specialized metabolism (Amdouni et al. 2016; Figueiredo et al. 2007; Gouinguené and Turlings 2002; Kamanula et al. 2017; Lima et al. 2014; Ormeno and Fernandez 2012).

In this chapter, we will emphasize some of these factors that interfere with the production and chemical composition of essential oils.

2.4.1 Genetic Diversity

According to Frankham et al. (2008), genetic diversity represents differences in the DNA sequence and may be expressed in the amino acid sequence of proteins encoded by the locus, leading to a variation of alleles and genotypes present in a population, species, or groups of species. Populations evolve through selection, mutation, migration (gene flow), and drift. Evolution at its simplest level involves any change in the frequency of an allele due to mutation, migration (gene flow),

selection, or drift. The patterns of genetic diversity in populations are the result of a variety of forces that act to eliminate or increase and disperse new mutant alleles and chromosome rearrangements between individuals and populations (Frankham et al. 2008).

The concern with the loss of variability and the extinction of species with potential biological activity, due to extractivism and deforestation among other causes, has generated demand for works that allow the study of these species in order to preserve them (Karp et al. 1997).

The study of population genetics allows the knowledge of the distribution, structure, and genetic diversity between and within populations, making possible the choice of efficient strategies for the conservation of the biodiversity of the species.

In this context, molecular markers are important tools for the calculation of population estimations (Allendorf et al. 2010; Haber 2008; Primack and Rodrigues 2001; Silva et al. 2013), allowing the evaluation of gene flow. These markers may be simple sequence repeats (SSRs), random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and single nucleotide polymorphisms (SNP) (Ferreira 2001; Milach 1998; Cho et al. 1999). In the sequence, we will present studies involving population genetics and the essential oils of native aromatic and medicinal species of the families Asteraceae (*Lychnophora pinaster* Mart., *Baccharis dracunculifolia* DC.) and Lamiaceae (*Ocimum selloi* Benth).

Haber (2008) characterized the chemical composition of essential oil and genetic diversity of four native populations of Lychnophora pinaster collected in Cerrado in the state of Minas Gerais, Brazil, from different geographical regions: Carrancas city (populations: ERE and ERE1), Lavras (population: ANT) and between the cities of Lavras and Ingaí (population: PBO). The author noted hight genetic diversity within populations (90.5%) and only 9.43% between them. The populations ERE, ERE1 and PBO presented a similar chemical profile, the major substance being methyl trans-cinnamate with a relative average abundance per population of 59.1%, 77.1%, and 79.7%, respectively. These populations differed from the population ANT, whose main constituent was cedr-8(15)-en-9-alpha-ol (27.9%), with the presence of methyl *trans*-cinnamate, with a significantly lower content (12.5%) than that observed for the other populations.The divergence in the chemical profile of essential oil of the population ANT is a result of the response of the genotypes to the environmental variations between the collection and cultivated regions.

Silva (2016) evaluated the characterization of genetic diversity patterns and population structure of seven other natural populations of *Lychnophora pinaster*, using a microsatellite marker (SSR), being a population of occurrence in the southeast region of the state of Minas Gerais, called Ouro Branco (OB), whose soil is rich in iron ore, and six populations in the south region, called *Poço Bonito (PB)*, *Serra do Sofá (SS)*, *Serra da Arnica (SA)*, *Serra do Salto (SSa)*, *Serra Branca (SB)*, and *Areia Branca (AB)*, a region whose soil is rich in aluminum. The author carried out a chemical characterization of the essential oils of the leaves of the populations *Ouro Branco (OB), Areia Branca (AB), and Serra do Salto (SSa)*. The results revealed the existence of genetic diversity within and between populations, with

special divergence in genetic diversity among the population of the southeast region (*Ouro Branco*) in relation to those occurring in the southern regions PB, SS, AS, SSa, SB, and AB. The chemical composition of the essential oils between the populations was divergent. The essential oils of populations of southern regions presented methyl *trans*-cinnamate (phenylpropanoid), as the most abundant substance (about 70% of essential oil), while in the essential oil of southeast region (OB), the presence of metabolites belonging to the phenylpropanoid class was not detected, with a greater abundance of sesquiterpenes (about 60% of essential oil). The observed divergence in the chemical composition of essential oils may be attributed to the genetic divergence and environmental conditions of the sites of occurrence of *L. pinaster*.

Vieira et al. (2014) evaluated the structure and genetic diversity of native populations of *Lychnophora* collected in the State Minas Gerais, with microsatellite marker (SSR). Three populations of *Lychnophora ericoides* were collected in the cities of São Roque de Minas (population P1) and Capitólio (populations P2 and P3). The geographic distance between the populations of *L. ericoides* was 80 km between P1 and P2, 40 km between P2 and P3, and 120 km between P1 and P3. The populations of *L. ericoides* showed a greater genetic diversity within than between populations (62% and 37%, respectively) and showed that the populations have a genetic structure (FST = 0,324), indicating a low gene flow among the populations evaluated. The author carried out the chemical characterization of essential oils of the leaves of the populations of São Roque de Minas (P1) and Capitólio (P2), with distance at 80 km and with altitude at 1118 m and 933 m, respectively (Vieira et al. 2017). Sesquiterpenes were the main chemical class identified in the essential oil of population P1 (São Roque de Minas), with the majority being spathulenol (mean value of the population, 5.6%), γ-eudesmol (9.2%), epi-α-cadinol (8.6%), α-muurolol (17.0%), and α-eudesmol (16.3%). On the other hand, essential oil of population P2 (Capitólio) presented monoterpenes and sesquiterpenes, the main substances being α-pinene (mean value, 4.6%), limonene (5.6%), terpinen-4-ol (9.3%), β-atlantol (18.8%), and *ortho*-acetoxy-bisabolol (21.4%). The divergence in the chemical composition of essential oils among the populations of *L. ericoides* may be associated with the modulation of the biosynthesis of the specialized metabolites due to the interaction genotype-environment of the different regions of the species occurrence.

The characterization of the chemical composition of essential oils and genetic diversity using RAPD marker was made with three native populations of *Ocimum selloi* occurring in contrasting climatic and altitude regions – the populations were collected in the cities of Iporanga (PQ; altitude, 192 m), Piquete (VR; altitude, 908 m) in the state of São Paulo, and Adrianópolis (ADR; altitude, 247 m) in the state of Paraná. The region VR was classified as Cwa (Köppen) with an average temperature of 21.3 °C, annual rainfall of 1672.5 mm, and dry and cold winter (average temperature of 11 °C), distinct from the two other regions (PQ and ADR) classified as Af (Köppen), with a mean temperature of 23.6 °C, annual rainfall of 2033.8 mm, and winter with average temperature of 20 °C, without dry season. The populations presented genetic divergence as a function of the geographical region, with a lower diversity between the populations ADR and PQ, whose regions are

geographically close, probably due to the occurrence of gene flow among the accessions. The major substances of the population Piquete (PQ) were germacrene D, elemicin, *trans*-α-bergamotene, and bicyclogermacrene. For the populations Adrianópolis and Iporanga, the major substances were elemicin, β-selinene, and β-4-copaen-α-ol. The results showed that the chemical composition of essential oils was influenced by geographical region and genetic factor (Facanali et al. 2015).

Belini (2015) evaluated the genetic diversity through a microsatellite marker (SSR) and the chemical composition of the essential oils of the native populations of *Baccharis dracunculifolia* DC., which occurs in regions with an altitudinal gradient in Brazil. The populations were collected in the cities of Campos do Jordão (population J; altitude, 1620 m), Ubatuba (population U; altitude, 2 m), and Campinas (population C; altitude, 680 m). The population Campos do Jordão (greatest altitude) presented greater genetic diversity when compared to the other populations. All populations presented *trans*-nerolidol as the major substance in essential oil, ranging from 21.6% to 40.8%. Despite this, the population Campos do Jordão diverged from the others as to the relative proportions of the substances in the essential oil. The results suggest the influence of the altitudinal gradient on the genetic and chemical diversity.

2.4.2 Mineral Nutrition

Plants are autotrophic organisms that remove CO_2 from the atmosphere and water and mineral nutrients from the soil. The mineral nutrients are absorbed by plants mainly by the root system in the inorganic ion form. The acquisition of nutrients by plants may result from the contribution of fungi (mycorrhizal) and nitrogen-fixing bacteria. The absorbed ions are transported to the various parts of the plant, where they are assimilated and used in important biological functions. Mineral nutrition is the study of how plants absorb, transport, assimilate, and utilize the ions, called essential elements, without which the plants may not complete their life cycle. These essential mineral elements are usually classified as macro- or micronutrients, according to their relative concentration in the tissue or according to the concentration required for proper growth of the plant. In general, the concentrations of the macronutrients (N, P, K, Si, Ca, Mg, and S) are higher than those of the micronutrients (Fe, Cu, Zn, Mn, Mo, B, Cl, and Ni) (Marschner 2012).

Inadequate supply of an essential element (excess or deficiency) results in nutritional disorder. These disorders are related to the physiological actions of the element in the normal functioning of the plant. Thus, mineral nutrition influences the primary and specialized metabolism (Marschner 2012).

Related to primary metabolism, Ye et al. (2014) reports that mineral nutrition plays a key role in photosynthesis since plants require macro- and micronutrients at some stage of the photosynthetic process (Marschner 2012; Maathuis 2009). Related to specialized metabolism, several studies have shown that macro- and micronutrients play an influence on the production and profile of volatile substances (Farzadfar et al. 2017; Pal et al. 2016).

Our studies, conducted with different plant species, have revealed the influence of macronutrients on plant growth and development, on gas exchange, and, as a consequence, on the yield and chemical composition of essential oils produced by them. Some of these studies are highlighted below.

Bueno (2004) evaluated the development, yield, and chemical composition of essential oils extracted from the leaves of *Thymus vulgaris* (thyme) grown in nutrient solution containing different levels of phosphorus (15.5, 31.0, and 46.5 mg.L^{-1}). The physiological indexes, length, and fresh mass of the aerial part, dry matter of the root, aerial and total part, aerial part/root relation, and absolute and relative growth rate were evaluated 62, 83, 104, 125, and 146 days after sowing, while the yield and chemical composition were determined 65, 95, and 125 days after sowing. The plants cultivated with the highest level of phosphorus in all crops presented, in general, greater length, fresh and dry mass of the aerial part, dry mass of the root, total dry mass, and yield of essential oil. The essential oil of thyme presented 29 substances, and thymol, which has great economic importance, was the main substance in plants grown with 31.0 mg.L^{-1} of phosphorus. Plants cultivated in 46.5 mg.L^{-1} of this nutrient presented higher carvacrol content, showing that the yield and quality of essential oil of thyme were influenced by an abiotic factor, represented in this study by phosphorus.

Menthol is the most abundant component of essential oil of adult mint leaves. The quality and commercial value of essential oils are determined by the balance between the quantities of their constituents. In general, in mint, quality oils contain higher amounts of menthol, intermediate amounts of menthone, and low levels of pulegone and menthofuran. In conditions of abiotic stress, including light, temperature, and relative humidity, plants of *Mentha x piperita* L. exhibit accumulation of pulegone and menthofuran (Brun et al. 1991; Voirin et al. 1990; Mahmoud and Croteau 2002). The metabolites and intermediate reactions are of great importance because they are the main determinants of the final production of menthol and its by-products (Bertea et al. 2001).

The evaluation of the plant development and yield and composition of essential oil of *Mentha x piperita* L. leaves grown in nutrient solution with nitrogen levels (abiotic factor that influences the essential oil) equal to 210 mg.L^{-1} (complete dose of N), 263 mg.L^{-1} (complete dose plus 25% of N), and 315 mg.L^{-1} (complete dose plus 50% of N) showed that the plants cultivated with lower nitrogen level (210/105 mg.L^{-1}) presented higher leaf area and total dry mass, which resulted in higher photosynthetic efficiency and better yield and quality of essential oil, which presented higher amount of menthol (Leal 2001).

Mentha x piperita was also grown in nutrient solution containing magnesium levels equal to 48.6 mg.L^{-1} (complete dose), 24.3 mg.L^{-1} (reduction of 50%), 12.1 mg.L^{-1} (reduction of 75%), and 2.4 mg.L^{-1} (reduction of 95%). Plant development and the essential oil yield were evaluated 21, 49, 63, 77, and 92 days after transplanting the seedlings to the nutrient solution. The gas exchanges were performed 9, 34, 91, and 109 days after transplanting the seedlings. Plants cultivated with a reduction of 50% in magnesium compared to the full-level treatment, equal to 24.3 mg.L^{-1}, presented satisfactory results. Reduction of magnesium to lower

levels (12.1 and 2.4 mg.L^{-1}), mainly from 77 days after transplantation, impaired development, gas exchange, and essential oil yield. It should be noted that 2.4 mg. L^{-1} of magnesium caused an increase in the essential oil yield before 63 days after transplantation, which may have occurred due to the low concentration of the mineral and consequent stress. In addition to the magnesium level, the harvesting period also influenced the essential oil yield (Vasques 2007).

David et al. (2007) evaluated the development and the yield of essential oil of *Mentha x piperita* L., grown in complete nutrient solution No. 2 of Hoagland and Arnon (1950) and in the same solution with decrease and increase of 50% of phosphorus. The results showed that the variations in phosphorus levels interfered with the development of *Mentha x piperita* L. Although the plants showed behavioral variation for many of the evaluated variables, when submitted to the different levels of phosphorus, they adapted to this condition, which may be confirmed by the relative growth rate, which reflects growth. However, those cultivated with the lowest level of phosphorus, indicative of stress due to the low concentration of this nutrient, presented higher essential oil yield 60 days after planting, a condition that once again may be indicative of stress due to low phosphorus concentration. The substances identified in essential oils in descending order of their contents were menthofuran, menthol, menthol acetate, menthone, 1,8-cineole, pulegone, limonene, β-pinene, isomenthol, α-pinene, and myrcene. The highest contents of menthone and menthofuran were obtained in cultivated plants at phosphorus levels of 23 and 46.5 mg.L^{-1}, respectively. The highest yield and content of menthol, menthyl acetate, and pulegone were obtained in plants grown at the lowest phosphorus level, equal to 7.5 mg.L^{-1} until the first harvest, 60 days after planting (David 2004, 2007; David, Boaro, and Marques 2006). Phosphorus as an abiotic factor interfered with the essential oil in mint plants.

Plant development, yield, and chemical composition of *Mentha x piperita* essential oil grown in a nutrient solution with N/P/K/Mg variation in different treatments (50% of N, P, K, and 25% of Mg, containing 94.0/15.5/107.5/12.15 mg.L^{-1}; 50% of N, P, K, and Mg, containing 94.0/15.5/107.5/24.3 mg.L^{-1}; 65% of N, 50% of P, 25% of K, and 100% of Mg, containing 124.0/15.5/53.6/48.6 mg.L^{-1}; and with complete solution containing 189.0/31.0/214.5/48.6 mg.L^{-1} of N/P/K/Mg) were evaluated 20, 35, 50, 65, and 85 days after transplanting (DAT) the seedlings for nutrient solution. Higher net assimilation rate (NAR) was verified in plants grown in the complete nutrient solution up to 35 DAT and lower specific leaf area (SLA) at the beginning of the cycle. The relative growth rate, in general, was the same in the plants grown with the different treatments. The highest essential oil yield was obtained in the plants grown in 65% of N, 50% of P, 25% of K, and 100% of Mg. The major substances identified in the essential oil were menthol, menthone, menthofuran, neomenthol, and menthyl acetate at 69 DAT, not differing among treatments, being, therefore, the best time for extracting essential oil. The results allow us to conclude that plants grown with 65% of N, 50% of P, 25% of K, and 100% of Mg showed a trend of higher mass production, essential oil yield, and menthol content, indicating that the cultivation of *Mentha x piperita* L. with these nutrient levels and harvest season was adequate (David 2007). This study demonstrated that the influence of

abiotic factors when considering mineral nutrition should take into account the relationship between nutrients for the fertilization of the species in the production and chemical composition of the essential oil.

Valmorbida et al. (2006) evaluated the influence of potassium level on the yield and chemical composition of essential oils extracted from the leaves of *Mentha x piperita* L. The plants were cultivated until the 21st day in a nutrient solution of Hoagland and Arnon (1950) diluted in 50%, passing the complete solution after this date. The treatments were as follows: T1, complete nutrient solution; T2, nutrient solution with reduction of 50%; and T3, nutrient solution with reduction of 75%, harvested at 60, 105, and 120 days after transplantation. Changes in potassium levels and harvesting times did not influence the yield and menthone content, while the relative percentage of menthol was influenced by potassium levels and harvest times, with lower content at 120 DAT at the concentration of 58.50/117.00 $mg.L^{-1}$ (T2, nutrient solution with reduction of 50%).

According to De Fazio (2007, 2011), calcium has been extensively studied, and the metabolism of this macronutrient needs to be better evaluated since this element acts as a specialized messenger in routes of signal transduction in plant cells, and, due to variations in its cellular concentration, acts through modulating proteins and its target molecules, regulating several cellular processes, from the control of ionic transport to gene expression.

In this context, there are doubts about the effect of calcium on plant development, gas exchange, and the route of production of essential oils, especially yield and chemical composition.

De Fazio (2007) evaluated the development, yield, and chemical composition of the essential oil of *Mentha x piperita* L. grown in a nutrient solution No. 2 of Hoagland and Arnon (1950) containing 160 $mg.L^{-1}$ of calcium and in the same solution with its reduction to 50%, 80 $mg.L^{-1}$ and 90%, 16 $mg.L^{-1}$ and subjected to leaf spraying with 100, 200, and 400 $mg.L^{-1}$ of ethephon, where they remained until the harvesting dates, which were performed at 46, 76, 106, and 136 DAT of seedlings to the nutrient solution. The length of the aerial part; leaf area; dry matter of leaf blades, petiole, stems and roots; leaf area ratio (LAR); specific leaf area (SLA); absolute growth rate (AGR); net assimilation rate (NAR) and relative growth rate (RGR) and essential oil yield were influenced by the decrease of the calcium level and by ethephon using. Plants grown with 160 $mg.L^{-1}$ of calcium showed higher essential oil yield. Ethephon used in the dosages of 200 and 400 $mg.L^{-1}$, associated with all levels of calcium and, mainly, lower and equal to 16 $mg.L^{-1}$, decreased all evaluated variables, impairing the development of mint and the yield of its essential oil, mainly from 106 DAT. Based on the results obtained, it is suggested that the influence of the calcium level associated with different doses of ethephon in the development and production of essential oil of mint plants may have occurred due to the relationship between the cellular concentration of this ion and ethylene biosynthesis, considering the participation of calcium in the activity of 1-aminocyclop ropane-1-carboxylic acid synthase and 1-aminocyclopropane-1-carboxylic acid enzymes, which play an important role in ethylene biosynthesis (Kende 1993). However, future studies should be carried out to confirm this hypothesis.

De Fazio (2011) evaluated the influence of the variation of calcium levels in physiological indexes, gas exchange, and yield and chemical composition of the oil of *Mentha x piperita* cultivated in nutrient solution. Using a nutrient solution No. 2 of Hoagland and Arnon, containing 160 $mg.L^{-1}$ of calcium and modified to supply 200, 120, 80, and 40 $mg.L^{-1}$, experiments were carried out and evaluated at 45, 65, 85, 105, and 140 DAT of seedlings into the culture solution. The physiological indexes showed that the increase of calcium was beneficial for leaf area development and dry matter production. There was a discrete influence of this element on the yield and chemical composition of essential oil, which also varied with the development of the species. Mint essential oil in quantity and quality may be obtained when plants are grown with 40 and 80 $mg.L^{-1}$ of calcium. Plants grown with 40 $mg.L^{-1}$ should be harvested at 65 DAT and those at 80 $mg.L^{-1}$ at 65 or 85 DAT when they had quality oil, high menthol content, menthone intermediate, and reduced menthofuran + neomenthol.

Carboni (2013) studied the variation of the nutrient solution of Hoagland and Arnon (1950) through the concentration of minerals that constitute it (nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, boron, chlorine, manganese, iron, molybdenum, nickel, zinc, and copper). The evaluated nutrient solutions were equal to 100% (complete), 75%, 50%, and 25% and in them the plants of *Origanum vulgare* L. *ssp. Vulgare* grown presented stimulation or inhibition of development. Plants grown in solutions with higher concentrations of nutrients presented lower productivity, as evaluated by carbon assimilation in the photosynthesis process. These plants presented an increase in their antioxidant potential. The major substances of essential oil, with the exception of germacrene D, were influenced by the concentration of the nutrient solution and the harvesting period during the development of the species. The sesquiterpene contents tended to increase in plants grown in more concentrated solutions (100% and 75%), suggesting possible stress caused by the increase of nutrients in the nutrient solution. Although lower concentrations have decreased the productivity (photosynthesis), they stimulated the production of essential oil (specialized metabolism) in oregano plants.

The cultivation with organic and conventional fertilization system of sweet basil (*Ocimum basilicum*) in a greenhouse with two nitrogen doses (150 and 250 $kg.ha^{-1}$) showed that plants grown with conventional fertilizer at the dose of 250 $kg.ha^{-1}$ presented a higher fresh mass, causing no change in the yield and chemical composition of essential oil, which presented linalool as major substance (Búfalo 2015).

2.4.2.1 Biosolids and Plant Growth

Biosolids are solid wastes produced by biological treatment of sewage sludge to reduce pathogenic organisms, which may be directly used in agricultural soils, as fertilizer or conditioner, aiming to give an adequate destination for these wastes. Its use allows taking advantage of the minerals and organic matter present in the sewage sludge, which may be beneficial to the soil structure and fertility and, consequently, to the growth of the plant species (Melo and Marques 2000; Tsutiya 2000).

When considering the possibility of agricultural use of industrial and urban wastes for the cultivation of plant species, the contents of heavy metals should be evaluated. In addition, in Brazil, the application of biosolids in agricultural areas depends on the prior consent of the governmental body responsible for local environmental control – *Companhia de Tecnologia de Saneamento Ambiental* (CETESB) is the agency of the state of São Paulo responsible for the standards and licensing of use.

Considering only the development of peppermint in the presence of biosolids, but not discussing the possibility of producing aromatic species in this substrate, Scavroni et al. (2005) evaluated the effects of biosolid concentrations (0, 28, 56, and 112 ton.ha^{-1}) from the Barueri Sewage Treatment Plant, state of São Paulo, on the yield and chemical composition of essential oils extracted from *Mentha x piperita* leaves at different stages of development. The treatments influenced discreetly the essential oil yield, increasing when the plants were cultivated with 28 ton.ha^{-1}, a condition that did not result in better quality, and the presence of biosolids favored the formation of menthofuran. Menthyl acetate was the major substance in all treatments and menthol was the second largest substance observed (42.3%) at 90 DAT in plants cultivated without biosolids. With the development of the plants, there was a decrease of menthol and menthone. It is recommended to harvest mint at 90 DAP, at which point the menthol level was higher. Although the cultivation with 28 ton.ha^{-1} is within the limits allowed by the legislation, this condition, which increased the oil yield, did not improve its quality. Thus, the biosolids of the Barueri Plant are not recommended for the cultivation of mint.

2.4.3 Water Stress and Aromatic Species

Water stress in plant species is an abiotic factor which influences the physiological processes, changes the metabolic homeostasis, and imposes adjustment of the metabolic routes (Shulaev et al. 2008; Ciarmiello et al. 2011). Water limitation has a negative effect on plant growth and development; however, moderate water deficiencies in some medicinal, aromatic, or spice species may result in the accumulation of bioactive substances due to the adverse condition. Under these conditions, the plant continues to perform photosynthesis, but its growth decreases and the excess photoassimilates produced are redirected to specialized metabolism (Marchese and Figueira 2005). Moreover, the detrimental effects of the stress may be compensated by the plants through several mechanisms that operate at different time scales, depending on the nature of the stress and the physiological processes that are affected (Lambers et al. 1998). In this way, evaluating and understanding the metabolic responses of medicinal species under the influence of water suppression may provide important information on the management of these plants under adverse conditions and enable the higher production of target substances.

Búfalo et al. (2016) evaluated the influence of osmotic stress by applying two concentrations of polyethylene glycol (50 g.L^{-1} and 100 g.L^{-1} of PEG) over a short period of time in the anatomy and leaf ultrastructure in the physiological pattern of

M. x piperita and in the essential oil profile. The results indicated that the responses to osmotic stress were dose-dependent, since the plants submitted to 50 $g.L^{-1}$ of PEG maintained the structural aspects and metabolic functions similar to the control treatment plants (0 $g.L^{-1}$ of PEG), without changes in structural characteristics and gas exchange. The increased activity of antioxidant enzymes reduced the presence of free radicals and protected the membranes, including those of chloroplasts and mitochondria. The osmotic stress caused by 100 $g.L^{-1}$ of PEG inhibited the gas exchange, reduced the yield of essential oil, and caused a change in its composition, with a decrease of menthone and increase of menthofuran.

Bolina (2015) evaluated the physiological, chemical, and biochemical responses of micropropagated plants of *Varronia curassavica* Jacq. (*erva baleeira*) in function of the suppression of irrigation. The treatments were represented by the control (daily irrigated) and by three levels of water deficiency, all expressed by water potential in the leaf xylem (Ψw): T1, control (Ψw~ −0.3 MPa), T2 (Ψw~ −1.0 MPa), T3 (Ψw~ −1.7 MPa), and T4 (Ψw~ −2.5 MPa). The results showed a reduction in plant height, leaf area and yield of fresh leaf, and stem and total mass, due to water suppression. The gaseous exchanges were similar between treatments. The relative water content decreased linearly in relation to the different levels of water potential, presenting a reduction of about 80%. The lower water potential (−2.5 MPa) provided a higher yield of essential oil (0.18%). The potential of −1.7 MPa led to the limit of activity of the enzyme superoxide dismutase and the greater accumulation of proline. The suppression of irrigation led to an increase in the essential oil of *V. curassavica*. There was no quantitative difference in essential oils between treatments, except for γ-(E)-bisabolene. The relative proportions of the active principles (*E*)-caryophyllene (25.2%) and α-humulene (4.4%) did not differ between treatments.

2.4.4 Allelopathy and Aromatic Species

Allelopathy may be defined as the science that studies the processes involving specialized metabolites produced by living organisms that have influence in inhibiting or stimulating the growth and development. Allelopathic substances synthesized by routes of specialized metabolism and released to the environment are called allelochemicals (Rice 1984) and may influence the development and establishment of agricultural crops and plant communities (Torres et al. 1996; Reigosa et al. 2013).

Considering the concentration and structure of the molecules, the use of allelopathic extracts in the cultivation of medicinal plants may provide different and advantageous responses in their development and in the yield and composition of the essential oil produced. The interaction of the molecules with the routes of the specialized metabolism may explain the effects of allelochemicals in the development of different species and elucidate the chemical-ecological mechanisms of the plant-plant interaction. On the other hand, nutritional conditions combined with allelopathic effects may shape the chemical profile of the substances of the

specialized metabolism, changing the concentration of the substances or driving the metabolic routes, with consequent modification of the final product naturally synthesized in these routes.

Leonurus sibiricus L. is known for the production of terpenoids and phenolic substances that exert allelopathic effects. Búfalo (2015) investigated the effects of the methanolic extract of *L. sibiricus* (25, 50, and 100 mg.L^{-1}) on the chemical composition of the essential oils of *Mentha* × *piperita* L. grown in the nutrient solution. Menthol was the main component of essential oil in all treatments. The relative relation of menthol and menthyl acetate was divergent in plants grown with 25 mg.L^{-1} of methanolic extract, where menthol was higher. Methyl acetate was higher when the plants were cultured in the presence of 50 and 100 mg.L^{-1} of methanolic extract of *L. sibiricus*.

2.4.5 Seasonality and Edaphoclimatic Factors

The seasonality and the edaphoclimatic factors (light, water, temperature, and soil) that characterize it may influence physiological and biochemical processes and/or metabolic routes, interfering with the synthesis of important specialized compounds, such as terpenes (Shao et al. 2001; Gobbo-Neto and Lopes 2007).

The period of the year in which a substance of specialized metabolism is identified is of great importance since it depends on the quantity and nature of these substances, not constant during the year. There are many reports of variations of classes of specialized metabolites, such as essential oils, due to seasonality and edaphoclimatic factors.

Isobe (2012) evaluated the influence of seasonality, summer and winter, on the yield and chemical composition of essential oils of three native populations of *Lychnophora pinaster* Mart., collected in the cities of Carrancas (population *Areia Branca*), Lavras (population *Poço Bonito*), and Itumirim (population *Serra do Sofá*), state of Minas Gerais. The two evaluated periods did not influence the average yield of essential oils within the three populations. Regardless of the season, the major substances in the leaves of essential oils of the three populations of *L. pinaster* were methyl *trans*-cinnamate and *trans*-caryophyllene, which presented different relative proportions between the populations. During summer, essential oils presented a higher relative proportion of methyl *trans*-cinnamate, and during winter the highest relative proportion was *trans*-caryophyllene for all populations.

Silva (2013) evaluated the influence of seasonality (summer, autumn, winter, and spring) on the yield and chemical composition of essential oils of the leaves from three native populations of *Lychnophora pinaster* – two collected in the city of Carrancas (MG), named *Serra Branca* and *Serra do Salto*, and one in the city of Ingaí (MG), named *Serra da Arnica*. The average yield of essential oil of the populations *Serra Branca* and *Serra do Salto* was not influenced by seasonality, and the population of *Serra do Salto* showed a higher average yield (0.43% vs. 0.61%) in all seasons during the year. The average yield of essential oil of the population *Serra da*

Arnica was influenced by seasonality, and the highest yield occurred in winter (0.79%). The major substances of essential oil in the three populations were methyl *trans*-cinnamate and *trans*-caryophyllene, and their relative proportions varied with seasonality. In *Serra Branca*, *trans*-caryophyllene presented the highest relative proportion during spring, in *Serra da Arnica* during autumn, and in *Serra do Salto* during summer (14.2%), which did not differ from autumn (12.3%).

It should be emphasized that the sites where the above-registered populations were evaluated have different edaphoclimatic conditions, which, although not considered in the studies, interact with seasonality and interfere with essential oils.

2.5 Biological Activity of the Essential Oil and Isolated Substances

The species *Mentha x piperita* L.; *Ocimum selloi* Benth, reclassified as *Ocimum carnosum* (Spreng.) Link and Otto ex Benth; *Ocimum basilicum* L.; *Origanum vulgare* L.; *Thymus vulgaris* L.; *Lychnophora ericoides* Mart.; *Lychnophora pinaster* Mart;, *Baccharis dracunculifolia* DC; and *Varronia curassavica* Jacq (synonym: *Cordia verbenacea* DC) were also evaluated by other authors, who evaluated the biological activities of their oils or chemical substances, and were isolated and observed their antibacterial, analgesic, antispasmodic, antiviral, anticancer, larvicidal, antifungal, and acaricidal actions, as presented in Table 2.1.

Besides these, other studies conducted may be identified in the specialized literature.

The biological activity of essential oils of several species and isolated substances has been described in the literature. The essential oil from *Mentha x villosa* Hudson and the substance rotundifolone showed larvicidal activity (Lima et al. 2014). Essential oil of *Croton heliotropiifolius,* Kunth revealed antibacterial activity (Araújo et al. 2017; Alencar Filho et al. 2017) and essential oil of *Schinus molle* an insecticidal activity (López et al. 2014).

Lima et al. (2014) evaluated the larvicidal activity of essential oil of *Mentha x villosa* and its major substance, rotundifolone (70%), against *Aedes aegypti* larvae. The essential oil had excellent larvicidal activity (LC_{50} = 45.0 ppm), while rotundifolone presented moderate larvicidal activity (LC_{50} = 62.5 ppm).

Essential oils of 11 species of *Piper* collected in Mata Atlântica, state of São Paulo, were evaluated for antimicrobial activity. Essential oils of most species showed up to 30% inhibitory activity against pathogenic in vitro bacteria (*E. coli, S. epidermidis*, *S. aureus*, and *C. xerosis*) in relation to commercial antibiotics. In these essential oils, the proportion of substances bicyclogermacrene and γ-muurolene were positively associated with inhibition of *E. coli*, whereas the proportions of limonene and *cis*-β-ocimene inhibited *Staphylococcus aureus*, and those of germacrene D and *trans*-caryophyllene were associated with inhibitory activity against all evaluated pathogens. The results demonstrated a chemical diversity of Piper's

essential oils and their potential as new antibacterial agents for several industrial applications (Perigo et al. 2016).

Essential oils extracted from the leaves of *Duguetia gardneriana* and *Duguetia moricandiana* were tested for antimicrobial activity against 11 pathogenic microorganisms using the standard gel diffusion method. The major substances identified in this essential oil of *D. gardneriana* leaves were germacrene D (28.1%), viridiflorene (24.0%), β-pinene (12.6%), α-pinene (9.1%), and β-caryophyllene (5.6%) and of *D. moricandiana* leaves were germacrene D (44.3%), α-pinene (13.0%), viridiflorene (9.3%), β-pinene (9.2%), and β-caryophyllene (6.8%). Essential oil of *D. gardneriana* showed activity against *Staphylococcus aureus* and *Candida guilliermondii*, and essential oil of *D. moricandiana* showed higher activity against *Staphylococcus aureus* and *Candida albicans* (Almeida et al. 2010).

2.6 Conclusions

The studies show that variations occur in the chemical compositions of essential oils of plant species cultivated or grown under conditions whose abiotic factors vary. In addition, the studies show a genetic and chemical diversity of essential oils among populations of native species of natural occurrence in different regions of Brazil.

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References

Abdelwahab SI, Faridah QZ, Mariod AA et al (2010) Chemical composition, antioxidant and antibacterial properties of the essential oils of Etlingera elatior and *Cinnamomum pubescens Kochummen*. J Sci Food Agric 90:2682–2688

Adams RP (2017) Identification of essential oil components by gas chromatography, Texensis Publishing Gruver, TX USA.

Ahmad A, Khan A, Akhtar F et al (2011) Fungicidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against Candida. Eur J Clin Microbiol Infect Dis 30:41–50

Akisue MK, Oliveira F, Moraes MS et al (1983) Caracterização farmacognóstica da droga e da tintura de *Cordia verbenacea* AL. DC. Boraginaceae. Revista de Ciências Farmacêuticas 5:69–82

Alencar Filho JMT, Araújo LC, Oliveira AP et al (2017) Chemical composition and antibacterial activity of essential oil from leaves of Croton heliotropiifolius in different seasons of the year. Rev Bras Farmacogn 27:440–444

Allendorf FW, Hohenlohe PA, Luikart G (2010) Genomics and the future of conservation genetics. Nat Rev Genet 11:697–709

Almeida JRGS, Facanali R, Vieira MAR (2010) Composition and antimicrobial activity of the leaf essential oils of *Duguetia gardneriana* Mart. and *Duguetia moricandiana* Mart. (Annonaceae). J Essent Oil Res 22:275–278

Al-Shehbaz IA (1991) The genera of Boraginaceae in the southeastern United States. J Arnold Arboretum Suppl 1:1–169

Amdouni T, Ben Abdallah S, Msilini N et al (2016) Effect of salt stress on the antimicrobial activity of *Ruta chalepensis* essential oils. Acta Physiol Plant 38:147

Antunes MDC, Cavaco AM (2010) The use of essential oils for postharvest decay control. A review. Flavour Fragr J 25:351–366

Antunes T, Sevinate-Pinto I, Barroso JG et al (2004) Micromorphology of trichomes and composition of essential oil of Teucrium capitatum. Flavour Fragr J 19:336–340

Araújo FM, Dantas MCSM, Silva LS et al (2017) Antibacterial activity and chemical composition of the essential oil of *Croton heliotropiifolius* Kunth from Amargosa, Bahia, Brazil. Ind Crop Prod 105:203–206

Aslam F, Khaliq A, Matloob A et al (2017) Allelopathy in agro-ecosystems: a critical review of wheat allelopathy-concepts and implications. Chemoecology 27:1–24

Aznar A, Fernandez PS, Periago PM et al (2015) Antimicrobial activity of nisin, thymol, carvacrol and cymene against growth of *Candida lusitaniae*. Food Sci Technol Int Ibaraki 21:72–79

Baldin ELL, Dal Pogetto MHFA, Pavarini DP et al (2010) Composição química e atividade acaricida do óleo essencial de *Lychnophora ericoides* Mart. sobre *Tetranychus urticae* Koch (Acari: *Tetranychidae*). Boletín de Sanidad Vegetal, Plagas 36:125–132

Belini CMB (2015) *Baccharis dracunculifolia* DC. (Asteraceae): composição do óleo essencial, diversidade e parâmetros genéticos. Thesis, Universidade Estadual Paulista "Júlio de Mesquita Filho" Faculdade de Ciências Agronômicas

Bertea CM, Schalk M, Karp F et al (2001) Demonstration that menthofuran synthase of mint (*Mentha*) is a cytochrome P450 monooxygenase: cloning, functional expression, and characterization of the responsible gene. Arch Biochem Biophys 390:279–286

Bird A (2007) Perceptions of epigenetics. Nature (London) 447:396–398

Blank AF, Carvalho Filho JLS, Neto S et al (2004) Caracterização morfológica e agronômica de acessos de manjericão e alfavaca. Hortic Bras 22:113–116

Bolina C (2015) Metabolismo, Desenvolvimento e composição química de *Varronia curassavica* Jacq. em função da supressão da irrigação. Thesis, Universidade Estadual Paulista "Júlio de Mesquita Filho" Faculdade de Ciências Agronômicas

Bowers WS, Nishida R (1980) Juvocimenes: potent juvenile hormones mimics from sweet basil. Science 209:1030–1332

Bozin B, Mimica-Dukic N, Simin N, Anackov G (2006) Characterization of the volatile composition of essential oils of some Lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils. J Agric Food Chem 54:1822–1828

Braga PC, Alfieri M, Culici M, Dal Sasso M (2007) Inhibitory activity of thymol against the formation and viability of *Candida albicans* hyphae. Mycoses 50:502–506

Brun N, Colson M, Perrin A, Voirin B (1991) Chemical and morphological studies ofThe effects of ageing on monoterpene composition in *Mentha x piperita* leaves. Can J Bot 69:2271–2278

Buchanan BB, Gruissem W, Jones RL (2015) Biochemistry & molecular biology of plants. Wiley, Chichester

Bueno MAS (2004) Níveis de fósforo no desenvolvimento e produção de óleo essencial de *Thymus vulgaris* L. cultivado em solução nutritiva. Dissertação, Universidade Estadual Paulista Júlio de Mesquita Filho

Búfalo J (2015) *Mentha* X *Piperita*, *Ocimum Basilicum* e *Salvia Deserta*, (Lamiaceae): Abordagens Fisiológicas E Fitoquímicas *Mentha X Piperita*, *Ocimum Basilicum* E *Salvia Deserta*, (Lamiaceae): Abordagens fisiológicas e fitoquímicas. thesis Universidade Estadual Paulista Júlio de Mesquita Filho

Búfalo J, Rodrigues TM, de Almeida LFR et al (2016) PEG-induced osmotic stress in *Mentha x* piperita: structural features and metabolic responses. Plant Physiol Biochem 105:174–184

Canceli RR, Evaldt ACP, Bauermann SG (2007) Contribuição da morfologia polínica da família Asteraceae Martinov. no Rio Grande do Sul – Parte I. Pesquisas Botânica São Leopoldo 58:347–374

Carboni TR (2013) Análise de crescimento, trocas gasosas, potencial antioxidante e óleo essencial de *Origanum vulgare* L. ssp. vulgare. 2013. Dissertation, Universidade Estadual Paulista Júlio de Mesquita Filho

Carneiro MAA, Fernandes GW (1996) As relações conflituosas entre plantas e insetos, Herbivoria. Ciência Hoje 20:32–35

Carreto CFP (2010) Atividade antimicrobiana de *Mentha piperita* L. sobre leveduras do gênero Candida. Dissertation, Faculdade de Odontologia de São José dos Campos

Carvalho Júnior PM, Rojasa LB, Velasco J et al (2004) Chemical composition and antimicrobial activity of the essentialoil of *Cordia verbenacea* D.C. J Ethnopharmacol 95:297–301

Cassel E, Frizzo CD, Vanderlinde R, Atti-Serafini L, Lorenzo D, Dellacassa E (2000) E. Extraction of *Baccharis* oil by supercritical CO2. Montevideo, Uruguai. Ind Eng Chem Res 39:4803–4805

Cattelan MG (2015) Atividade antibacteriana de óleo essencial de orégano (*Origanum vulgare*): ações in vitro e in situ para preservação de alimento. Thesis, Universidade Estadual Paulista "Júlio de Mesquita Filho"

Chacón IV, Riley-Saldaña CA, González-Esquinca AR (2013) Secondary metabolites during early development in plants. Phytochemistry Reviews 12(1):47–64

Chezem WR, Clay NK (2016) Regulation of plant secondary metabolism and associated specialized cell development by MYBs and bHLHs. Phytochemistry 131:26–43

Cho RJ, Mindrinos M, Richards DR et al (1999) Genome-wide mapping with biallelic markers in *Arabidopsis thaliana*. Nat Genet 23:203–207

Ciarmiello LF, Woodrow P, Fuggi A et al (2011) Chapter 13: Abiotic stress in plants – mechanisms and adaptations. In: Plant Genes for abiotic stress. InTech, Rijeka

Coile NC, Jones SB (1981) *Lychnophora* (Compositae: Vernonieae), a genus endemic to the Brazilian Planalto. Briffonia 33:528–542

Cola M et al (2003) Óleo essencial de *Ocimum selloi* Benth.: atividade antiulcerogênica. Documentos IAC 74:120

David EFS (2004) Níveis de fósforo no desenvolvimento e produção de óleo essencial de *Mentha piperita* L. cultivada em solução nutritiva. Dissertation, Universidade Estadual Paulista Júlio de Mesquita Filho

David EFS (2007) Desenvolvimento, trocas gasosas, rendimento e composição de óleo essencial de *Mentha Piperita* L cultivada em solução nutritiva com variação dos níveis de N, P, K E Mg. 2007. Thesis, Universidade Estadual Paulista Júlio de Mesquita Filho

David EF, Boaro CSF, Marques MOM (2006) Rendimento e composição do óleo essencial de *Mentha piperita* L., cultivada em solução nutritiva com diferentes níveis de fósforo. Rev Bras Plantas Med 8:183–188

David EFS, Pirozzi DCZ, Braga JF et al (2007) Desenvolvimento do manjericão (*Ocimum basilicum* L.) cultivado em solução nutritiva com diferentes níveis de magnésio. Revista Brasileira de Plantas Medicinais 9:15–22

De Fazio JL (2007) Influência de cálcio e de ethephon no desenvolvimento e produção de óleo essencial de menta (*Mentha piperita* L.), cultivada em solução nutritiva 2007. Dissertation, Universidade Estadual Paulista Júlio de Mesquita Filho

De Fazio JL (2011) *Mentha piperita* cultivada com variação de cálcio. Trocas gasosas e óleo essencial. 2011. Thesis, Universidade Estadual Paulista Júlio de Mesquita Filho

De Queiroz ACM, Rabello AM, Braga DL et al (2017) Cerrado vegetation types determine how land use impacts ant biodiversity. Biodivers Conserv 26:1–18

De Vries J, Evers JB, Poelman EH (2017) Dynamic plant-plant-herbivore interactions govern plant growth–defence integration. Trends Plant Sci 22:329–337

Degenhardt J, Hiltpoldb I, Köllner TG et al (2009) Restoring a maize root signal that attracts insectkilling nematodes to control a major pest. Proc Natl Acad Sci U S A 106:13213–13218

Dewick PM (2009) Medicinal natural products: a biosynthetic approach, 3rd edn. Wiley, Chichester

Dickison WC (2000) A Intergrative plant anatomy. Academic Press, London

Dorman HJD, Deans SG (2000) Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J Appl Microbiol 88:308–316

Duarte AR, Naves RR, Santos SC et al (2010) Genetic and environmental influence on essential oil composition of *Eugenia dysenterica*. J Braz Chem Soc 21:1459–1467

Edris AE, Farrag ES (2003) Antifungal activity of peppermint and sweet basil essential oils and their major aroma constituents on some plant pathogenic fungi from the vapor phase. Nahrung 47:117–121

Elgayyar M, Draughon FA, Golden DA, Mount JR (2001) Antimicrobial activity of essential oils from plants selected pathogenic and saprophytic microorganisms. J Food Prot 64:1019–1024

Facanali R, Colombo CA, Teixeira JPF et al (2015) Genetic and chemical diversity of native populations of Ocimum selloi Benth. Ind Crop Prod 76:249–257

Farzadfar S, Zarinkamar F, Hojati M (2017) Magnesium and manganese affect photosynthesis, essential oil composition and phenolic compounds of *Tanacetum parthenium*. Plant Physiol Biochem 112:207–217

Fernandes ES, Passos GF, Medeiros R et al (2007) Anti-inflammatory effects of compounds *alpha*-humulene and (−)-*trans*-caryophyllene isolated from the essential oil of *Cordia verbenacea*. Eur J Pharmacol 569:228–236

Fernandes MG, Gomes RA, Brito-Filho SG (2014) Characterization and anti-staphylococcal activity of the essential oil from *Turnera subulata* Sm. Revista Brasileira de Plantas Medicinais 16:534–538

Ferraz RPC, Cardoso GMB, Silva TB et al (2013) Antitumour properties of the leaf essential oil of *Xylopia frutescens* Aubl. (Annonaceae). Food Chem 141:542–547

Ferreira ME (2001) Técnicas e estratégias para a caracterização molecular e uso de recursos genéticos. In: Garray I, Dias BFS (eds) Conservação da Biodiversidade em ecossistemas tropicais: avanços conceituais e revisão de novas metodologias de avaliação e monitoramento. Petrópolis, Vozes, p 2001

Figueiredo C, Barroso J, Pedro L et al (2007) Factors affecting secondary metabolite production in plants: volatile components and essential oils. Flavour Fragr J 22:206–213

Figueiredo A, Barroso JG, Pedro LG et al (2008) Portuguese *Thymbra* and *Thymus* species volatiles: chemical composition and biological activities. Curr Pharm Des 14:3120–3140

Flora do Brasil (2020) Flora do Brasil. http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB110219. Accessed 31 July 2017

Frankham R, Ballou JD, Briscoe DA, Mclnnes KH (2008) Fundamentos de Genética da Conservação. Sociedade Brasileira de Genética, Ribeirão Preto.

Funk VA, Bayer RJ, Keeley S et al (2005) Everywhere but Antarctica: using a super tree to understand the diversity of the Compositae. Biologiske Skrifter 55:343–374

Galindez G, Biganzoli F, Ortega-Baes P et al (2009) Fire responses of three co-occurring Asteraceae shrubs in a temperature savanna in South America. Plant Ecol 202:149–158

Giordani R, Regli P, Kaloustian J et al (2004) Antifungal effect of various essential oils against *Candida albicans*. Potentiation of antifungal action of amphotericin B by essential oil from *Thymus vulgaris*. Phytother Res 18:990–995

Gobbo-Neto L, Lopes NP (2007) Plantas Medicinais: Fatores de Influência no Conteúdo de Metabólitos Secundários. Química Nova 30:374–381

Goggin FL (2007) Plant-aphid interactions: molecular and ecological perspectives. Curr Opin Plant Biol 10:399–408

Gouinguené SP, Turlings TCJ (2002) The effects of abiotic factors on induced volatile emissions in corn plants. Plant Physiol 129:1296–1307

Goutham MP (1980) Activity of some essential oils against dermatophytes. In: Lorenzi H, Matos FJA (eds) Plantas medicinais do Brasil: nativas e exóticas cultivadas. Nova Odessa: Instituto Plantarum, 2002

Guenther E (1948) The essential oils: history-origin in plants production-analysis. Krieger Publishing, New York

Gupta R (1991) Agrotechnology of medicinal plants. In: Wijessekera ROB (ed) The medicinal plant industry. CRC Press, Boca Raton

Gupta AK, Mishra R, Singh AK et al (2016) Genetic variability and correlations of essential oil yield with agro-economic traits in Mentha species and identification of promising cultivars. Ind Crop Prod 95:726–732

Haber LL (2008) Caracterização da diversidade genética , via marcador microssatélite, e constituintes do óleo essencial de *lychnophora pinaster* mart. Thesis, Universidade Estadual Paulista Júlio de Mesquita Filho

Harley R, França F, Santos EP, Santos JS, Pastore JF (2015) Lamiaceae in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB142. Accessed: 18 outubro 2016.

Heiden G, Barbieri RL, Wasum RA et al (2007) A família Asteraceae em São Mateus do Sul, Paraná. Revista Brasileira de Biociências 5:249–251

Hoagland DR, Arnon DI (1950) The water: culture method for growing plants without soil. Berkeley, California

Hussain AI, Anwar F, Sherazi STH, Przybylski R (2008) Chemical composition, antioxidant and antimicrobial activities of basil (Ocimum basilicum) essential oils depends on seasonal variations. Food Chemistry 108(3):986–995

Ietswaart JH (1980) A taxonomic revision of the genus Origanum (Labiatae). Leiden University, Leiden

Isobe MTC (2012) Lychnophora pinaster: estudo anatômico, propagação e composição química dos óleos essenciais de populações. Dissertation, Universidade Estadual Paulista Júlio de Mesquita Filho

Jamieson MA, Burkle LA, Manson JS et al (2017) Global change effects on plant–insect interactions: the role of phytochemistry. Curr Opin Insect Sci 23:70–80

Joly AB (1993) Botânica: introdução à taxonomia vegetal. Companhia Editora Nacional, São Paulo

Kamanula JF, Belmain SR, Hall DR et al (2017) Chemical variation and insecticidal activity of *Lippia javanica* (Burm. f.) Spreng essential oil against *Sitophilus zeamais* Motschulsky. Ind Crops Prod 110:75–82

Karp A, Kresovich S, Bhat KV et al (1997) Molecular tools in plant genetic resources conservation a guide in the technologies. IPGRI, Rome

Keita SM, Vincent C, Schmit JP et al (2001) Efficacy of essential oil of *Ocimum basilicum* L. and *O. gratissimum* L. applied as an insecticidal fumigant and powder to control *Callosobruchus maculatus* (Fab) (Coleoptera: Bruchidae). J Stored Prod Res 37:339–349

Kende H (1993) Ethylene biosynthesis. Annu Rev Plant Physiol Plant Mol Biol 44:283–307

Kim SH, Lee S, Piccolo SR et al (2012) Menthol induces cell-cycle arrest in PC-3 cells by down-regulating G2/M genes, including polo-like kinase 1. Biochem Biophys Res Commun 422:436–441

Klaric MS, Kosalec I, Mastelic J et al (2007) Antifungal activity of thyme (*Thymus vulgaris* L.) essential oil and thymol against moulds from damp dwellings. Lett Appl Microbiol 44:36–42

Klimánková E, Holadová K, Hajslová J et al (2008) Aroma profiles of five basil (*Ocimum basilicum* L.) cultivars grown under conventional and organic conditions. Food Chem 107:464–472

Kopsell DA, Kopsell DE, Curran-Celentano J (2005) Carotenoid and chlorophyll pigments in sweet basil grown in the field and greenhouse. Hortscience 40:1230–1233

Kroymann J (2011) Natural diversity and adaptation in plant secondary metabolism. Curr Opin Plant Biol 14:246–251

Kumar S, Wahab N, Warikoo R (2011) Bioefficacy of Mentha piperita essential oil against dengue fever mosquito *Aedes aegypti* L. Asian Pac J Trop Biomed 1:85–88

Lambers H, Chapin FS, Pons TL (1998) Plant physiological ecology. Springer, New York

Langenheim JH (2003) Plant Resins: Chemistry, Evolution, Ecology, and Ethnobotany. Timber Press, Oregon

Lawrence BM (1995) The isolation of aromatic materials from natural plant products. In: Tuley de Silva K (ed) A manual on the essential oil industry. Proceedings of the 3rd UNIDO workshop on essential oil and aroma chemical industries, pp 57–154

Leal FP (2001) Desenvolvimento, produção e composição de óleo essencial da *Mentha piperita* L., cultivada em solução nutritiva com diferentes níveis de nitrogênio. Dissertation, Universidade Estadual Paulista Júlio de Mesquita Filho

Li Q, Wang X, Yang Z et al (2009) Menthol induces cell death via the TRPM8 channel in the human bladder cancer cell line T24. Oncology 77:335–341
Lima TC, Silva TKM, Silva FL et al (2014) Larvicidal activity of Mentha × villosa Hudson essential oil, rotundifolone and derivatives. Chemosphere 104:37–43
Loewenfeld C, Back F (1980) Guia de las hierbas y especias. Ediciones Omega, Barcelona
López A, Castro S, Andina MJ et al (2014) Insecticidal activity of microencapsulated *Schinus molle* essential oil. Ind Crop Prod 53:209–216
Lorenzi H, Matos FJA (2008) Plantas medicinais no Brasil: nativas e exóticas, 2nd edn. Instituto Plantarum, Nova Odessa
Maathuis FJM (2009) Physiological functions of mineral macronutrients. Curr Opin Plant Biol 12:250–258
Mahmoud SS, Croteau RB (2002) Strategies for transgenic manipulation of monoterpene biosynthesis in plants. Trends Plant Sci 7:366–373
Marchese JÁ, Figueira GM (2005) O uso de tecnologias pré e pós-colheita e boas práticas agrícolas na produção de plantas medicinais e aromáticas. Revista Brasileira de Plantas Medicinais 7:86–96
Marin M, Koko V, Duletić-Laušević S et al (2006) Glandular trichomes on the leaves of *Rosmarinus officinalis*: morphology, stereology and histochemistry. S Afr J Bot 72:378–382
Marotti M, Piccaglia R, Giovanelli E (1996) Differences in essential oil composition of Basil (*Ocimum basilicum* L.) Italian cultivars related to morphological characteristics. J Agric Food Chem 44:3926–3929
Marques MOM, Facanali R, Haber LL et al (2012) Essential oils: history, biosynthesis, and agronomic aspects. In: Medicinal essential oils: chemical, pharmacological and therapeutic aspects. Nova Science Publishers, New York
Marques MOM, Facanali R, Haber LL et al (2013) Óleos essenciais. In: Haber LL, Clemente FMVT (eds) Plantas aromáticas e condimentares: uso aplicado na horticultura. Embrapa, Brasília
Marschner H (2012) Mineral nutrition of higher plants, 3rd edn. Academic Press, San Diego
Martins ER, Casali VWD, Barbosa LCA et al (1997) Essential oil in the taxonomy of Ocimum selloi Benth. J Braz Chem Soc 8:29–32
Matos-Rocha T, Cavalcanti MGS, Barbosa-Filho JM et al (2013) In vitro evaluation of schistosomicidal activity of essential oil of *Mentha* × *villosa* and some of its chemical constituents in adult worms of *Schistosoma mansoni*. Planta Med 79:1307–1312
Matos-Rocha TJ, Cavalcanti MGS, Veras DL et al (2016) Ultrastructural changes in *Schistosoma mansoni* male worms after in vitro incubation with the essential oil of Mentha × villosa Huds. Revista Do Instituto de Medicina Tropical de Sao Paulo 58:2–7
Matos-Rocha TJ, Cavalcanti MGS, Barbosa-Filho JM et al (2017) Ultrastructural study of morphological changes in *Schistosoma mansoni* after in vitro exposure to the monoterpene rotundifolone. Sociedade Brasileira De Medicina Tropical 50:86–91
Medeiros R, Passos GF, Vitor CE et al (2007) Effect of two active compounds obtained from the essential oil of *Cordia verbenacea* on the acute inflammatory responses elicited by LPS in the rat paw. Br J Pharmacol 151:618–627
Meldau S, Erb M, Baldwin IT (2012) Defence on demand: mechanisms behind optimal defence patterns. Ann Bot 110:1503–1514
Melo WJ, Marques MOM (2000) Potencial do lodo de esgoto como fonte de nutrientes para as plantas. In: Bettiol W, Camargo OA (eds) Impacto ambiental do uso agrícola do lodo de esgoto. Embrapa Meio Ambiente, Jaguariúna, pp 109–141
Milach SCK (1998) Principais tipos de marcadores moleculares e suas características. In: Milach SCK (ed) Marcadores moleculares em plantas. UFRGS, Porto Alegre
Miller JS, Gottschling M (2007) Generic classification in the Cordiaceae (Boraginales): resurrection of the genus *Varronia* P Br. Taxon 56:163–169
Millezi FM, Pereira MO, Batista NN et al (2012) Susceptibility of monospecies and dual-species biofilms Staphylococcus aureus and *Escherichia coli* to essential oils. J Food Saf 32:351–359
Millezi FM, Cardoso MG, Alves E et al (2013) Reduction of *Aeromonas hydrophila* biofilm on stainless steel surface by essential oils. Braz J Microbiol 44:73–80

Mockute D, Bernotiene G, Judzentiene A (2001) The essential oil of *Origanum vulgare* L. ssp. *vulgare* growing wild in Vilnius district (Lithuania). Phytochemistry 57:65–69
Mockute D, Bernotiene G, Judzentiene A (2003) The β-ocimene chemotype of essential oils of the inflorescences and the leaves with stems from *Origanum vulgare ssp. vulgare* growing wild in Lithuania. Biochem Syst Ecol 31:269–278
Molt O, Trka A (1983) Parfum Kostmet 64: 488. apud Verdi LG 2005.
Montanari I Jr (2000) Cultivo comercial de erva-baleeira. Revista Agroecologia Hoje 3:14–15
Monteith GR, McAndrew D, Faddy HM et al (2007) Calcium and cancer: targeting Ca2+ transport. Nat Rev Cancer 7:519–530
Morhy L (1973) Metil-chavicol, *cis* e *trans*-Anetol no Óleo Essencial de *Ocimum selloi* Benth. An Acad Bras Ciênc 45:401–412
Morris JA, Khettry A, Seitz EWM (1979) Antimicrobial activity of aroma chemicals and essential oils. J Am Oil Chem Soc 56:595–603
Mors WB, Rizzini CT, Pereira NA (2000) Medicinal plants of Brazil. Reference Publications, Algonac
Morshedloo MR, Craker LE, Salami A et al (2017) Effect of prolonged water stress on essential oil content, compositions and gene expression patterns of mono- and sesquiterpene synthesis in two oregano (*Origanum vulgare* L.) subspecies. Plant Physiol Biochem 111:119–128
Munsi OS (1992) Nitrogen and phosphorus nutrition response in Japanese mint cultivation. Acta Hortic 306:436–443
Nagegowda DA (2010) Plant volatile terpenoid metabolism: biosynthetic genes, transcriptional regulation and subcellular compartmentation. FEBS Letters 584(14):2965–2973
Naghibi F, Mosaddegh M, Motamed SM et al (2005) Labiatae Family in folk medicine in Iran: from Ethnobotany to pharmacology. Iran J Pharm Res 2:63–79
Nakajima J, Loeuille B, Heiden, G et al (2015) Asteraceae in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB55 Accessed 03 out. 2017
Nascimento KF, Moreira FMF, Santos JA, Kassuia CAL, Croda JHR, Cardoso CAL, Vieira MC, Ruiz ALTG, Foglio MA, de Carvalho JE, Formagio ASN (2017) Antioxidant, anti-inflammatory, antiproliferative and antimycobacterial activities of the essential oil of *Psidium guineense* Sw and spathulenol. J Ethnopharmacol 210:351–358
Oliveira VB, Yamada LT, Fagg CW, Brandão MGL (2012) Native foods from Brazilian biodiversity as a source of bioactive compounds. Food Res Int 48:170–179
Oliveira MMM, Brugnera DF, Piccoli RH (2013) Essential oils of thyme and Rosemary in the control of *Listeria monocytogenes* in raw beef. Braz J Microbiol 44:1181–1188
Ormeno E, Fernandez C (2012) Effect of soil nutrient on production and diversity of volatile terpenoids from plants. Curr Bioact Compd 8:71–79
Pal PK, Mahajan M, Agnihotri VK (2016) Foliar application of plant nutrients and kinetin modifies growth and essential oil profile in Rosa damascena under acidic conditions. Acta Physiol Plant 38:176
Pandotra P, Gupta AP, Gandhiram et al (2013) Genetic and chemo-divergence in eighteen core collection of *Zingiber officinale* from North-West Himalayas. Sci Hortic 160:283–291
Park YK, Paredes-Guzman JF, Aguiar CL et al (2004) Chemical constituents in *Baccharis dracunculifolia* as the main botanical origin of southeastern Brazilian propolis. J Agric Food Chem 52:1100–1103
Park MJ, Gwak KS, Yang I et al (2009) Effect of citral, eugenol, nerolidol and alpha-terpineol on the ultrastructural changes of Trichophyton mentagrophytes. Fitoterapia 80:290–296
Pascual-Villalobos MJ, Ballesta-Acosta MC (2003) Chemical variation in an *Ocimum basilicum germplasm* collection and activity of the essential oils on *Callosobruchus maculatus*. Biochem Syst Ecol 31:673–679
Passos GF, Fernandes ES, da Cunha FM et al (2007) Anti-inflammatory and anti-allergic properties of the essential oil and active compounds from Cordia verbenacea. J Ethnopharmacol 110:323–333

Pavarini DP, Nogueira EF, Callejon DR et al (2013) Novel bisabolane derivative from "arnica-da-serra" (Vernonieae: Asteraceae) reduces pro-nociceptive cytokines levels in LPS-stimulated rat macrophages. J Ethnopharmacol 148:993–998

Perigo CV, Torres RB, Bernacci LC et al (2016) The chemical composition and antibacterial activity of eleven Piper species from distinct rainforest areas in Southeastern Brazil. Ind Crop Prod 94:528–539

Prasad G, Kuman A, Singh AK, Bhattacharya AK et al (1986) Antimicrobial activity of essential oils of some *Ocimum* species and clove oil. Fitoterapia 57:429–432

Primack RB, Rodrigues E (2001) Biologia da conservação. Planta, Londrina

Queiroz VS (2012) Avaliação in vitro do efeito dose-resposta da nanoemulsão do extrato etanólico de *Lychnophora* pinaster sobre células planctônicas e biofilme de *Streptococcus mutans* e sobre a desmineralização do esmalte dental ao redor de braquetes ortodônticos. Thesis, Universidade Estadual de Campinas

Rana IS, Rana AS, Rajak RC (2011) Evaluation of antifungal activity in essential oil of the *Syzygium aromaticum* (L.) by extraction, purification and analysis of its main component eugenol. Braz J Microbiol 42:1269–1277

Rehman R, Hanif MA (2016) Biosynthetic factories of essential oils: the aromatic plants. Nat Prod Chem Res 04(04)

Reigosa M, Gomes AS, Ferreira AG, Borghetti F (2013) Allelopathic research in Brazil. Acta Botanica Brasilica 27(4):629–646

Richards EJ (2006) Inherited epigenetic variation-revisiting soft inheritance. Mycorrhiza 7:395–401

Rodrigues VEG (1988) Levantamento florístico e etnobotânico de plantas medicinais dos cerrados na região do Alto Rio Grande – Minas Gerais. Dissertation, Universidade Federal de Lavras

Rice EL (1984) Alelopatia. Academic Press, New York

Saggiorato AG, Gaio I, Treichel H et al (2012) Antifungal activity of basil essential oil (*Ocimum basilicum* L.): evaluation in vitro and on an Italian-type sausage surface. Food Bioproc Technol 5:378

Scavroni J, Boaro CSF, Marques MOM, Ferreira LC (2005) Yield and composition of the essential oil of *Mentha piperita* L. (Lamiaceae) grown with biosolid. Braz J Plant Physiol 17:345–352

Schilmiller AL, Last RL, Pichersky E (2008) Harnessing plant trichome biochemistry for the production of useful compounds. Plant J 54:702–711

Semir J (1991) Revisão taxonômica de *Lychnophora* Mart. (Vernoniaceae: Compositae). Thesis, Universidade de Campinas

Semir J, Rezende AR, Monge M, Lopes NP (2011) As arnicas endêmicas das Serras do Brasil: Uma visão sobre a biologia e a química das espécies de *Lychnophora* (Asteraceae). UFOP, Ouro Branco (MG)

Sforcin JM, Souza JPB, Silva Filho AA et al (2012) *Baccharis dracunculifolia*: Uma das principais fontes vegetais da própolis brasileira. Editora UNESP, São Paulo

Shao M, Czapiewski KV, Heiden AC et al (2001) Volatile organic compound emissions from Scots pine: mechanisms and description by algorithms. J Geophys Res 106:483–491

Shao H, Guo Q, Chu L et al (2007) Understanding molecular mechanism of higher plant plasticity under abiotic stress. Colloids Surf B: Biointerfaces 54:37–45

Shulaev V, Cortes D, Miller G, Mittler R (2008) Metabolomics for plant stress response. Physiol Plant 132:199–208

Sienkiewicz M, Lysakowska M, Denys P, Kowalczyk E (2012) The antimicrobial activity of *Thyme* essential oil against multidrug resistant clinical bacterial strains. Microb Drug Resist 18:137–148

Sifola MI, Barbieri G (2006) Growth, yield and essential oil content of three cultivars of basil grown under different levels of nitrogen in the field. Sci Hortic 108:408–413

Silva PSS (2013) Caracterização da composição química dos óleos essenciais de Lychnophora pinaster Mart. em função da sazonalidade Dissertation, Universidade Estadual Paulista Júlio de Mesquita Filho.

Silva PSS (2016) Caracterização da diversidade genética e composição química de *Lychnophora pinaster* Mart. Thesis, Universidade Estadual Paulista Júlio de Mesquita Filho

Silva LE, Reis RA, Moura EA et al (2015) Plantas do Gênero *Xylopia*: Composição Química e Potencial Farmacológico. Revista Brasileira de Plantas Medicinais 17:814–826

Silvas PKJ, Syed NH, Valliyodan B, Nguyen HT (2013) Understanding abiotic stress tolerance mechanisms in soybean (*Glycine max*). Plant Physiol Biochem 85:1–16

Simões CMO, Spitzer V (2000) Óleos voláteis. In: Farmacognosia: da planta ao medicamento. Universidade Federal do Rio Grande do Sul; Universidade Federal de Santa Catarina

Simões LN, Meideiros LCC, Heinzmann BM et al (2017) Essential oil of *Lippia alba* as a sedative and anesthetic for the sea urchin *Echinometra lucunter* (Linnaeus, 1758). Mar Freshw Behav Physiol 50:205–217

Singh B, Sharma RA (2015) Plant terpenes: defense responses, phylogenetic analysis, regulation and clinical applications. Biotech 5:129–151

Sousa AAS, Soares PMG, Almeida ANS et al (2010) Antispasmodic effect of *Mentha piperita* essential oil on tracheal smooth muscle of rats. Rev Ethnopharmacol Commun 130:433–436

Souza VC, Lorenzi H (2008) Botânica Sistemática – Guia ilustrado para identificação das famílias de angiospermas da flora brasileira, baseado em APG II. Plantarum

Stengel M, Binder A, Klebe O et al (2007) Topical menthol: stability of a sensory profile in a human surrogate model. Eur J Pain 11:59–S207

Stojkovic D, Soković M, Glamočlija J et al (2011) Chemical composition and antimicrobial activity of *Vitex agnus-castus* L. fruits and leaves essential oils. Food Chem 128:1017–1022

Tabarelli M, Mantovani W (1999) A regeneração de uma floresta tropical montana após corte e queima, São Paulo. Rev Bras Biol 59:239–250

Taiz L, Zeiger E (2010) Plant physiology, 5th edn. Sinauer Associates Inc, Sunderland

Torres A et al (1996) A science of the future. In: World congress of Allelopa, Cadiz. Annals… Cidade: University of Cadiz, 1996. p. 16–20.

Trapp SC, Croteau RB (2001) Genomic organization of plant terpene synthases and molecular evolutionary implications. Genetics 158:811–832

Trombetta D, Castelli F, Sarpietro MG et al (2005) Mechanisms of antibacterial action of three monoterpenes. Antimicrob Agents Chemother 49:2474–2478

Tsutiya MT (2000) Alternativas de disposição final de biossólidos gerados em estações de tratamentos de esgotos. In*:* Bettiol W, Camargo OA (eds) Impacto ambiental do uso agrícola do lodo de esgoto EMBRAPA Meio Ambiente, pp 69–105

Umerie SC, Anaso HU, Anyasoro JC (1998) Insecticidal potentials of *Ocimum basilicum* leaf-extract. Biores Technol 64:237–239

Valeriano C, Oliveira TLC, Carvalho SM et al (2012) The sanitizing action of essential oil-based solutions against *Salmonella enterica serotype Enteritidis* S64 biofilm formation on AISI 304 stainless steel. Food Control 25:673–677

Valmorbida J, Boaro CSF, Marques MOM, Ferri AF (2006) Rendimento e composição química de óleos essenciais de *Mentha piperita* L . cultivada em solução nutritiva com diferentes concentrações de potássio. Rev Bras Plantas Med 8:56–61

Vanderlinde FA, Costa EA, D'Angelo LCA (1994) Atividades farmacológicas gerais e atividade anti-espasmódica do extrato etanólico de *Ocimum selloi* Benth. (elixir paregórico). In: Simpósio de Plantas Medicinais do Brasil

Vasques MCP (2007) Influência do magnésio no desenvolvimento, trocas gasosas e rendimento de óleo essencial de *Mentha piperita* L. cultivada em solução nutritiva. Dissertation, Universidade Estadual Paulista, Botucatu

Vaz APA, Scaranari C, Rocha LA et al (2006) Biomassa e composição química de genótipos melhorados de espécies medicinais cultivadas em quatro municípios paulistas. Pesquisa Agropecuária Brasileira, Brasília 41:69–872

Verpoorte R (2000) Engineering the plant cell factory for secondary metabolite production. Trangenic Res 9:323–343

Vieira MAR, Marques MOM, Haber LL et al (2014) New loci of *Lychnophora ericoides* and transferability to *Lychnophora pinaster*, endangered medicinal species from Brazil. Genet Mol Res 13:10878–10882

Vieira MAR, Marques MOM, Haber LL et al (2017) Perfil do óleo essencial de folhas de *Lychnophora ericoides* Mart coletadas em duas localidades de Minas Gerais. In: 9° Simpósio Brasileiro de Óleos Essenciais

Voirin B, Brun N, Bayet C (1990) Effects of daylength on the monoterpene composition of leaves of *Mentha x piperita*. Phytochemistry 29:749–755

Wang Y, Wang X, Yang Z et al (2012) Menthol inhibits the proliferation and motility of prostate cancer DU145 cells. Pathol Oncol Res 18:903–910

Wink M (2016) Secondary metabolites, the role in plant diversification of Encyclopedia of. Evol Biol 4:1–9

Ye Y, Liang X, Chen Y et al (2014) Carbon, nitrogen and phosphorus accumulation and partitioning, and C: N: P stoichiometry in late-season rice under different water and nitrogen managements. PLoS One 9:7

Zucchi MI (2009) Diversidade genética em espécies medicinais. http://www.infobibos.com/Artigos/2009_4/DiversidadeGenetica/index.htm. Accessed: 11/6/2018

Chapter 3
Hedychium Essential Oils: Composition and Uses

Hamidou F. Sakhanokho and Kanniah Rajasekaran

3.1 Introduction

Hedychium species are perennial monocotyledonous plants belonging to the Zingiberaceae family, which consists of 52 genera and close to 2000 species known for their various uses, including in medicine, cosmetics, fragrance, ornamental, paper, and food industries. The genus *Hedychium*, often referred to as "butterfly ginger," "garland lily," or "ginger lily," is one of the most popular genera of the Zingiberaceae family because of its attractive foliage, diverse and showy flowers, and sweet fragrance. Currently, there is little consensus on the number of *Hedychium* species, which is estimated to vary from 50 to 80, and more species continue to be discovered and described (Ding et al. 2018). The word "*Hedychium*" is from Greek and derived from "hedys" and "chion" meaning "sweet" and "snow," respectively (Branney 2005). All *Hedychium* species, except *H. peregrinum* – an endemic species to Madagascar – are native to Central and Southeastern Asia, with centers of origin and diversity in Southern China and Northeastern India (Branney 2005; Sarangthem et al. 2013). *Hedychium* species are widely cultivated for their perfume essences. The scents range from the rich gardenia-like fragrance of *H. coronarium* to scents reminiscent of citrus, clove, and even coconut (Wood 1999). *Hedychium* rhizomes and aerial stems contain a starch similar to arrowroot which is a useful raw material for manufacturing paper (Mukherjee 1970). Because of their attractive foliage, diverse and showy flowers, and sweet fragrance, *Hedychium* species are increasing in popularity as ornamental plants (Fig. 3.1).

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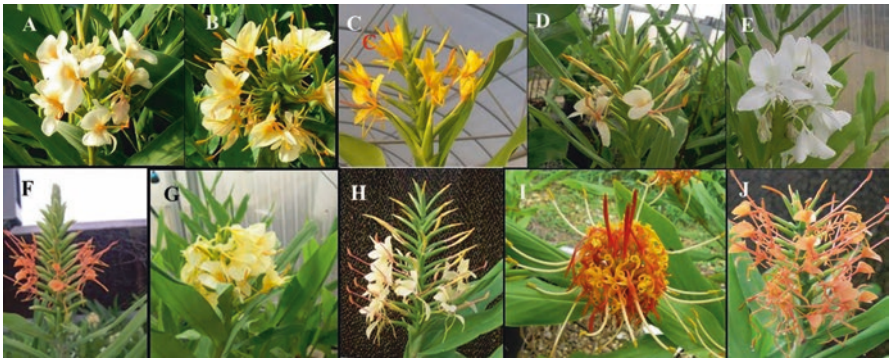

Fig. 3.1 *Hedychium* species are used for multiple purposes, one of which is their ornamental value. *Hedychium* species are gaining popularity as ornamental plants because of their attractive foliage, diverse and showy flowers, and sweet fragrance. (**a**) *Hedychium* "Kinkaku"; (**b**) *Hedychium* "Dr. Moy"; (**c**) *Hedychium* "Betty Ho"; (**d**) *H. anguistifolium*; (**e**) *H. coronarium*; (**f**) *H. coccineum*; (**g**) *Hedychium* "Daniel Weeks"; (**h**) *H. gardnerianum*; (**i**) *H. longicornutum*; (**j**) *Hedychium* "Orange Brush"

In addition to the beneficial traits mentioned above, *Hedychium* spices, like many members of Zingiberaceae, are widely used in ethnomedicine to treat various ailments including nausea, asthma, flu, diarrhea, snake bites, and leishmaniasis (Chen et al. 2008; Valadeau et al. 2009; Tushar et al. 2010; Tiuman et al. 2011). Further, the essential oils extracted from various *Hedychium* plant parts have shown antimicrobial activities in various studies, so they offer the potential to be used as safer alternatives to synthetic antibiotics, antifungals, and insecticides. For example, essential oils in the leaves and flowers of *H. gardnerianum* have been found to have antimicrobial activity against certain bacteria, namely, *Staphylococcus aureus* and *S. epidermis* (Medeiros et al. 2003), while Gopanraj et al. (2005) reported antibacterial activity of the essential oil from the rhizomes of *H. larsenii* against both Gram-positive and Gram-negative bacteria. Others have reported antifungal, insecticidal, and antioxidant properties of essential oils from various *Hedychium species* (Rajasekaran et al. 2012; Sakhanokho et al. 2013; Ray et al. 2018).

3.2 Chemical Constituents of *Hedychium* Essential Oils

Essential oils can be defined as a mixture of volatile and natural substances, characterized by a strong odor and produced by aromatic plants as secondary metabolites (Sá et al. 2013). Increasingly, plant essential oils are recognized as important sources of biopesticides, against which insects and microbes do not acquire induced resistance and have limited toxic effects on human or animal health and non-target organisms (AlShebly et al. 2017). The antimicrobial properties of *Hedychium* essential oils, in particular, have been well documented, and their use as "biocides" is gaining popularity (Sakhanokho et al. 2013). Listed in Table 3.1 are *Hedychium*

Table 3.1 Major chemical constituents of *Hedychium* essential oils

Taxa	Major compound	Compound percentage (w/w)				Reference
		Leaves	Flowers	Rhizomes	Stems	
Hedychium coronarium	Caryophyllene	43.9		1.1		Rodrigues et al. (2013)
	1,8-Cineole	0.7		31.7		
Hedychium coronarium	1,8-Cineole	–		40.2		Lechat-Vahirua et al. (1993)
	β-Pinene	–		24.8		
Hedychium acuminatum	1,8-Cineole			76		Weyerstahl et al. (1995)
Hedychium coccineum	(E)-Nerolidol			44.4		Gurib-Fakim et al. (2002)
	trans-Sesquisabinene hydrate			24.2		
Hedychium flavesens	Linalool			35.0		Gurib-Fakim et al. (2002)
	1,8-Cineole			15.3		
	β-Pinene			14.7		
	α-Terpineol			14.5		
	α-Pinene			5.3		
Hedychium coronarium	α-Muurolol			16.8		Gurib-Fakim et al. (2002)
	α-Terpineol			15.9		
	1,8-Cineole			11.2		
Hedychium cylindricum	Terpinen-4-ol			40.5		Ahmad et al. (2004)
	Sabinene			9.9		
	p-Cymene			8.5		
	Limonene			6		
	β-Pinene			5.6		
	γ-Yerpinene			4.5		
	α-Terpineol			2.2		
Hedychium coronarium	β-Pinene			42.74		Ray et al. (2018)
	Eucalyptol			40.59		
	Linalool			45.11		
	Coronarin-E			39.56		
	α-Pinene			16.60		
	p-Cymene			8.89		
	γ-Terpinene			5.82		
	10-Epi-γ-eudesmol			4.86		
Hedychium greenii	Bomyl acetate			31.32		Ray et al. (2017a, b)
	α-Pinene			14.49		
	Camphene			12.81		
	Limonene			10.55		
Hedychium gracile	β-Pinene			25.24		Ray et al. (2017a, b)
	γ-Terpinene			24.62		
	Terpinen-4-ol			14.87		
	1,8-Cineole			7.51		

(continued)

Table 3.1 (continued)

Taxa	Major compound	Compound percentage (w/w)				Reference
		Leaves	Flowers	Rhizomes	Stems	
Hedychium coronarium	Linalool			29.3		Prakash et al. (2010)
	Limonene			20.3		
	trans-Meta-mentha,2,8-diene			12.9		
	γ-Terpinene			8.9		
	10-Epi-γ-eudismol			3.8		
Hedychium spicatum	1,8-Cineole			17.6		Prakash et al. (2010)
	α-Eudesmol			17		
	10-Epi-γ-eudismol			9.7		
	δ-Cadinene			7.5		
	Eugenol			6.9		
	Germacrene D-4-ol			6.8		
	γ-Cadinene			5.4		
Hedychium coronarium	β-Pinene	33.9		23.0		Ho (2011)
	α-Pinene	14.7				
	1,8-Cineole	13.3		37.3		
	r-Elemene	11.0				
	Carotol	9.1				
	α-Terpineol			10.4		
	α-Pinene			9.9		
Hedychium coronarium	β-Pinene	46.9		41.5		Miranda et al. (2014)
	1,8-Cineole			23.6		
	α-Pinene	19.2		13.1		
	β-Caryophyllene	13.2				
Hedychium stenopetalum	α-Pinene	52.5		5.0		Van Thanh et al. (2014)
	β-Pinene	31.8				
	Linalool			45.2		
	(E)-Nerolidol			8.7		
Hedychium coronarium	β-Pinene	20.0		23.6		Van Thanh et al. (2014)
	Linalool	15.8				
	1,8-Cineole	10.7				
	α-Pinene	10.1				
	α-Terpineol	8.6				
	α-Humulene			17.1		
	β-Caryophyllene			13.0		
Hedychium flavum	β-Pinene	22.5		21.8	11.2	Van Thanh et al. (2014)
	α-Humulene	15.7			18.9	
	β-Caryophyllene	10.4			11.8	
	Linalool			17.5		
	1,8-Cineole			13.5		

(continued)

Table 3.1 (continued)

Taxa	Major compound	Compound percentage (w/w)				Reference
		Leaves	Flowers	Rhizomes	Stems	
Hedychium ellipticum	(E)-Nerolidol	15.9				Van Thanh et al. (2014)
	β-Pinene	11.8		11.0		
	Bomyl acetate	9.2				
	1,8-Cineole			40.8		
	α-Pinene			18.3		
Hedychium malayanum	1,8-Cineole			37.7		Abdo et al. (2015)
	β-Pinene			35.2		
	α-Pinene			10.9		
Hedychium forrestii	β-Pinene			18.3		Thomas and Mani (2016)
	β-Linalool			17.8		
	1,8-Cineole			12.0		
	4-Terpineol			5.5		
Hedychium aurantiacum	Linalool			83.01		Kumar et al. (2017)
	Limonene			4.81		
	α-Terpinene			2.69		
	trans-Linalool oxide			1.55		
	cis-Linalool oxide			1.53		
Hedychium matthewiii	Linalool			45.6		Thomas and Mani (2018)
	β-Pinene			6.5		
	Camphene			3.3		
	α-Pinene			2.5		
Hedychium roxburghii	α-Fenchyl acetate			45.85		Hartati et al. (2015)
	Alloaromadendrene			8.83		
	β-Maaliene			4.88		
	Spathulenol			4.42		
Hedychium aurantiacum	(+)-Linalool			80.6		Pant et al. (1992)
Hedychium coronarium	β-Caryophyllene	43.0				Dos Santos et al. (2010)
	Caryophyllene oxide	12.1				
	β-Pinene	11.6		16.7		
	1,8-Cineole			34.8		
	α-Terpineol			13.1		
Hedychium spicatum	1,8-Cineole			30.84		Semwal et al. (2015)
	β-Eudesmol			14.50		
	β-Pinene			9.24		
	Limonene			6.42		
	Linalool			5.29		
Hedychium "Dr. Moy"	1,8-Cineole			42		Sakhanokho et al. (2013)
Hedychium forrestii	Linalool			56		Sakhanokho et al. (2013)

(continued)

Table 3.1 (continued)

Taxa	Major compound	Compound percentage (w/w)				Reference
		Leaves	Flowers	Rhizomes	Stems	
Hedychium "Tai Emperor"	α-Pinene			17		Sakhanokho et al. (2013)
Hedychium bousigonianum	β-Pinene			31		Sakhanokho et al. (2013)
Hedychium "Dave Case"	(E)-Nerolidol			20		Sakhanokho et al. (2013)
Hedychium gardnerianum	α-Cadinol		26.22			Medeiros et al. (2003)
	α-Pinene		18.37			
	β-Pinene		14.53			
Hedychium larsenii	Linalool		62.26			Gopanraj et al. (2005)
	1,8-Cineole		14.41			
Hedychium larsenii	ar-Curcumene			28.6		AlShebly et al. (2017)
	epi-β-Bisabolol			10.3		

taxa along with the major constituents of their essential oils, but this list is far from being exhaustive. In addition to published chemical constituents as cited in Table 3.1, other minor chemical compounds were also identified but not listed here. Plant parts used for essential oil extraction include leaves, stems (pseudo-stems), flowers, roots, and rhizomes (Arruda et al. 2012; Sakhanokho et al. 2013; Verma and Padalia 2010). The rhizomes appear to be the plant material most often used for *Hedychium* essential oil extraction. This is most likely because once *Hedychium* plants are grown and well established, their rhizomes offer readily available vegetal parts throughout the year. Among the taxa listed in Table 3.1 are the variegated *Hedychium* cultivar "Dr. Moy" (Fig. 3.1b), *H. coronarium* (Fig. 3.1e), *H. coccineum* (Fig. 3.1f), and *H. gardnerianum* (Fig. 3.1h).

Monoterpenes and sesquiterpenes constitute the main compounds as reported in most of the studies on *Hedychium* essential oils (Table 3.1). This is particularly true for essential oil studies in *H. coronarium* where the monoterpene 1,8-cineole is ubiquitous and found in both rhizomes and leaves. In an essential oil study involving 19 *Hedychium* genotypes, the compound 1,8-cineole was, by far, the most ubiquitous constituent of the oils being found in 16 out of the 19 *Hedychium* genotypes. In 13 of those genotypes, 1,8-cineol was the dominant constituent (Sakhanokho et al. 2013). Other monoterpene compounds found by the same authors included linalool (<0.1–56%), α-pinene (3–17%), and β-pinene (4–31%) followed by sesquiterpene constituents such as (*E*)-nerolidol (0.1–20%). Further, Abdo et al. (2015) reported that 99% of the essential oil compounds found in *Hedychium malayanum* rhizomes were monoterpenes, including 1,8-cineole, β-pinene, and α-pinene. Similarly, Gopanraj et al. (2005) found that 99% of the essential oils found in *Hedychium larsenii* were monoterpenes, with linalool and 1,8-cineole as major constituents. Recently, however, AlShebly et al. (2017) found that, instead of monoterpene compounds, the major compounds found in essential oils of the same species, *H. larsenii*, were the sesquiterpene compounds ar-curcume (28.6%) and epi-β-bisabolol (10.3%).

Differences in oil constituents of the same *Hedychium* species have been reported before (Verma and Padalia 2010; Ray et al. 2018), and these differences are attributed to various factors. Chemical composition of the essential oil of the same plant species can vary depending on factors such as genotype, season, vegetal parts, and environment. For example, Zheljazkov et al. (2008) reported differences in oil constituents of the same cultivars of two other species, *Ocimum basillicum* L. and *O. sanctum* L., grown in two different locations and attributed these differences to different environmental conditions such as temperature, soil characteristics, and production system at the two locations of their study. Ray et al. (2018) studied the chemical diversity, antioxidant, and antimicrobial activities of the essential oils from various Indian populations of *Hedychium coronarium* and found that geographic origin greatly influenced not only the chemical composition of essential oils but also their associated bioactivities. Furthermore, production of a particular type of oil is highly influenced by the physiology of plant, which undergoes, for example, crucial changes during the transition from the vegetative stage to flowering (Sangwan et al. 2001; Argyropoulou et al. 2007).

3.3 Antimicrobial Activities

The antimicrobial and antioxidant activities of essential oils of various *Hedychium* species have been investigated (Medeiros et al. 2003; Verma and Padalia 2010; Rajasekaran et al. 2012; Sakhanokho et al. 2013; Ray et al. 2018; Table 3.2). As stated earlier, sesquiterpenes and, particularly, monoterpenes are the major components of *Hedychium* essential oils. The mechanism of action of monoterpenes is possible breakdown of cytoplasm and organelle members exposed to volatile oils, and this loss of membrane integrity results in leakage of intracellular materials and antimicrobial activity of essential oils (Trombetta et al. 2005; Nazzoro et al. 2013). Essential oils are generally more effective against Gram-positive bacteria than Gram-negative bacteria because of the differing structures of the cell walls of the two types of bacteria, and the structure of the Gram-positive bacteria cell wall allows hydrophobic molecules to easily penetrate the cells and act on both the cell wall and within the cytoplasm (Trombetta et al. 2005). In a study of the antimicrobial activity of essential oil of *Hedychium larsenii* on three Gram-positive and seven Gram-negative bacteria, Gopanraj et al. (2005) found moderate antibacterial activity of the oil against all the bacteria except the Gram-positive *Klebsiella pneumoniae* (Table 3.2). However, other studies such as those reported by Rajasekaran et al. (2012) and Ray et al. (2018) showed more effective antibacterial and antifungal effect of *Hedychium* essential oils.

The effectiveness of essential oils depends on several factors, including the percentage and composition of the chemical constituents, as well as the interaction between these constituents; and these interactions may lead to an additive, indifferent synergistic, or antagonistic effect (Marino et al. 2001; Delaquis et al. 2002; Ray et al. 2018). Several studies have shown that essential oils as a whole have a more

Table 3.2 Antimicrobial and insecticidal activities of *Hedychium* essential oils

Taxa	Organism	Efficacy[a]	Reference
Hedychium spicatum	*Staphylococcus aureus*	+	Semwal et al. (2015)
	Bacillus subtilis	+	
	Pseudomonas aeruginosa	+	
	Pseudomonas aeruginosa	+	
	Escherichia coli	+	
Hedychium coronarium	*Staphylococcus aureus*	+	Chimnoi et al. (2008)
	Bacillus subtilis	+	
	Escherichia coli	+	
	Pseudomonas aeruginosa	+	
	Salmonella typhi	+	
Hedychium spicatum	*Pseudomonas vulgaris*	+	Prakash et al. (2010)
	Staphylococcus aureus	+	
	Salmonella typhi	+	
	Escherichia coli	+	
	Pseudomonas aeruginosa	+	
Hedychium coronarium	*Pseudomonas vulgaris*	+	Prakash et al. (2010)
	Staphylococcus aureus	+	
	Salmonella typhi	+	
	Escherichia coli	+	
	Pseudomonas aeruginosa	+	
Hedychium spicatum	*Aspergillus niger*	–	Pawar and Thaker (2006)
Hedychium gardnerianum	*Staphylococcus aureus*	+	
	Staphylococcus epidermis	+	
	Pseudomonas aeruginosa	–	
Hedychium coronarium	*Staphylococcus aureus*	+	Ray et al. (2018)
	Bacillus subtilis	+	
	Enterococcus faecalis	+	
	Escherichia coli	+	
	Pseudomonas aeruginosa	+	
	Aspergillus niger	+	
	Aspergillus flavus	+	
	Candida albicans	+	
	Fusarium oxysporum	+	
Hedychium larsenii	Early third instars of *Anopheles stephensi*	+	AlShebly et al. (2017)
	Early third instars of *Aedes aegypti*	+	
	Early third instars of *Culex quinquefasciatus*	+	

(continued)

Table 3.2 (continued)

Taxa	Organism	Efficacy[a]	Reference
Hedychium larsenii	*Bacillus cereus*	+	Gopanraj et al. (2005)
	Bacillus subtilis	+	
	Staphylococcus aureus	+	
	Escherichia coli	+	
	Klebsiella pneumoniae	–	
	Proteus vulgaris	+	
	Pseudomonas aeruginosa	+	
	Pseudomonas fluorescens	+	
	Salmonella typhii	+	
	Serratia marcescens	+	
Hedychium spicatum	*Pediculus humanus capitis*	+	Jadhav et al. (2007)
	Pthirus pubis	+	
	Pediculus humanus	+	
Hedychium forrestii	*Stephanitis pyrioides*	+	Sakhanokho et al. (2013)
Hedychium elatum	*Stephanitis pyrioides*	+	
Hedychium bousigonianum	*Stephanitis pyrioides*	+	
Hedychium "Tai Golden Goddes"	*Stephanitis pyrioides*	–	
Hedychium bousigonianum	First instar larvae of *Aedes aegypti*	+	Sakhanokho et al. (2013)
Hedychium coccineum	First instar larvae of *Aedes aegypti*	+	
Hedychium "Kinkaku"	First instar larvae of *Aedes aegypti*	+	
Hedychium "Tai Golden Goddess"	First instar larvae of *Aedes aegypti*	+	
Hedychium forrestii	*Colletotrichum gloeosporioides*	–	Sakhanokho et al. (2013)
	Colletotrichum fragariae	–	
	Colletotrichum acutatum	–	
Hedychium elatum	*Colletotrichum gloeosporioides*	–	
	Colletotrichum fragariae	–	
	Colletotrichum acutatum	–	
Hedychium gardnerianum	*Staphylococcus aureus*	+	Medeiros et al. (2003)
	Staphylococcus epidermis	+	
	Pseudomonas aeruginosa	–	
Hedychium forrestii	*Aspergillus flavus*	+	Rajasekaran et al. (2012)
	Fusarium verticillioides	+	
Hedychium elatum	*Aspergillus flavus*	+	Rajasekaran et al. (2012)
	Fusarium verticillioides	+	
Hedychium "Tai Monarch"	*Aspergillus flavus*	+	Rajasekaran et al. (2012)
	Fusarium verticillioides	+	

[a]The + sign indicates the essential oil compound is effective or partially effective against the targeted organism; the – sign signifies that the essential oil compound has no effect on the targeted organism

potent antimicrobial activity than the individual main components mixed together, proving the significant role played by the minor compounds in the antimicrobial effectiveness of essential oils (Marino et al. 2001; Delaquis et al. 2002; Gill et al. 2002; Mourey and Canillac 2002; Ait-Ouazzou et al. 2012).

3.4 Conclusions

Hedychium species are multiple-purpose plants that are generally grown as ornamental plants, but they are also used for their industrial (perfumery, manufacturing paper) and medicinal (antimicrobial and oxidant properties) properties. In addition, *Hedychium* extracts are widely used in traditional medicine to treat a multitude of ailments. Furthermore, *Hedychium* essential oils have been shown to inhibit fungal growth and aflatoxin secretion, so they can potentially be used in formulations of plant-based preservatives for improvement of stored foods.

References

Abdo MY, Ahmad WYW, Din LB, Ibrahim N (2015) Composition of *Hedychium malayanum* rhizome oil and its antioxidant activity. Malays J Anal Sci 19:1147–1155

Ahmad BF, Jantan BI, Jalil J (2004) Constituent of the rhizome oil of *Hedychium cylindricum* Ridl. J Essent Oil Res 16:299–301

Ait-Ouazzou A, Loran S, Arakrak A, Laglaoui A, Rota C, Herrera A, Pagán R, Conchello P (2012) Evaluation of the chemical composition and antimicrobial activity of *Mentha pulegium*, *Juniperus phoenicea*, and *Cyperus longus* essential oils from Morocco. Food Res Int 45:313–319

AlShebly MM, AlQahtani FS, Govindarajan M, Gopinah K, Vijayan P, Benelli G (2017) Toxicity of ar-curcumene and epi-ß-bisabolol from *Hedychium larsenii* (Zingiberaceae) essential oil on malaria, chikungunya and St. Louis encephalitis mosquito vectors. Ecotoxicol Environ Saf 137:149–157

Argyropoulou C, Daferera D, Tarantilis PA, Fasseas C, Polissiou M (2007) Chemical composition of the essential oil from leaves of *Lippia citriodora* H.B.K. (Verbenaceae) at two developmental stages. Biochem Syst Ecol 35:831–837

Arruda M, Viana H, Rainha N, Neng NR, Rosa JS, Nogueira JMF, Barreto MC (2012) Anti-acetylcholinesterase and antioxidant activity of essential oils from *Hedychium gardnerianum* Sheppard ex Ker-Gawl. Molecules 17:3082–3092

Branney TME (2005) Hardy gingers: including *Hedychium*, *Roscocea*, and *Zingiber*, Royal Horticultural Society Plant Collector Guide. Timber Press, Portland, 267p. ISBN: 0881926779

Chen IN, Chang CC, Ng CC, Wang CY, Shyu YT, Chang TL (2008) Antioxidant and antimicrobial activity of Zingiberaceae plants in Taiwan. Plant Foods Hum Nutr 63:15–20

Chimnoi N, Pisutiaroenpong S, Ngiwsara L, Dechtrirut D, Chokchaichamnanki D, Khunnawutmanotham N, Mahidol C, Techasakul S (2008) Labdane diterpenes from rhizomes of *Hedychium coronarium*. Nat Prod Res 22:1249–1255

Delaquis PJ, Stanich K, Girard B, Mazza G (2002) Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. Int J Food Microbiol 74:101–109

Ding HB, Bin Y, Zhou SS, Li R, Maw MB, Kyaw WM, Tan YH (2018) Hedychium putaoense (Zingiberaceae), a new species from Putao, Kachin State, Northern Myanmar. PhytoKeys 94:51–57

dos Santos BCB, Barata LES, Marques FA, Baroni ACM, Kanos BAC, de Oliveira PR, Guerrero PG Jr (2010) Composition of leaf and rhizome essential oils of *Hedychium coronarium* Koen. from Brazil. J Essent Oil Res 22:305–306

Gill AO, Delaquis P, Russo P, Holley RA (2002) Evaluation of antilisterial action of cilantro oil on vacuum packed ham. Int J Food Microbiol 73:83–92

Gopanraj G, Dan M, Shihuraj S, Sethuraman MG, George V (2005) Chemical composition and antibacterial activity of the rhizome oil of *Hedychium larsenii*. Acta Pharma 55:315–320

Gurib-Fakim A, Maudarbaccus N, Leach D, Doimo L, Vohlmuth H (2002) Essential oil composition of Zingiberaceae species from Mauritius. J Essent Oil Res 14:271–273

Hartati R, Suganda AG, Fidrianny I (2015) Chemical composition and antimicrobial activity of diterpene and essential oils of *Hedychium roxburghii* Blume rhizome. Asian J Pharm Clin Res 8:209–214

Ho J-C (2011) Antimicrobial, mosquito larvicidal and antioxidant properties of the leaf and rhizome of *Hedychium coronarium*. J Chin Chem Soc 58:563–567

Jadhav V, Kore A, Kadam VJ (2007) *In-vitro* pediculicidal activity of *Hedychium spicatum* essential oil. Fitoterapia 78:470–473

Kumar R, Prakash O, Singh SP, Pant AK, Isidorov V, Szczepaniak L (2017) Chemical composition, antioxidant and myorelaxant activity of essential oil of *Hedychium aurantiacum*. Asian J Chem 29:2587–2591

Lechat-Vahirua I, François P, Menut C, Lamaty G, Bessiere J-M (1993) Aromatic plants of French Polynesia. I. Constituents of the essential oils of rhizomes of three Zingiberaceae: *Zingiber zerumbet* Smith, *Hedychium coronarium* Koenig and *Etlingera cevuga* Smith. J Essen Oil Res 5:55–59

Marino M, Bersani C, Comi G (2001) Impedance measurements to study the antimicrobial activity of essential oils from Lamiacea and Compositae. Int J Food Microbiol 67:187–195

Medeiros J, Campos LB, Mendonca SC, Davin LB, Lewis NG (2003) Composition and antimicrobial activity of the essential oils from invasive species of the Azores, *Hedychium gardnerianum* and *Pittosporum undulatum*. Phytochemistry 64:561–565

Miranda CASF, Cardoso MG, Mansanares ME, Gomes MS, Marcussi S (2014) Preliminary assessment of *Hedychium coronarium* essential oil on fibrinogenolytic and coagulant activity induced by *Bothrops* and *Lachesis* snake venoms. J Venom Anim Toxins Incl Trop Dis 20:39

Mourey A, Canillac N (2002) Anti-Listeria monocytogenes activity of essential oils components of conifers. Food Control 13:289–292

Mukherjee I (1970) Chromosome studies of some species of *Hedychium*. Bot Mag Tokyo 83:237–241

Nazzoro F, Fratianni F, De Martino L, Coppola R, De Feo V (2013) Effect of essential oils on pathogenic bacteria. Pharmaceuticals 6:1461–1474

Pant AK, Mathela CS, Dev V, Bottini AT (1992) Rhizome essential oil of *Hedychium aurantiacum* (Zingiberaceae), a potential source of (+)-linalool. J Essent Oil Res 4:129–131

Pawar VC, Thaker VS (2006) *In vitro* efficacy of 75 essential oils against *Aspergillus niger*. Mycoses 49:316–323

Prakash O, Rajput M, Kumar M, Pant AK (2010) Chemical composition and antibacterial activity of rhizome oils from *Hedychium coronarium* Koenig and *Hedychium spicatum* Buch-Ham. J Essent Oil Bearing Plants 13:250–259

Rajasekaran K, Sakhanokho HF, Tabanca N (2012) Antifungal activities of *Hedychium* essential oils and plant extracts against mycotoxigenic fungi. J Crop Improv 26:389–396

Ray A, Dash B, Sahoo A, Nasim N, Panda PC, Patnaik J, Ghosh B, Nayak S, Kar B (2017a) Assessment of the terpenic composition of *Hedychium coranarium* oil from eastern India. Ind Crop Prod 97:49–55

Ray A, Jena S, Kar B, Patnaik J, Panda PC, Nayak S (2017b) Chemical composition and antioxidant activities of essential oil *Hedychium greenii* and *Hedychium gracile* from India. Nat Prod Res:1–4. https://doi.org/10.1080/14786419.2017.1416384

Ray A, Jena S, Dash B, Kar B, Halder T, Chatterjee T, Ghos B, Panda PC, Nayak S, Mahapatra N (2018) Chemical diversity, antioxidant and antimicrobial activities of the essential oils from Indian populations of *Hedychium coronarium* Koen. Ind Crop Prod 112:353–362

Rodrigues DF, Velasquez AMA, Cavaleiro C, Salgueiro L, Martins GZ, Magalhaes NO, Martins MBG, Cicarelli RMB, Moreira RRD (2013) Chemical composition and trypanocidal activity of the essential oils from *Hedychium coronarium* J. Koenig (Zingiberaceae). ISRN Infect Dis 2013:1–6. https://doi.org/10.5402/2013/639275

Sá RCS, Andrade LN, Sousa DP (2013) A review on ant-inflammatory activity of monoterpenes. Molecules 18:1227–1254

Sakhanokho HF, Sampson BJ, Tabanca N, Wedge DE, Demirci B, Baser KHC, Bernier UR, Tsikolia M, Agramonte NM, Becnel JJ, Chen J, Rajasekaran K, Spiers JM (2013) Chemical composition, antifungal and insecticidal activities of *Hedychium* essential oils. Molecules 18:4308–4327

Sangwan NS, Farroqi AHA, Shabih F, Sangwan RS (2001) Regulation of essential oil production in plants. Plant Growth Regul 34:3–21

Sarangthem N, Talukdar NC, Thongan B (2013) Collection and evaluation of *Hedychium* species of Manipur, Northeast India. Genet Resour Crop Evol 60:13–21

Semwal RB, Semwal DK, Mishra SP, Semwal R (2015) Chemical composition and antibacterial potential of essential oils from *Artemisia capillaris*, *Artemisia nilagirica*, *Citrus limon*, *Cymbopogon flexuous*, *Hedychium spicatum* and *Ocimum tenuiflorum*. Nat Prod J 5:199–205

Thomas S, Mani B (2016) Chemical composition, antibacterial and antioxidant properties of essential oil from rhizomes of *Hedychium forrestii* var. *planiense* Sanoj and M. Sabu. Ind J Pharm Sci 78:452–457

Thomas S, Mani B (2018) Composition, antibacterial and anti-oxidant potentials of the essential oil of *Hedychium matthewii*. Bangladesh J Pharmacol 12:173–179

Tiuman TS, Santos AO, Ueda-Nakamura T, Filho BPD, Nakamura CV (2011) Recent advances in leishmaniasis treatment. Int J Infect Dis 15:e525–e532

Trombetta D, Castelli F, Sarpietro MG, Venuti V, Cristani M, Daniele C, Saja A, Mazzanti G, Bisignano G (2005) Mechanisms of antibacterial action of three monoterpenes. Antimicrob Agents Chemother 49:2474–2478

Tushar BS, Sarma GC, Rangan L (2010) Ethnomedical uses of zingiberaceous plants of Northeast India. J Ethnopharmacol 132:286–296

Valadeau C, Pabon A, Deharo E, Alban-Castillo J, Estevez Y, Lores FA, Rojas R, Gamboa D, Sauvain M, Castillo D, Bourdy G (2009) Medicinal plants from the Yanesha (Peru): evaluation of the leishmanicidal and antimalarial activity of selected extracts. J Ethnopharmacol 123:413–422

Van Thanh B, Dai DN, Thang TD, Binh NQ, Anh LDN, Ogunwande IA (2014) Composition of essential oils of four *Hedychium* species from Vietnam. Chem Cent J 8:54. https://doi.org/10.1186/s13065-014-0054-3

Verma RS, Padalia RC (2010) Comparative essential oil composition of different vegetative parts of *Hedychium spicatum* Smith from Uttarakhand, India. Int J Green Pharm 4:292–295

Weyerstahl P, Marshall H, Schneider S (1995) Constituents of the essential oil from the rhizomes of *Hedychium acuminatum* Roscoe. Flavour Fragr J 10:179–185

Wood T (1999) Ginger lilies. The American Gardener 78:40–45

Zheljazkov V, Cantrell CL, Tekwani B, Khan SI (2008) Content, composition, and bioactivity of the essential of three basil genotypes as a function of harvesting. J Agric Food Chem 56:380–385

Chapter 4
The Essential Oils of the Burseraceae

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Abbreviations

DCM	Dichloromethane
HD	Hydrodistillation
IC_{50}	Median inhibitory concentration
LC_{50}	Median lethal concentration
LD_{50}	Median lethal dose
MeOH	Methanol
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
SAFE	Solvent-assisted flavor evaporation
SC CO_2	Supercritical carbon dioxide extraction
SD	Steam distillation
SPME	Solid-phase micro extraction

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4.1 Introduction: Overview of the Burseraceae

The Burseraceae is a family of angiosperms in the order Sapindales and comprises about 18 genera and 640 species of aromatic resinous trees and shrubs (Mabberley 2008). This pan-tropical family is characterized by smooth, flakey, aromatic bark and the presence of resin ducts in the bark and leaves (Daly et al. 2010). The resins are generally composed of nonvolatile triterpenoids, lignans, and coumarins, along with volatile components, monoterpenoids and sesquiterpenoids, making up the essential oils (EOs) (Khalid 1983; Hanuš et al. 2005; Rüdiger et al. 2007; Moussaieff and Mechoulam 2009; Murthy et al. 2016). The Burseraceae is divided into three tribes: Bursereae, which includes the genera *Bursera*, *Boswellia*, and *Commiphora*; Canarieae, with *Canarium*, *Dacryodes*, *Haplolobus*, *Santiria*, and *Trattinnickia*; and Protieae, including *Crepidospermum*, *Protium*, and *Tetragastris* (Weeks et al. 2005).

The most celebrated Old World resins of the Burseraceae are frankincense (*Boswellia* spp.), myrrh [*Commiphora myrrha* (T. Nees) Engl.] (Tucker 1986), and balm of Gilead [*Commiphora gileadensis* (L.) C. Chr.] (Ben Yehoshua et al. 2015), used in biblical times as incense, as perfumes, for embalming, and as medicines. Important New World members of the family are the sources of copal and breu (mainly *Protium* spp.) (Rüdiger et al. 2007). Other ethnobotanically important Neotropical Burseraceae include tabonuco (*Dacryodes excelsa*) (Lugo and Wadsworth 1990) and gumbo limbo [*Bursera simaruba* (L.) Sarg.] (Eldridge 1975; Higgs 1978).

4.2 Tribe Bursereae

4.2.1 The Genus Bursera

The genus *Bursera* comprises around 100 species of deciduous aromatic trees and shrubs distributed from southern United States through Peru (Espinosa et al. 2006; Mabberley 2008; De-Nova et al. 2011; Gigliarelli et al. 2015). *Bursera* species have played a prominent role in the ethnomedicine throughout their ranges (Morton 1981; Duke et al. 2009). Members of *Bursera* produce oleoresins and essential oils that not only serve as protection of the plants against herbivory and infection but can also be attributed to the numerous medicinal properties of this genus, including antimicrobial, antineoplastic, anti-inflammatory, and analgesic (Noguera et al. 2004; Murthy et al. 2016). The resins, known as “copal,” have been burned as incense since pre-Columbian times. *Bursera* resins are also used for making ointments, treating scorpion bites, relieving cold symptoms, and as a remedy for headaches (Peters et al. 2003). The essential oil compositions and biological activities of *Bursera* species are summarized in Table 4.1.

Table 4.1 Chemical compositions and biological activities of *Bursera* essential oils

Bursera species[a]	Collection site	Essential oil	Major components (>5%)	Bioactivity of essential oil	Reference
B. aloexylon (Schiede ex Schltdl.) Engl. [syn. *Bursera linanoe* (La Llave) Rzed., Calderón & Medina]	Guerrero, Mexico	Bark (SD)	Linalool (49.3%), α-terpineol (20.2%), linalyl anthranilate (14.5%), neryl acetate (5.1%)	–	Zúñiga et al. (2005)
	Zapotitlan, Puebla, Mexico	Leaf (DCM extract)	Linalyl acetate (92.2%)	–	Becerra and Noge (2010)
	Tucson, Arizona (greenhouse)	Leaf (DCM extract)	(*R*)-(−)-Linalyl acetate (57.6%), (*S*)-(−)-germacrene D (39.3%)	–	Noge et al. (2010)
	Campinas, SP Brazil	Leaf (HD)	Linalool (96.7%)	Antibacterial, broth microdilution assay (*Rhodococcus equi*, MIC 600 μg mL^{-1}; *Staphylococcus epidermidis*, MIC = 150 μg mL^{-1})	Queiroga et al. (2007)
B. aptera Ramírez	Puebla, Mexico	Bark (SD)	Limonene (94.6%), kaur-16-ene (5.4%)	Anti-inflammatory (mouse ear edema, 10.0% inhibition with a dose of 2.5 μg ear^{-1})	Zúñiga et al. (2005)
B. aromatica Proctor	Trelawny, Jamaica	Bark (HD)	Nonane (5.2%), α-copaene (23.7%), β-caryophyllene (12.8%), δ-cadinene (21.5%), viridiflorol (11.8%)	–	Junor et al. (2010b)
	Trelawny, Jamaica	Fruit (HD)	Nonane (23.7%), β-pinene (7.0%), limonene (8.0%), α-copaene (14.0%), β-caryophyllene (6.8%), caryophyllene oxide (5.2%), viridiflorol (7.9%)	–	Junor et al. (2010b)

(continued)

Table 4.1 (continued)

Bursera species[a]	Collection site	Essential oil	Major components (>5%)	Bioactivity of essential oil	Reference
	Trelawny, Jamaica	Leaf (HD)	Nonane (14.7%), α-copaene (15.8%), β-caryophyllene (21.7%), δ-cadinene (11.3%), viridiflorol (5.9%)	–	Junor et al. (2010b)
B. bipinnata (DC.) Engl.	Commercial	Oleoresin (HD)	α-Copaene (14.5%), β-bourbonene (6.1%), β-caryophyllene (8.5%), germacrene D (13.8%), spathulenol (5.1%)	–	Case et al. (2003)
B. chemapodicta Rzed. & Evangelina Ortiz	Guerrero, Mexico	Leaf (SPME)	Heptane (22.5%), 2-heptanol (26.4%), 2-heptyl acetate (40.0%)	–	Evans and Becerra (2006)
	Guerrero, Mexico	Twig (SPME)	Heptane (19.4%), nonane (6.0%), 4-methyl-3-hexyl acetate (15%), 2-heptyl acetate (51.0%)	–	Evans and Becerra (2006)
B. copallifera (DC) Bullock	Tucson, Arizona (greenhouse)	Leaf (DCM extract)	β-Caryophyllene (9.6%), α-humulene (12.5%), germacrene D (56.2%), bicyclogermacrene (6.2%)	–	Noge and Becerra (2009)
B. delpechiana Poisson, Henri Louis ex Engl. [syn *B. citronella* McVaugh & Rzed.]	Bangalore, India	Leaf (DCM extract)	Linalyl acetate (90.3%)	–	Becerra and Noge (2010)
B. excelsa (Kunth) Engl.	Tucson, Arizona (greenhouse)	Leaf (DCM extract)	β-Caryophyllene (15.0%), germacrene D (50.5%), bicyclogermacrene (8.8%)	–	Noge and Becerra (2009)

B. fagaroides var. *purpusii* (Brandegee) McVaugh & Rzed.	Tucson, Arizona (greenhouse)	Leaf (DCM extract)	α-Pinene (67.8%), β-pinene (5.7%), germacrene D (15.1%)	–	Noge and Becerra (2009)
B. glabrifolia (Kunth) Engl.	Guerrero, Mexico	Bark (SD)	α-Thujene (7.4%), limonene (13.4%), terpinen-4-ol (34.5%), α-terpineol (31.6%), verbenone (7.5%)	–	(Zúñiga et al. (2005)
B. grandifolia (Schltdl.) Engl.	Guerrero, Mexico	Bark (SD)	α-Thujene (23.5%), limonene (64.0%)	Anti-inflammatory (mouse ear edema, 2.0% inhibition with a dose of 2.5 μg ear^{-1})	Zúñiga et al. (2005)
B. graveolens (Kunth) Triana & Planch.	Limones, Ecuador	Fruit (HD)	α-Phellandrene (37.6%), limonene (49.9%), menthofuran (6.1%)	Acaricidal (*Rhipicephalus* (*Boophilus*) *microplus* larvae, IC_{50} 0.87%)	Rey-Valeirón et al. (2017)
	Havana, Cuba	Leaf (HD)	Limonene (26.5%), (*E*)-β-ocimene (13.0%), menthofuran (5.1%), β-elemene (14.1%)	Antiprotozoal (*Leishmania amazonensis* amastigotes, IC_{50} 36.7 μg mL^{-1})	Monzote et al. (2012)
	San José de Ancon, Ecuador	Stem (HD)	Viridiflorol (70.8%), α-cadinol (5.5%)	–	Manzano Santana et al. (2009)
	Piura, Peru	Wood (SD)	Limonene (9.1%), α-terpineol (8.1%), β-bisabolene (5.7%)	–	Yukawa et al. (2006)
	Puerto Lopez, Ecuador	Wood (SD)	Limonene (58.6%), menthofuran (6.6%), α-terpineol (10.9%)	–	Young et al. (2007)
	Commercial (Young Living Essential Oils, Lehi, Utah)	Wood (HD)	Limonene (67%), α-terpineol (10%)	–	Satyal (unpublished)
	Commercial (Santoil S.A., Quito, Ecuador)	Oleoresin (HD)	Limonene (41%), menthofuran (35%), germacrene D (16%)	–	Satyal (unpublished)

(continued)

Table 4.1 (continued)

Bursera species[a]	Collection site	Essential oil	Major components (>5%)	Bioactivity of essential oil	Reference
B. hollickii Fawc. & Rendle	St. Andrew, Jamaica	Bark (HD)	α-Pinene (34.8%), β-pinene (10.6%), terpinolene (13.4%), α-terpineol (8.9%)	Insecticidal (*Cylas formicarius elegantulus*, LD_{50} = 11 μg g^{-1} insect)	Junor et al. (2008b)
	St. Andrew, Jamaica	Leaf (HD)	α-Pinene (49.8%), β-pinene (11.0%), α-terpineol (5.7%)	Insecticidal (*Cylas formicarius elegantulus*, LD_{50} = 59 μg g^{-1} insect)	Junor et al. (2008b)
B. lancifolia (Schltdl.) Engl.	Guerrero, Mexico	Bark (SD)	Limonene (14.5%), terpinen-4-ol (7.3%), α-terpineol (15.2%) elemol (6.0%), agarospirol (6.1%), β-eudesmol (14.4%), 3,8-dimethylundecane (5.6%), docosane (18.0%)	Anti-inflammatory (mouse ear edema, 16.6% inhibition with a dose of 2.5 μg ear^{-1})	Zúñiga et al. (2005)
B. lunanii (Spreng.) C.D. Adams & Dandy ex Proctor	St. Andrew, Jamaica	Bark (HD)	α-Pinene (51.2%), α-terpineol (6.5%)	–	Junor et al. (2010a)
	St. Andrew, Jamaica	Fruit (HD)	*trans*-Pinocarveol (7.0%), *trans*-verbenol (13.6%), myrtenal (8.7%), verbenone (14.7%), caryophyllene oxide (5.6%)	–	Junor et al. (2010a)
	Trelawny, Jamaica	Fruit (HD)	–	Antibacterial, disk diffusion assay (*Escherichia coli*, *Staphylococcus aureus*, MRSA)	Junor et al. (2007)
	St. Andrew, Jamaica	Leaf (HD)	α-Pinene (42.7%), β-caryophyllene (14.2%), caryophyllene oxide (12.2%)	–	Junor et al. (2010a)

B. microphylla A. Gray	South Mountain Park, Arizona	Oleoresin (HD)	δ-3-Carene (0.1–8.3%), myrcene (0.4–14.4%), β-caryophyllene (35.7–72.9%), caryophyllene oxide (4.8–8.8%)	–	Tucker et al. (2009)
B. mirandae C.A. Toledo	Tucson, Arizona (greenhouse)	Leaf (DCM extract)	α-Pinene (6.6%), α-phellandrene (15.0%), β-caryophyllene (14.4%), germacrene D (36.6%)	–	Noge and Becerra (2009)
B. morelensis Ramirez	Puebla, Mexico	Bark (SD)	α-Thujene (14.9%), limonene (56.5%), terpinen-4-ol (8.4%), α-terpineol (7.2%), carvotanacetone (6.5%)	–	Zúñiga et al. (2005)
	Cañada ragion, Teotitlán de Flores Magón, Oaxaca, Mexico	Aerial parts (HD)	α-Pinene (8.3%), α-phellandrene (51.9%), *p*-cymene (5.0%), β-phellandrene (10.8%), β-caryophyllene (5.6%)	Anti-inflammatory (rat paw edema, 86.8% inhibition with dose of 0.5 mg kg^{-1})	Carrera-Martinez et al. (2014)
	San Rafael, Coxcatlan, Mexico	Stems and bark (HD)	α-Pinene (5.8%), α-phellandrene (32.7%), *o*-cymene (8.7%), β-phellandrene (14.8%), β-caryophyllene (7.5%)	Antibacterial, broth microdilution assay (*Streptococcus pneumoniae*, MIC 125 μg mL^{-1}; *Vibrio cholerae*, MIC 125 μg mL^{-1}; *Escherichia coli*, MIC 125 μg mL^{-1})	Canales-Martinez et al. (2017)
B. rupicola León de la Luz	Tucson, Arizona (greenhouse)	Leaf (DCM extract)	α-Thujene (5.3%), α-pinene (10.3%), β-pinene (21.9%), β-caryophyllene (18.3%), germacrene D (31.9%)	–	Noge and Becerra (2009)

(continued)

Table 4.1 (continued)

Bursera species[a]	Collection site	Essential oil	Major components (>5%)	Bioactivity of essential oil	Reference
B. serrata Wall. ex Colebr.	Lucknow, India	Leaf (HD)	Linalool (53.4%), α-terpineol (26.4%)	–	Sharma et al. (1996)
B. simaruba (L.) Sarg.	Monteverde, Costa Rica	Bark (HD)	α-Thujene (11.9%), α-phellandrene (29.1%), *o*-cymene (13.1%), β-caryophyllene (19.3%)	–	Setzer (2014)
	St. Andrew, Jamaica	Bark (HD)	α-Pinene (32.1%), β-pinene (13.5%), *p*-mentha-1(7),8-diene (5.6%), viridiflorol (7.1%)	–	Junor et al. (2008a, b)
	Costa Rica	Fruit (HD)	α-Terpinene (26.2%), δ-terpinene (20.4%), α-pinene (18.2%), *p*-cymene (15.9%)	–	Rosales-Ovares and Cicció-Alberti (2002)
	Trelawny, Jamaica	Fruit (HD)	–	Antibacterial, disk diffusion assay (*Staphylococcus aureus*, MRSA)	Junor et al. (2007)
	St. Andrew, Jamaica	Fruit (HD)	α-Pinene (27.6%), sabinene (8.1%), β-pinene (24.1%), terpinen-4-ol (13.3%)	–	Junor et al. (2008a)
	Monteverde, Costa Rica	Leaf (HD)	α-Phellandrene (6.3%), *o*-cymene (65.2%), germacrene D (5.3%)	–	Setzer (2014)
	St. Andrew, Jamaica	Leaf (HD)	α-Pinene (10.2%), myrcene (5.2%), β-elemene (5.6%), β-caryophyllene (9.0%), γ-muurolene (6.2%), trans-cadina-1(6),4-diene (9.7%)	–	Junor et al. (2008a)

	Fouillole, Pointe-à-Pître, Guadeloupe	Leaf (HD)	Limonene (46.7%), β-caryophyllene (14.7%), α-humulene (13.3%), germacrene D (7.6%)	Cytotoxic (A-549 human adenocarcinomic alveolar basal epithelial cells, IC_{50} 42 μg mL^{-1}; DLD-1 human colon adenocarcinoma cells, IC_{50} 48 μg mL^{-1}). α-Humulene is the active agent (IC_{50} 62 μM and 71 μM on A-549 and DLD-1 cells, respectively)	Sylvestre et al. (2007)
	Trelawny, Jamaica	Stem (HD)	–	Antibacterial, disk diffusion assay (*Staphylococcus aureus*, MRSA)	Junor et al. (2007)
B. submoniliformis Engl.	Puebla, Mexico	Bark (SD)	*cis-p*-Menth-2-en-1-ol (6.9%), cuminol (5.4%), docosane (8.0%)	–	Zúñiga et al. (2005)
B. tomentosa (Jacq.) Triana & Planch.	Cabudare, Lara, Venezuela	Bark (HD)	Nonane (6.4%), (*Z*)-β-ocimene (7.3%), bicyclogermacrene (6.6%), spathulenol (11.4%), globulol (8.9%), τ-cadinol (8.8%)	–	Moreno et al. (2010b)
	Cabudare, Lara, Venezuela	Fruit (HD)	Nonane (28.2%), (*Z*)-β-ocimene (47.6%), undecane (5.5%), germacrene D (11.1%)	Antibacterial, disk diffusion assay (*Staphylococcus aureus*, MIC 80 μg mL^{-1}; *Enterococcus faecalis*, MIC 120 μg mL^{-1}; *Staphylococcus typhi*, MIC 100 μg mL^{-1})	Moreno et al. (2010a)

(continued)

Table 4.1 (continued)

Bursera species[a]	Collection site	Essential oil	Major components (>5%)	Bioactivity of essential oil	Reference
B. velutina Bullock	Guerrero, Mexico	Bark (SD)	Limonene (15.7%), α-terpineol (10.7%), *trans*-carveol (5.5%), carvone (5.7%), spathulenol (12.5%), β-eudesmol (12.9%)	–	Zúñiga et al. (2005)
	Altamirano, Guerrero, Mexico	Leaf (DCM extract)	α-Phellandrene (28.8%), β-phellandrene (11.0%), 2-phenylethanol (29.5%), phytol (5.3%)	–	Noge et al. (2011)

[a]There is apparently some confusion regarding synonymous *Bursera* taxa (*B. aloexylon*, *B. linanoe*, *B. delpechiana*, *B. citronella*); the synonyms reported in this table are based on those reported by the Missouri Botanical Garden (Missouri Botanical Garden 2017)

Bursera aloexylon (Schiede ex Schltdl.) Engl. is now considered to be synonymous with *B. linanoe* (La Llave) Rzed., Calderón & Medina (Missouri Botanical Garden 2017) and is likely the same species as the Indian lavender, *B. delpechiana* Poisson, Henri Louis ex Engl. (Becerra and Noge 2010). However, *B. delpechiana* is considered to be synonymous with *B. citronella* McVaugh & Rzed. (Missouri Botanical Garden 2017). Nevertheless, *B. aloexylon* (*B. linanoe*) and *B. delpechiana* (*B. citronella*) are both rich in either linalool (Zúñiga et al. 2005; Queiroga et al. 2007) or linalyl acetate (Becerra and Noge 2010; Noge et al. 2010).

Bursera graveolens (Kunth) Triana & Planch., "palo santo," is native to the Yucatan, Central America, and Colombia south to Peru and Venezuela (Morton 1981). The wood has a strong woody odor and has been used by indigenous people of South America for incense. The sesquiterpenoids junenol, jinkoheremol, valerianol, and 6,10-epoxy-7(14)-isodaucene have been determined to be the odiferous woody components by gas chromatography–olfactometry (Yukawa et al. 2006). The resin of the tree has been used medicinally to treat pain and for rheumatism (Yukawa et al. 2004; Young et al. 2007). A decoction of the bark is taken as a sudorific (Yucatan) and as a remedy for stomachache (Peru); in Costa Rica and Cuba, an alcohol infusion of the bark is used as a liniment for rheumatism; a poultice of the bark is used in Peru as an analgesic (Morton 1981; Duke et al. 2009). The high percentage of viridiflorol in the stem bark of *B. graveolens* (Manzano Santana et al. 2009) may be responsible for the analgesic effect of the extract; viridiflorol has shown antioxidant and anti-inflammatory activities (Trevizan et al. 2016). Interestingly, viridiflorol is also an effective inhibitor of acetylcholinesterase (Miyazawa et al. 1998), so *B. graveolens* bark essential oil may be helpful in treating Alzheimer's disease (Nordberg and Svensson 1998).

Bursera microphylla A. Gray, "torote blanco," is distributed in the Sonoran Desert of Mexico. In Seri traditional medicine, the plant is steeped in alcohol to make a tincture to treat sore gums, cold sores, and abscessed teeth; a decoction of the stems and leaves is taken to relieve painful urination and to treat symptoms of bronchitis, while the resin is used to treat venereal diseases (Adorisio et al. 2017). The oleoresin essential oil of *B. microphylla* is rich in β-caryophyllene and caryophyllene oxide (Tucker et al. 2009). β-Caryophyllene has shown numerous biological activities, including antinociceptive and anesthetic activities (Ghelardini et al. 2001; Bakır et al. 2008; Katsuyama et al. 2013; Paula-Freire et al. 2014). This sesquiterpene is also an important clinical drug for treatment of bronchitis (Xie et al. 2008). Interestingly, β-caryophyllene has been shown to be a selective cannabinoid receptor type 2 (CB_2) agonist (Gertsch et al. 2008), which probably mediates many of the pharmacological effects. The biological properties of β-caryophyllene likely account for the traditional uses of *B. microphylla*.

Bursera morelensis Ramírez, "aceitillo," is endemic to Mexico. The bark infusion is used by inhabitants of San Rafael, Coxcatlan, Puebla, and Mexico, to treat skin infections and to aid wound healing (Canales-Martinez et al. 2017). The antibacterial properties of the essential oil, rich in α- and β-phellandrene (see Table 4.1), likely contribute to the traditional uses of this tree. α-Phellandrene has shown antimicrobial properties (İşcan et al. 2012).

Bursera simaruba (L.) Sarg., "gumbo limbo," "indio desnudo," ranges from south Florida, the Florida Keys, and the Bahamas throughout the West Indies and Central America from southern Mexico to Colombia (Morton 1981). The bark, gum, and leaves of this tree are used as traditional medicines throughout its range (Morton 1981; Duke et al. 2009). For example, the plant is used in Belize to treat wounds, insect bites, and skin sores (Arvigo and Balick 1993), leaves are used as a bath by the Yucatec Maya to treat fever (Ankli et al. 1999), a leaf decoction is taken in Cuba as a carminative (Beyra et al. 2004), and the leaf decoction is used in the Bahamas to treat poisonwood (*Metopium toxiferum*) dermatitis (Higgs 1978). The tree is commonly used as living fence posts in Costa Rica (Budowski and Russo 1993). There is wide variation in the essential oils of *B. simaruba*, depending not only on the plant tissue but also the geographical origin of the plant (see Table 4.1). Thus, for example, the leaf essential oil from Monteverde, Costa Rica, was dominated by *o*-cymene (65.2%) (Setzer 2014), but the leaf oil from Fouillole, Pointe-à-Pître, Guadeloupe, was rich in limonene (46.7%) (Sylvestre et al. 2007). The leaf oil from St. Andrew, Jamaica, on the other hand, had α-pinene (10.2%), β-caryophyllene (9.0%), and *trans*-cadina-1(6),4-diene (9.7%), as major components (Junor et al. 2008a, b). Similarly, the bark essential oil from Costa Rica had abundant α-phellandrene (29.1%) and β-caryophyllene (19.3%) (Setzer 2014), while the bark oil from Jamaica showed α- and β-pinenes as major components (32.1% and 13.5%, respectively) (Junor et al. 2008a, b). Clearly the geographical location plays a role in the chemistry of these essential oils and must affect the biological activities and likely affects the traditional uses as well.

Bursera tomentosa (Jacq.) Triana & Planch., "bálsamo de incienso," is native to the Lesser Antilles, Central America, northern Venezuela, and Colombia (Morton 1981; Moreno et al. 2010a, b). In northern South America, a decoction of the bark is used to treat sciatica, pulmonary complaints, asthma, epilepsy, and venereal diseases; the oleoresin is applied to ulcers and wounds (Morton 1981).

4.2.2 The Genus Boswellia

The genus *Boswellia* (Burseraceae) is a group of deciduous resiniferous trees and shrubs distributed across Africa, Arabia, and India that is characterized by papery bark and compound leaves. The genus comprises approximately 17 species, although the exact number is in dispute, with multiple revisions removing species from the *Boswellia* (Thulin 1999; Thulin et al. 2008) and questioning whether species should be considered legitimate or are hybrids or differential growth forms (Thulin and Warfa 1987; Thulin 1999; Woolley et al. 2012; Miller 2015; Eslamieh 2017). Perhaps unsurprisingly, even relatively, recent papers report the number of species as diversely as 15–43 species (Weeks et al. 2005; Camarda et al. 2007; Al-Harrasi and Al-Saidi 2008; Mertens et al. 2009; Roy et al. 2016; Missouri Botanical Garden 2017). The genus is best known for producing an aromatic terpenoid gum-oleoresin known as frankincense. The resins serve to protect the

trees from infection, herbivory, and insect attack, but they have been prominent aspects of human ethnobotanical medicine and religious practice throughout their ranges for millennia (Langenheim 2003; Pichersky and Raguso 2018). Frankincense features prominently in Indian Ayurvedic medicine and Chinese traditional medicine, as well as having been used locally for oral hygiene, dressing wounds, calming/psychoactive effects, and anti-inflammatory treatment, among a variety of other uses (Getahon 1976; Michie and Cooper 1991; Thulin 1999; Burkill 2000; Mies et al. 2000; Frawley and Lad 2001; Dannaway 2010; Price et al. 2016; Acıduman et al. 2017).

In modern times the resins and essential oils are still used in religious ceremonies and are appreciated for their antiseptic, antimicrobial, anti-inflammatory, and psychoactive properties yielded by 300+ chemical constituents (Mertens et al. 2009; Moussaieff and Mechoulam 2009). The resin is also of considerable modern interest as the basis for cancer therapies (Roy et al. 2016). The most extensive studies on components of frankincense have focused on the boswellic acids contained in the resin. These boswellic acids have been shown to have effective anti-inflammatory properties. Similar studies have shown the components of frankincense resin to have a sedative activity, significantly reduce inflammation markers, induce apoptosis in human leukemia and prostate cells, and improve arthritis and many pharmacological therapies (Moussaieff and Mechoulam 2009). Recent studies have provided scientific justification regarding the analgesic effects of frankincense extracts and essential oils. This research concludes that frankincense is as effective as the commercially available painkillers when comparing their analgesic properties and bioactivity (Al-Harrasi et al. 2014). The essential oil compositions and biological activities of each *Boswellia* species are summarized in Table 4.2.

Resins excreted from the wounds of the *Boswellia* species, often called olibanum, incense, or perhaps most commonly known as frankincense, have been traded in the Arabic and African regions for more than 5000 years (Sultana et al. 2013; Pickenhagen 2017). Frankincense has been used in numerous religious and cultural ceremonies throughout the documented trade history of this traditionally important commodity, and these uses of frankincense continue today. Frankincense was burned in Assyria and Egypt as early as 3000 BC (Pickenhagen 2017). Chemical analyses of archaeological samples taken from Egyptian tombs confirm the identity of frankincense (Archier and Vieillescazes 2000; Mathe et al. 2004); between 2500 BC and 600 BC, frankincense was a major object of trade between Egypt and the mysterious Land of Punt, likely located in the Horn of Africa (Kitchen 1971; Phillips 1997). Following the decline of Punt, the center of trade shifted to Arabian trade routes (*B. sacra*) and the Axumite Empire (*B. papyrifera*) (Butzer 1981; Hull 2008). Frankincense was considered the "scent of the gods" and was widely used in wealthy households (Pickenhagen 2017). The same was true of Greece and Rome, where it became hugely popular (Groom 1981). Frankincense also appears as a component of medical and religious practice throughout the Middle East and in Chinese medicine.

Boswellia sacra Flueck. is native to southern Oman, Yemen, Somaliland, and Somalia (Thulin and Warfa 1987). The African populations are frequently referred

Table 4.2 Chemical compositions and biological activities of *Boswellia* essential oils

Boswellia species	Collection site	Essential oil	Major components (> 5%)	Bioactivity of essential oil	Reference
B. ameero Balf. f.	Soqotra Island, Yemen	Oleoresin (HD)	α-Campholenal (13.4%), cosmene (34.9%), 3,4-dimethylstyrene (17.3%), α-terpineol (12.4%), 1-(2,4-dimethylphenyl)ethanol (20.3%)	Inhibitor of acetylcholinesterase (IC_{50} 217 μg mL^{-1})	Ali et al. (2008)
	Soqotra Island, Yemen	Oleoresin (MeOH extract)	α-Thujene (37.5%), α-pinene (20.5%), sabinene (6.8%), *p*-cymene (6.0%)	–	Maděra et al. (2017)
	Soqotra Island, Yemen	Oleoresin (MeOH extract)	α-Thujene (26.4%), α-pinene (7.2%), terpinen-4-ol (26.1%), (*E*)-β-farnesene (6.1%)	–	Maděra et al. (2017)
	Soqotra Island, Yemen	Oleoresin (MeOH extract)	α-Thujene (41.6%), terpinen-4-ol (14.1%), (*E*)-β-farnesene (8.9%), cembrene (9.2%)	–	Maděra et al. (2017)
	Soqotra Island, Yemen	Oleoresin (MeOH extract)	α-Thujene (72.1%), sabinene (6.1%), (*E*)-β-farnesene (5.0%), cembrene (5.2%)	–	Maděra et al. (2017)
B. bullata Thulin	Soqotra Island, Yemen	Oleoresin (MeOH extract)	β-Caryophyllene (7.2%), (*E*)-β-farnesene (7.5%), δ-cadinene (8.6%), guaiol (8.4%), α-cadinol (6.9%)	–	Maděra et al. (2017)
B. carteri Birdw.	Banaras Hindu University, Varanasi, India	Bark (HD)	δ-3-Carene (5.6%), 2-phenylethanol (12.3%), benzyl acetate (13.4%), limonene (9.5%), citronellol (8.0%)	Antifungal (*Aspergillus niger*, IC_{50} 616 μg mL^{-1}; *Alternaria alternata*, IC_{50} 354 μg mL^{-1}; *Cladosporium cladosporioides*, IC_{50} 848 μg mL^{-1}; *Curvularia lunata*, $IC_{50} < 250$ μg mL^{-1}; *Fusarium oxysporum*, $IC_{50} < 250$ μg mL^{-1})	Prakash et al. (2014)

	Commercial (drug market in Cairo, Egypt; from Somalia)	Oleoresin (HD)	Octanol (12.7%), octyl acetate (60.0%)[a]	Antibacterial, disk diffusion assay (*Staphylococcus aureus*, *Sarcina lutea*, *Mycobacterium phlei*)	Abdel Wahab et al. (1987)
	Not reported	Oleoresin (HD)	–	Spasmogenic effect on guinea pig ileum smooth muscle	Lis-Balchin and Hart (1997)
	Commercial (Sunspirit Oils Pty Ltd., Byron Bay, NSW, Australia)	Oleoresin (HD)	–	Weakly antimicrobial (*Staphylococcus aureus*, MIC 5000 μg mL^{-1}; *Candida albicans*, MIC 5000 μg mL^{-1})	Hammer et al. (1999)
	Ethiopia	Oleoresin (HD)	α-Pinene, 1,8-cineole, (*E*)-β-ocimene, octanol, linalool, octyl acetate, (*E*)-nerolidol (percentages not reported)	–	Basar et al. (2001)
	Commercial (market in Cairo, Egypt)	Oleoresin (HD)	Limonene (7.6%), octyl acetate (13.4%), biformene (5.7%), 4-acetoxy-2,6,11-cembratriene-8-ol (21.4%)	Immunostimulant (T-lymphocyte proliferation) activity	Mikhaeil et al. (2003)
	Ethiopia	Oleoresin (HD)	α-Pinene (10.9%), octanol (11.9%), octyl acetate (39.3%), verticilla-4(20),7,11-triene (6.0%)[a]	–	Basar (2005)
	Commercial (Spinnrad, Germany; from Somalia)	Oleoresin (HD)	–	Mosquito repellent (*Aedes aegypti*, *Anopheles stephensi*, *Culex quinquefasciatus*), 20% essential oil	Amer and Mehlhorn (2006)
	Commercial (Binardi, Bagnacavallo-Ravenna, Italy)	Oleoresin (HD)	Octyl acetate (45.2%), (3*E*)-cembrene A (5.5%), phyllocladene (13.2%), incensole (6.1%), incensole acetate (13.0%)[a]	–	Marongiu et al. (2006)

(continued)

Table 4.2 (continued)

Boswellia species	Collection site	Essential oil	Major components (> 5%)	Bioactivity of essential oil	Reference
	Commercial (Kyungdong medical market, Seoul, Korea)	Oleoresin (HD)	–	Nematicidal (*Bursaphelenchus xylophilus*, LC_{50} 2100 μg mL^{-1})	Choi et al. (2007)
	Commercial (White Lotus Aromatics Ltd., Port Angeles, WA, USA; from Somalia)	Oleoresin (HD)	α-Thujene (7.3%), α-pinene (15.1%), myrcene (8.2%), *p*-cymene (6.2%), limonene (18.2%), α-cedrene (6.1%)	Antibacterial, broth dilution assay (*Staphylococcus aureus*, MIC 3.52–54.6 μg mL^{-1}; *Staphylococcus epidermidis*, MIC 17.6 μg mL^{-1}; *Escherichia coli*, MIC 54.6 μg mL^{-1}; *Pseudomonas aeruginosa*, MIC 6.6 μg mL^{-1}); antifungal (*Candida albicans*, MIC 6.16 μg mL^{-1}; *Candida tropicalis*, MIC 6.16 μg mL^{-1})[b]	Camarda et al. (2007)
	Commercial ("various herbal shops")	Oleoresin (HD)	α-Pinene (17.0%), myrcene (6.8%), *p*-cymene (6.2%), limonene (14.9%), β-caryophyllene (7.0%)	Antibacterial, broth dilution assay (*Staphylococcus aureus*, MIC 5000 μg mL^{-1}; *Bacillus cereus*, MIC 4000 μg mL^{-1}; *Escherichia coli*, MIC 8000 μg mL^{-1}; *Proteus vulgaris*, MIC 3000 μg mL^{-1}; *Candida albicans*, MIC 12000 μg mL^{-1})	Van Vuuren et al. (2010)
	Commercial ("various herbal shops")	Oleoresin (HD)	α-Thujene (8.3%), α-pinene (22.8%), myrcene (5.5%), limonene (14.9%), β-caryophyllene (8.0%)	Antibacterial, broth dilution assay (*Staphylococcus aureus*, MIC 8000 μg mL^{-1}; *Bacillus cereus*, MIC 1500 μg mL^{-1}; *Escherichia coli*, MIC 8000 μg mL^{-1}; *Proteus vulgaris*, MIC 12800 μg mL^{-1}; *Candida albicans*, MIC 8000 μg mL^{-1})	Van Vuuren et al. (2010)

	Commercial ("various herbal shops")	Oleoresin (HD)	α-Pinene (30.1%), myrcene (6.0%), limonene (20.4%), β-caryophyllene (6.9%)	Antibacterial, broth dilution assay (*Staphylococcus aureus*, MIC 8000 μg mL^{-1}; *Bacillus cereus*, MIC 4000 μg mL^{-1}; *Escherichia coli*, MIC 8000 μg mL^{-1}; *Proteus vulgaris*, MIC 3000 μg mL^{-1}; *Candida albicans*, MIC 6600 μg mL^{-1})	Van Vuuren et al. (2010)
	Commercial ("various herbal shops")	Oleoresin (HD)	α-Thujene (7.9%), α-pinene (27.6%), myrcene (6.8%), limonene (14.6%), β-caryophyllene (5.6%)	Antibacterial, broth dilution assay (*Staphylococcus aureus*, MIC 6000 μg mL^{-1}; *Bacillus cereus*, MIC 2000 μg mL^{-1}; *Escherichia coli*, MIC 12000 μg mL^{-1}; *Proteus vulgaris*, MIC 3000 μg mL^{-1}; *Candida albicans*, MIC 8000 μg mL^{-1})	Van Vuuren et al. (2010)
	Commercial ("various herbal shops")	Oleoresin (HD)	α-Thujene (7.7%), α-pinene (40.4%), myrcene (8.8%), *p*-cymene (5.3%), limonene (15.8%)	Antibacterial, broth dilution assay (*Staphylococcus aureus*, MIC 6000 μg mL^{-1}; *Bacillus cereus*, MIC 3000 μg mL^{-1}; *Escherichia coli*, MIC 6000 μg mL^{-1}; *Proteus vulgaris*, MIC 3000 μg mL^{-1}; *Candida albicans*, MIC 8000 μg mL^{-1})	Van Vuuren et al. (2010)
	Commercial ("various herbal shops")	Oleoresin (HD)	α-Thujene (52.4%), sabinene (5.6%)	Antibacterial, broth dilution assay (*Staphylococcus aureus*, MIC 16000 μg mL^{-1}; *Bacillus cereus*, MIC 4000 μg mL^{-1}; *Escherichia coli*, MIC 6000 μg mL^{-1}; *Proteus vulgaris*, MIC 3000 μg mL^{-1}; *Candida albicans*, MIC 8000 μg mL^{-1})	Van Vuuren et al. (2010)
	Commercial ("various herbal shops")	Oleoresin (HD)	α-Pinene (22.3%), limonene (11.9%), β-caryophyllene (7.8%)	Antibacterial, broth dilution assay (*Staphylococcus aureus*, MIC 10400 μg mL^{-1}; *Bacillus cereus*, MIC 4000 μg mL^{-1}; *Escherichia coli*, MIC 6000 μg mL^{-1}; *Proteus vulgaris*, MIC 3000 μg mL^{-1}; *Candida albicans*, MIC 5300 μg mL^{-1})	Van Vuuren et al. (2010)

(continued)

Table 4.2 (continued)

Boswellia species	Collection site	Essential oil	Major components (> 5%)	Bioactivity of essential oil	Reference
	Commercial ("various herbal shops")	Oleoresin (HD)	α-Thujene (12.0%), α-pinene (23.6%), limonene (18.3%), β-caryophyllene (6.3%)	Antibacterial, broth dilution assay (*Staphylococcus aureus*, MIC 8000 μg mL^{-1}; *Bacillus cereus*, MIC 8300 μg mL^{-1}; *Escherichia coli*, MIC 4000 μg mL^{-1}; *Proteus vulgaris*, MIC 3000 μg mL^{-1}; *Candida albicans*, MIC 8000 μg mL^{-1})	Van Vuuren et al. (2010)
B. carteri Birdw.	Commercial ("various herbal shops")	Oleoresin (HD)	α-Thujene (5.3%), α-pinene (12.0%), myrcene (9.9%), limonene (12.7%), β-caryophyllene (10.5%)	Antibacterial, broth dilution assay (*Staphylococcus aureus*, MIC 10400 μg mL^{-1}; *Bacillus cereus*, MIC 4000 μg mL^{-1}; *Escherichia coli*, MIC 6000 μg mL^{-1}; *Proteus vulgaris*, MIC 3000 μg mL^{-1}; *Candida albicans*, MIC 5300 μg mL^{-1})	Van Vuuren et al. (2010)
	Commercial (Skinmate, Puchon, Gyeonggido, Korea)	Oleoresin (HD)	α-Terpinene (7.9%), *p*-cymene (11.8%)[c], γ-terpinene (16.9%), *p*-menth-2-en-1-ol (34.5%)	–	Yang et al. (2010)
	Ethiopia	Oleoresin (HD)	Octyl acetate (34.7%), nardoeudesmol C (5.6%), nerolidol isobutyrate (18.3%)	Cytotoxic (MCF-7, IC50 40.7 μg mL^{-1}; HepG2, IC_{50} 57.0 μg mL^{-1}; HeLa, IC_{50} 55.5 μg mL^{-1}; HS-1, IC50 39.7 μg mL^{-1}; A459, IC_{50} 60.3 μg mL^{-1})	Chen et al. (2013)
	Commercial (Young Living Essential Oils; from Somalia)	Oleoresin (HD)	–	Cytotoxic (J82 human bladder tumor cells, IC_{50} 910 μg mL^{-1})	Dozmorov et al. (2014)
	Commercial (herbal pharmacy, Rotterdam, Netherlands)	Oleoresin (HD)	α-Pinene (23.0%), limonene (10.9%), β-caryophyllene (8.3%), δ-cadinene (5.8%), caryophyllene oxide (11.1%)	Weak anticandidal activity (*Candida albicans*, MIC 1250 μg mL^{-1})	Nikolić et al. (2016)

	Commercial (Sensient Essential Oils, Bremen, Germany)	Oleoresin (HD)	α-Thujene (12.0%), α-pinene (31.8%), sabinene (5.4%), *p*-cymene (6.0%), limonene (17.9%), β-caryophyllene (5.4%)	Weak anticandidal activity (*Candida albicans*, MIC 2500 μg mL^{-1})	Nikolić et al. (2016)
	Commercial (Tehran market)	Oleoresin (HD)	Dihydrocitronellyl acetate (55.6%), α-santonin (9.0%)	–	Dounchaly et al. (2016)
	Commercial (Ameo, Zija International)	Oleoresin (HD)	*p*-Cymene (10.0%), limonene (22.4%), α-copaene (5.8%), β-caryophyllene (22.2%), δ-cadinene (9.4%)	–	Setzer (unpublished)
	Commercial (Scents of the Earth, Sun City, USA; from Somalia)	Oleoresin (headspace SPME)	α-Pinene (23.2%), limonene (22.4%), β-caryophyllene (6.9%)	–	Hamm et al. (2005)
	Commercial (Scents of the Earth, Sun City, USA; from Aden)	Oleoresin (headspace SPME)	α-Pinene (6.3%), limonene (10.2%), β-caryophyllene (66.9%), α-humulene (5.2%), 1(10,5-germacradien-4-ol (5.7%), caryophyllene oxide (13.1%)	–	Hamm et al. (2005)
B. dalzielii Hutch.	Gombi, Adamawa state, Nigeria	Leaf (HD)	α-Pinene (45.7%), γ-terpinene (11.5%)	–	Kubmarawa et al. (2006)
	Ségbana region, Benin	Leaf (HD)	α-Pinene (15.2%), myrcene (5.7%), δ-3-carene (27.7%), *p*-cymene (9.5%), β-phellandrene (8.5%), isolongifolene (6.2%)	Enzyme inhibition (acetylcholinesterase, IC_{50} 67.1 μg mL^{-1}; 5-lipoxygenase, IC_{50} 70.0 μg mL^{-1})	Kohoude et al. (2017)
B. dioscoridis Thulin	Soqotra Island, Yemen	Bark (HD)	α-Thujene (9.3%), α-pinene (8.3%), camphor (5.5%), β-caryophyllene (5.5%), caryophyllene oxide (5.0%)	Antibacterial, broth dilution assay (*Staphylococcus aureus*, MIC 3620 μg mL^{-1}; *Bacillus subtilis*, MIC 3620 μg mL^{-1})	Mothana et al. (2011)
	Soqotra Island, Yemen	Oleoresin (MeOH extract)	α-Thujene (56.6%), α-pinene (8.3%), sabinene (10.0%), myrcene (6.0%), terpinen-4-ol (14.0%)	–	Maděra et al. (2017)

(continued)

Table 4.2 (continued)

Boswellia species	Collection site	Essential oil	Major components (> 5%)	Bioactivity of essential oil	Reference
	Soqotra Island, Yemen	Oleoresin (MeOH extract)	α-Thujene (60.3%), terpinen-4-ol (26.5%)	–	Maděra et al. (2017)
	Soqotra Island, Yemen	Oleoresin (MeOH extract)	α-Thujene (69.5%), α-pinene (10.5%), sabinene (7.4%), β-pinene (6.0%)	–	Maděra et al. (2017)
B. elongata Balf. f.	Soqotra Island, Yemen	Bark (HD)	Verticilla-4(20),7,11-triene (8.2%), incensole (14.8%), incensole acetate (7.4%)	Antibacterial, broth dilution assay (*Staphylococcus aureus*, MIC 4310 μg mL^{-1}; *Bacillus subtilis*, MIC 4310 μg mL^{-1})	Mothana et al. (2011)
	Soqotra Island, Yemen	Oleoresin (HD)	β-Caryophyllene (39.1%), methyl cycloundecanecarboxylate (7.9%)[d], verticillol (52.4%)	–	Ali et al. (2008)
	Soqotra Island, Yemen	Oleoresin (MeOH extract)	α-Thujene (59.1%), (*E*)-β-farnesene (5.9%), cembrene (13.1%)	–	Maděra et al. (2017)
	Soqotra Island, Yemen	Oleoresin (MeOH extract)	α-Pinene (34.3%), β-pinene (12.5%), *p*-cymene (6.4%), terpinen-4-ol (15.8%), (*E*)-β-farnesene (7.7%)	–	Maděra et al. (2017)
	Soqotra Island, Yemen	Oleoresin (MeOH extract)	α-Thujene (49.2%), myrcene (9.4%), terpinen-4-ol (13.1%)	–	Maděra et al. (2017)
	Soqotra Island, Yemen	Oleoresin (MeOH extract)	α-Thujene (16.2%), sabinene (16.1%), cembrene (28.5%)	–	Maděra et al. (2017)
B. frereana Birdw.	Commercial (Willy Benecke GmbH, Hamburg, Germany)	Oleoresin (HD)	α-Thujene (8.1%), α-pinene (38.0%), *p*-cymene (11.0%), β-caryophyllene (5.3%)	–	Basar (2005)

	Commercial ("various herbal shops")	Oleoresin (HD)	α-Pinene (64.7%), sabinene (7.0%), *p*-cymene (5.4%)	Antibacterial, broth dilution assay (*Staphylococcus aureus*, MIC 8000 μg mL^{-1}; *Bacillus cereus*, MIC 3000 μg mL^{-1}; *Escherichia coli*, MIC 6000 μg mL^{-1}; *Proteus vulgaris*, MIC 3000 μg mL^{-1}; *Candida albicans*, MIC 6000 μg mL^{-1})	Van Vuuren et al. (2010)
	Commercial ("various herbal shops")	Oleoresin (HD)	α-Thujene (19.9%), α-pinene (24.7%), *p*-cymene (12.3%), β-caryophyllene (5.3%)	Antibacterial, broth dilution assay (*Staphylococcus aureus*, MIC 4000 μg mL^{-1}; *Bacillus cereus*, MIC 1500 μg mL^{-1}; *Escherichia coli*, MIC 4000 μg mL^{-1}; *Proteus vulgaris*, MIC 3000 μg mL^{-1}; *Candida albicans*, MIC 6000 μg mL^{-1})	Van Vuuren et al. (2010)
	Commercial ("various herbal shops")	Oleoresin (HD)	α-Thujene (33.1%), sabinene (5.1%), *p*-cymene (16.9%), β-caryophyllene (6.9%)	Antibacterial, broth dilution assay (*Staphylococcus aureus*, MIC 12000 μg mL^{-1}; *Bacillus cereus*, MIC 6600 μg mL^{-1}; *Escherichia coli*, MIC 4000 μg mL^{-1}; *Proteus vulgaris*, MIC 12800 μg mL^{-1}; *Candida albicans*, MIC 12000 μg mL^{-1})	Van Vuuren et al. (2010)
	Commercial (Scents of the Earth, Sun City, USA; from Somalia)	Oleoresin (headspace SPME)	α-Thujene (9.8%), α-pinene (12.4%), *p*-cymene (7.8%)	–	(Hamm et al. (2005)
B. nana Hepper	Soqotra Island, Yemen	Oleoresin (MeOH extract)	α-Thujene (57.6%), α-pinene (5.9%), sabinene (10.4%), β-pinene (10.2%)	–	Maděra et al. (2017)
B. neglecta S. Moore	Dibluk, Sidamo province, Ethiopia	Oleoresin (HD)	α-Pinene (16.7%), α-thujene (19.2%), *p*-cymene (9.5%), terpinen-4-ol (12.5%)	–	Başer et al. (2003)

(continued)

Table 4.2 (continued)

Boswellia species	Collection site	Essential oil	Major components (> 5%)	Bioactivity of essential oil	Reference
	Ethiopia	Oleoresin (HD)	α-Thujene (21.3%), α-pinene (21.3%), *p*-cymene (11.8%), terpinen-4-ol (5.3%)	–	Basar (2005)
	Commercial ("various herbal shops")	Oleoresin (HD)	α-Pinene (43.4%), β-pinene (13.1%), *p*-cymene (8.6%), terpinen-4-ol (12.5%)	Antibacterial, broth dilution assay (*Staphylococcus aureus*, MIC 6000 μg mL^{-1}; *Bacillus cereus*, MIC 2000 μg mL^{-1}; *Escherichia coli*, MIC 6000 μg mL^{-1}; *Proteus vulgaris*, MIC 3000 μg mL^{-1}; *Candida albicans*, MIC 6600 μg mL^{-1})	Van Vuuren et al. (2010)
	Borena region, southern Ethiopia	Oleoresin (HD)	–	Antibacterial, broth dilution assay (*Pseudomonas aeruginosa*, MIC 1300 μg mL^{-1}); antifungal (*Candida albicans*, MIC 1800 μg mL^{-1}; *Cryptococcus neoformans*, MIC 1300 μg mL^{-1})	de Rapper et al. (2012)
	Dubuluk, Borana zone, Oromiya region Ethiopia	Oleoresin (HD)	α-Thujene (16.5%), α-pinene (42.0%), terpinen-4-ol (28.2%)	–	Bekana et al. (2014)
	Mega, Borana zone, Oromiya region, Ethiopia	Oleoresin (HD)	α-Thujene (13.0%), α-pinene (32.6%), *p*-cymene (5.1%), terpinen-4-ol (29.9%)	–	Bekana et al. (2014)
	Wachile, Borana zone, Oromiya region, Ethiopia	Oleoresin (HD)	α-Thujene (12.7%), α-pinene (50.7%), terpinen-4-ol (17.5%), verbenone (6.6%)	–	Bekana et al. (2014)
B. papyrifera Hochst.	Metema, north Ethiopia	Oleoresin (HD)	Limonene (6.5%), octanol (8.0%), octyl acetate (56.0%)	–	Dekebo et al. (1999)

	Commercial (White Lotus Aromatics Ltd., Port Angeles, WA, USA; from Ethiopia)	Oleoresin (HD)	α-Pinene (5.2%), sabinene (6.0%), octanol (17.8%), octyl acetate (63.5%)	Antibacterial, broth dilution assay (*Staphylococcus aureus*, MIC 22.6–27.0 μg mL^{-1}; *Staphylococcus epidermidis*, MIC 22.6 μg mL^{-1}; *Escherichia coli*, MIC 27.0 μg mL^{-1}; *Pseudomonas aeruginosa*, MIC 27.0 μg mL^{-1}); antifungal (*Candida albicans*, MIC 6.09 μg mL^{-1}; *Candida tropicalis*, MIC 6.09 μg mL^{-1})[b]	Camarda et al. (2007)
	Northern Ethiopia	Oleoresin (HD)	–	Antibacterial, broth dilution assay (*Staphylococcus aureus*, MIC 1500 μg mL^{-1}; *Bacillus cereus*, MIC 1600 μg mL^{-1}; *Pseudomonas aeruginosa*, MIC 1500 μg mL^{-1}); antifungal (*Candida albicans*, MIC 1400 μg mL^{-1}; *Cryptococcus neoformans*, MIC 1400 μg mL^{-1})	de Rapper et al. (2012)
	Metema, Amhara region, Ethiopia	Oleoresin (HD)	Octyl acetate (65.7%)	–	Bekana et al. (2014)
B. papyrifera Hochst.	Humera, Tigray regional state, Ethiopia	Oleoresin (HD)	Octanol (8.8%), octyl acetate (60.4%)	–	Bekana et al. (2014)
	Metekel, Benishangul-Gomuz regional state, Ethiopia	Oleoresin (HD)	Octyl acetate (57.1%)	–	Bekana et al. (2014)
	Commercial (White Lotus Aromatics Ltd., Port Angeles, WA, USA	Oleoresin (HD)	Octanol (17.8%), octyl acetate (63.5%)	Anti-biofilm activity (*Staphylococcus epidermidis*, *Staphylococcus aureus*, $IC_{50} < 750$ μg mL^{-1})[b]	Schillaci et al. (2008)

(continued)

Table 4.2 (continued)

Boswellia species	Collection site	Essential oil	Major components (> 5%)	Bioactivity of essential oil	Reference
	Commercial (Scents of the Earth, Sun City, USA; from Ethiopia)	Oleoresin (headspace SPME)	Octanol (13.9%), octyl acetate (64.6%), incensole acetate (10.8%)	–	Hamm et al. (2005)
B. pirottae Chiov.	Gibe Valley, Ethiopia	Oleoresin (HD)	Terpinen-4-ol (14.6%), *trans*-verbenol (15.5%)	–	Başer et al. (2003)
B. popoviana Hepper	Soqotra Island, Yemen	Oleoresin (MeOH extract)	α-Thujene (55.3%), *p*-cymene (9.0%), terpinen-4-ol (6.4%), cembrene (12.8%)	–	Maděra et al. (2017)
	Soqotra Island, Yemen	Oleoresin (MeOH extract)	α-Thujene (29.9%), *p*-cymene (10.9%), terpinen-4-ol (8.0%)	–	Maděra et al. (2017)
	Soqotra Island, Yemen	Oleoresin (MeOH extract)	α-Thujene (51.5%), *p*-cymene (9.0%), terpinen-4-ol (7.0%), (*E*)-β-farnesene (9.2%)	–	Maděra et al. (2017)
	Soqotra Island, Yemen	Oleoresin (MeOH extract)	α-Thujene (67.3%), cembrene (6.3%)	–	Maděra et al. (2017)
B. rivae Engl.	Bulagere, Ogaden Region, Ethiopia	Oleoresin (HD)	α-Pinene (5.3%), δ-3-carene (9.6%), limonene (14.8%), *trans*-verbenol (6.8%)	–	Başer et al. (2003)
	Ethiopia	Oleoresin (HD)	α-Pinene (16.7%), δ-3-carene (17.3%), limonene (21.1%)	–	Basar (2005)

	Commercial (White Lotus Aromatics Ltd., Port Angeles, WA, USA; from Ethiopia)	Oleoresin (HD)	α-Pinene (13.3%), δ-3-carene (15.7%), *p*-cymene (7.1%), limonene (28.0%), *trans*-verbenol (5.8%)	Antibacterial, broth dilution assay (*Staphylococcus aureus*, MIC 13.3–54.8 μg mL^{-1}; *Staphylococcus epidermidis*, MIC 23.0 μg mL^{-1}; *Escherichia coli*, MIC 3.53 μg mL^{-1}; *Pseudomonas aeruginosa*, MIC 54.8 μg mL^{-1}); antifungal (*Candida albicans*, MIC 2.65 μg mL^{-1}; *Candida tropicalis*, MIC 27.4 μg mL^{-1})[b]	Camarda et al. (2007)
	Ogaden region, southeastern Ethiopia	Oleoresin (HD)	–	Antibacterial, broth dilution assay (*Pseudomonas aeruginosa*, MIC 1000 μg mL^{-1}); antifungal (*Cryptococcus neoformans*, MIC 800 μg mL^{-1})	de Rapper et al. (2012)
	Filtu district, Somalia regional state, Ethiopia	Oleoresin (HD)	α-Pinene (37.3%), *o*-cymene (5.6%), δ-3-carene (6.7%), *p*-cymene (9.8%), limonene (9.7%)	–	Bekana et al. (2014)
	Chereti district, Somalia regional state, Ethiopia	Oleoresin (HD)	α-Pinene (32.5%), δ-3-carene (6.2%), *p*-cymene (21.1%), limonene (19.6%)	–	Bekana et al. (2014)
	Dolo Odo district, Somalia regional state, Ethiopia	Oleoresin (HD)	α-Thujene (10.0%), α-pinene (66.2%), *p*-cymene (5.7%)	–	Bekana et al. (2014)
B. rivae Engl.	Commercial (White Lotus Aromatics Ltd., Port Angeles, WA, USA	Oleoresin (HD)	α-Pinene (13.3%), δ-3-carene (15.7%), *p*-cymene (7.1%), limonene (28.0%), *trans*-verbenol (5.8%)	Anti-biofilm activity (*Candida albicans*, IC_{50} 13,900 μg mL^{-1})[b]	Schillaci et al. (2008)
B. sacra Flueck.	Dhofar region, Oman	Oleoresin (HD)	α-Pinene (5.3%), myrcene (6.9%), α-thujene (6.6%), (*E*)-β-ocimene (32.3%), sabinene (5.2%), limonene (33.5%)	–	Al-Harrasi and Al-Saidi (2008)

(continued)

Table 4.2 (continued)

Boswellia species	Collection site	Essential oil	Major components (> 5%)	Bioactivity of essential oil	Reference
	Commercial ("various herbal shops")	Oleoresin (HD)	α-Pinene (22.5%), myrcene (5.5%), sabinene (6.9%), *p*-cymene (5.9%), limonene (11.2%), β-caryophyllene (7.6%)	Antibacterial, broth dilution assay (*Staphylococcus aureus*, MIC 4000 μg mL^{-1}; *Bacillus cereus*, MIC 3000 μg mL^{-1}; *Escherichia coli*, MIC 4000 μg mL^{-1}; *Proteus vulgaris*, MIC 3000 μg mL^{-1}; *Candida albicans*, MIC 8000 μg mL^{-1})	Van Vuuren et al. (2010)
	Commercial ("various herbal shops")	Oleoresin (HD)	α-Thujene (11.2%), α-pinene (18.3%), limonene (13.1%), β-caryophyllene (7.2%)	Antibacterial, broth dilution assay (*Staphylococcus aureus*, MIC 8000 μg mL^{-1}; *Bacillus cereus*, MIC 2000 μg mL^{-1}; *Escherichia coli*, MIC 6000 μg mL^{-1}; *Proteus vulgaris*, MIC 3000 μg mL^{-1}; *Candida albicans*, MIC 8000 μg mL^{-1})	Van Vuuren et al. (2010)
	Hasik area, Oman	Oleoresin (HD)	α-Pinene (59.4–65.5%), myrcene (5.4–7.5%), limonene (8.4–9.0%)	Cytotoxic to breast tumor cells (MCF10-2A, IC_{50} 1470 μg mL^{-1}; T47D IC_{50} 690 μg mL^{-1}; MCF7, IC_{50} 556 μg mL^{-1}; MDA-MB-231 IC_{50} 769 μg mL^{-1})	Suhail et al. (2011)
	Hasik area, Oman	Oleoresin (HD)	α-Pinene (59.4–78.5%), myrcene (2.6–5.4%), limonene (5.6–9.0%)	Cytotoxic to pancreatic tumor cells (MIA MaCa-2, IC_{50} 833 μg mL^{-1}; Panc-28, IC_{50} 813 μg mL^{-1}; DANG, IC_{50} 641 μg mL^{-1}; BxPC-3, IC_{50} 741 μg mL^{-1})	Ni et al. (2012)
B. sacra Flueck.	Dhofar region, Oman	"Hoojri" oleoresin (HD)	α-Pinene (76.0%)	Antibacterial to Gram-positive organisms (*Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*, MIC 5000 μg mL^{-1}); inactive to Gram-negative bacteria	Al-Saidi et al. (2012)
	Dhofar region, Oman	"Shaabi" oleoresin (HD)	α-Pinene (68.5%)	Antibacterial to Gram-positive organisms (*Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*, MIC 5000 μg mL^{-1}); inactive to Gram-negative bacteria	Al-Saidi et al. (2012)

	Dhofar region, Oman	"Najdi" oleoresin (HD)	α-Pinene (46.8%), myrcene (8.9%), limonene (15.9%)	Antibacterial to Gram-positive organisms (*Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*, MIC 5000 μg mL^{-1}); inactive to Gram-negative bacteria	Al-Saidi et al. (2012)
	Dhofar region, Oman	"Shathari" oleoresin (HD)	α-Pinene (64.7%), limonene (8.1%)	Antibacterial to Gram-positive organisms (*Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*, MIC 5000 μg mL^{-1}); inactive to Gram-negative bacteria	Al-Saidi et al. (2012)
	Commercial (J. Ertelt, AureliaSan, Bisingen, Germany; from Oman)	Oleoresin (SAFE distillation)	α-Pinene (11.4%), myrcene (8.0%), limonene (12.8%)	–	Niebler and Buettner (2015)
	Commercial (Anandam GmbH, Hamburg, Germany; from Oman)	Oleoresin (SAFE distillation)	α-Pinene (14.2%), myrcene (7.6%), *p*-cymene (6.5%), limonene (10.9%)	–	Niebler and Buettner (2015)
B. sacra Flueck.	Commercial (J. Ertelt, AureliaSan, Bisingen, Germany; from Somalia)	Oleoresin (SAFE distillation)	α-Pinene (24.3%), myrcene (6.6%), *p*-cymene (5.2%), limonene (18.9%)	–	Niebler and Buettner (2015)
	Commercial (J. Ertelt, AureliaSan, Bisingen, Germany; from Somalia)	Oleoresin (SAFE distillation)	α-Pinene (12.6%), myrcene (7.1%), limonene (12.8%)	–	Niebler and Buettner (2015)

(continued)

Table 4.2 (continued)

Boswellia species	Collection site	Essential oil	Major components (> 5%)	Bioactivity of essential oil	Reference
	Commercial (J. Ertelt, AureliaSan, Bisingen, Germany; from Somalia)	Oleoresin (SAFE distillation)	α-Pinene (24.1%), *p*-cymene (7.0%), limonene (11.3%)	–	Niebler and Buettner (2015)
	Commercial (C.E. Roeper, Hamburg, Germany; from Somalia)	Oleoresin (SAFE distillation)	α-Pinene (13.8%), myrcene (5.4%), *p*-cymene (9.7%), limonene (10.4%)	–	Niebler and Buettner (2015)
	Salalah, Oman	Oleoresin (hexane extract)	α-Pinene (61.6%)	–	Hakkim et al. (2015)
	Commercial (Premaveral Life GmbH, Mittelberg, Germany; from Somalia)	Oleoresin (HD)	–	Rotundone (woody, coniferous) and mustakone (spicy, woody) identified as highly potent odorants	Niebler et al. (2016)
	Commercial (Scents of the Earth, Sun City, USA; from Oman)	Oleoresin (headspace SPME)	α-Pinene (13.9%), myrcene (5.2%), *p*-cymene (5.5%), limonene (6.9%),	–	Hamm et al. (2005)
B. serrata Roxb. ex Colebr.	Sokoto state, Nigeria	Bark (HD)	α-Pinene (73.3%)	–	Kasali et al. (2002)
	Commercial (Willy Benecke GmbH, Hamburg, Germany)	Oleoresin (HD)	α-Thujene (12.0%), α-pinene (8.0%), myrcene (38.0%), estragole (11.6%)	–	Basar (2005)

	Jabalpur area, Madhya Pradesh, India	Oleoresin (HD)	α-Thujene (22.7%), tetrahydrolinalool (10.6%)[e], benzyl tiglate (5.5%), *epi*-cubenol (5.2%), 10-*epi*-γ-eudesmol (5.3%)	–	Singh et al. (2007)
	Commercial (market in Amritsar, Punjab India)	Oleoresin (HD)	α-Thujene (47.4%), δ-3-carene (9.6%), tetrahydrolinalool (7.0%)[e], *epi*-cubenol (9.1%)	–	Singh et al. (2007)
	Commercial (market in Khari Baoli, New Delhi, India)	Oleoresin (HD)	α-Thujene (26.2%), δ-3-carene (7.9%), limonene (6.3%), tetrahydrolinalool (8.8%)[e], α-terpineol (5.8%)	–	Singh et al. (2007)
	Commercial (market in Karol Bagh, New Delhi, India)	Oleoresin (HD)	α-Pinene (11.2%), α-thujene (29.5%), δ-3-carene (7.6%), limonene (8.5%), tetrahydrolinalool (7.8%)[e]	–	Singh et al. (2007)
	Commercial (White Lotus Aromatics Ltd., Port Angeles, WA, USA; from India)	Oleoresin (HD)	α-Thujene (29.7%), sabinene (7.4%), δ-3-carene (7.5%), *p*-cymene (12.5%), estragole (6.7%)	Antibacterial, broth dilution assay (*Staphylococcus aureus*, MIC 53.2–107 μg mL^{-1}; *Staphylococcus epidermidis*, MIC 89.2 μg mL^{-1}; *Escherichia coli*, MIC 107.2 μg mL^{-1}; *Pseudomonas aeruginosa*, MIC 12.9 μg mL^{-1}); antifungal (*Candida albicans*, MIC 12.9 μg mL^{-1}; *Candida tropicalis*, MIC 12.9 μg mL^{-1})[b]	Camarda et al. (2007)
B. serrata Roxb. ex Colebr.	Commercial (Egyptian herbal store, Cairo, Egypt)	Oleoresin (HD)	Sabinene (19.1%), terpinen-4-ol (14.6%), *cis*-carveol (6.3%), α-terpinyl acetate (13.0%), elemicin (7.1%), β-copaen-4α-ol (10.2%), germacrene D (12.6%)	Cytotoxic (HepG2 human hepatocellular carcinoma cells, IC_{50} 5.5 μg mL^{-1}; HCT 116 human colon cancer cells, IC_{50} 6.2 μg mL^{-1})	Ahmed et al. (2015)

(continued)

Table 4.2 (continued)

Boswellia species	Collection site	Essential oil	Major components (> 5%)	Bioactivity of essential oil	Reference
	Commercial (Konark Herbals and Health Care)	Oleoresin (HD)	α-Thujene (43.5%), α-pinene (7.2%), sabinene (7.8%), *p*-cymene (8.7%), thujol (7.2%)	Antimicrobial, disk diffusion assay (*Propionibacterium acnes*, *Malassezia furfur*, *Malassezia globosa*, *Trichophyton rubrum*, *Trichophyton mentagrophytes*)	Sadhasivam et al. (2016)
	Shivpuri forest area, Madhya Pradesh, India	Oleoresin (HD)	α-Thujene (22.5%), α-pinene (10.9%), myrcene (8.9%), α-terpineol (7.8%), terpinyl isobutyrate (5.1%), eudesmol (11.5%)	Antibacterial (*Klebsiella pneumoniae*, MIC 18.8 μg mL^{-1}; *Escherichia coli*, MIC 300 μg mL^{-1}; *Salmonella typhi* MIC 37.5 μg mL^{-1}; *Streptococcus mutans*, MIC 300 μg mL^{-1}; *Pseudomonas aeruginosa* MIC 37.5 μg mL^{-1}; *Staphylococcus aureus*, MIC 37.5 μg mL^{-1})	Gupta et al. (2017)
	Commercial (market, Mandsaur district, Madhya Pradesh, India	Oleoresin (HD)	α-Thujene (61.4%), sabinene (5.5%)	Antibacterial (*Klebsiella pneumoniae*, MIC 150 μg mL^{-1}; *Escherichia coli*, MIC 150 μg mL^{-1}; *Salmonella typhi* MIC 150 μg mL^{-1}; *Streptococcus mutans*, MIC 150 μg mL^{-1}; *Pseudomonas aeruginosa* MIC 37.5 μg mL^{-1}; *Staphylococcus aureus*, MIC 37.5 μg mL^{-1})	Gupta et al. (2017)
	Commercial (market, Mandsaur district, Madhya Pradesh, India	Oleoresin (HD)	α-Thujene (63.6%), α-pinene (5.5%), sabinene (5.9%)	Antibacterial (*Klebsiella pneumoniae*, MIC 37.5 μg mL^{-1}; *Escherichia coli*, MIC 300 μg mL^{-1}; *Salmonella typhi* MIC 300 μg mL^{-1}; *Streptococcus mutans*, MIC 300 μg mL^{-1}; *Pseudomonas aeruginosa* MIC 75 μg mL^{-1}; *Staphylococcus aureus*, MIC 75 μg mL^{-1})	Gupta et al. (2017)

B. serrata Roxb. ex Colebr.	Commercial (market, Neemuch district, Madhya Pradesh, India)	Oleoresin (HD)	α-Thujene (65.6%), α-pinene (8.1%), sabinene (5.1%)	Antibacterial (*Klebsiella pneumoniae*, MIC 75 μg mL^{-1}; *Escherichia coli*, MIC 300 μg mL^{-1}; *Salmonella typhi* MIC 150 μg mL^{-1}; *Streptococcus mutans*, MIC 300 μg mL^{-1}; *Pseudomonas aeruginosa* MIC 150 μg mL^{-1}; *Staphylococcus aureus*, MIC 37.5 μg mL^{-1})	Gupta et al. (2017)
	Commercial (market, Neemuch district, Madhya Pradesh, India)	Oleoresin (HD)	α-Thujene (69.8%)	Antibacterial (*Klebsiella pneumoniae*, MIC 300 μg mL^{-1}; *Escherichia coli*, MIC 300 μg mL^{-1}; *Salmonella typhi* MIC 300 μg mL^{-1}; *Streptococcus mutans*, MIC 300 μg mL^{-1}; *Pseudomonas aeruginosa* MIC 300 μg mL^{-1}; *Staphylococcus aureus*, MIC 300 μg mL^{-1})	Gupta et al. (2017)
	Commercial (Scents of the Earth, Sun City, USA; from India)	Oleoresin (headspace SPME)	α-Thujene (11.7%), myrcene (7.0%), kessane (8.0), incensole (6.9)	–	Hamm et al. (2005)
B. socotrana Balf. f.	Soqotra Island, Yemen	Oleoresin (HD)	*p*-Cymene (7.1%), 2-thujen-4-ol (31.3%), (*E*)-2,3-epoxycarene (51.8%)	Inhibitor of acetylcholinesterase (IC_{50} 141 μg mL^{-1})	Ali et al. (2008)
	Soqotra Island, Yemen	Bark (HD)	α-Thujene (7.6%), *p*-cymene (13.0%), camphor (11.6%), terpinen-4-ol (6.1%), 2′-hydroxy-5′-methoxyacetophenone (16.3%)	Antibacterial, broth dilution assay (*Staphylococcus aureus*, MIC 1870 μg mL^{-1}; *Bacillus subtilis*, MIC 1870 μg mL^{-1})	Mothana et al. (2011)

(continued)

Table 4.2 (continued)

Boswellia species	Collection site	Essential oil	Major components (> 5%)	Bioactivity of essential oil	Reference
	Soqotra Island, Yemen	Oleoresin (MeOH extract)	α-Pinene (55.2%), myrcene (11.4%), limonene (8.0%), terpinen-4-ol (6.8%)	–	Maděra et al. (2017)
	Soqotra Island, Yemen	Oleoresin (MeOH extract)	α-Thujene (46.0%), α-pinene (5.4%), myrcene (18.5%), *p*-cymene (7.1%), terpinen-4-ol (15.8%)	–	Maděra et al. (2017)
	Soqotra Island, Yemen	Oleoresin (MeOH extract)	α-Pinene (92.4%)	–	Maděra et al. (2017)
B. thurifera Roxb. ex Fleming	Commercial (Lothian Herbs, Edinburgh, UK)	Oleoresin (HD)	α-Thujene (5.8%), α-pinene (41.2%), *p*-cymene (5.6%), limonene (16.7%), *trans*-sabinene hydrate (10.8%)	Antibacterial, agar diffusion assay (*Beneckea natriegens*, *Citrobacter freundii*, *Salmonella pullorum*); antifungal (*Aspergillus niger*)	Baratta et al. (1998)
	Commercial ("various herbal shops")	Oleoresin (HD)	α-Pinene (28.0%), myrcene (5.6%), limonene (14.6%), β-caryophyllene (5.8%)	Antibacterial, broth dilution assay (*Staphylococcus aureus*, MIC 10000 μg mL^{-1}; *Bacillus cereus*, MIC 4000 μg mL^{-1}; *Escherichia coli*, MIC 6000 μg mL^{-1}; *Proteus vulgaris*, MIC 2000 μg mL^{-1}; *Candida albicans*, MIC 6000 μg mL^{-1})	Van Vuuren et al. (2010)

[a]The high concentrations of octanol and octyl acetate in this sample suggest that the plant may be *B. papyrifera* rather than *B. carteri*

[b]It is likely that the MIC determinations have incorrect units, reported as μg mL^{-1} rather than mg mL^{-1} (see Schillaci et al. 2008), and that may account for the large discrepancies in antimicrobial activities

[c]This compound was reported as 1-ethyl-3,5-dimethylbenzene, but it is probably incorrect; 1-ethyl-3,5-dimethylbenzene is not a natural product and not listed in the *Dictionary of Natural Products* (Dictionary of Natural Products 2017)

[d]This component is likely incorrect; methyl cycloundecane carboxylate is not known to be a natural product and is not listed in the *Dictionary of Natural Products* (Dictionary of Natural Products 2017)

[e]This component is likely incorrect; dihydrolinalool is not known to be a natural product and is not listed in the *Dictionary of Natural Products* (Dictionary of Natural Products 2017)

to as *Boswellia carteri* and occasionally *Boswellia bhau-dajiana*, *Boswellia undocrenulata*, and *Boswellia thurifera*, due both to historical placement as separate species and to some differences in growth form. However, while there may be some differences in the resin chemotypes produced in the African versus Arabian populations (Woolley et al. 2012), it is generally recognized as a single species. The resin contains nonvolatiles like boswellic acid and incensole, while the essential oil is primarily composed of α-pinene, α-thujene, limonene, sabinene, myrcene, β-caryophyllene, and *p*-cymene (Hamm et al. 2005; Camarda et al. 2007; Al-Harrasi and Al-Saidi 2008; Suhail et al. 2011; Woolley et al. 2012; Niebler and Buettner 2015). However, chemotypes in the literature vary widely, with (*E*)-β-ocimene-, methoxydecane-, and octyl acetate-dominant chemotypes reported (Basar 2005; Marongiu et al. 2006; Al-Harrasi and Al-Saidi 2008; Satyal and Pappas 2016). Much of this may be due to differential geography, environment, and tree management, although some variation is likely due to misidentification of the source trees and lack of testing resins directly collected from identified trees (Basar 2005; Marongiu et al. 2006). The resin has been traded for thousands of years throughout Egypt, the Middle East, and Europe, originally via Egyptian, Axumite, and then Nabatean trade routes (Tyldesley 1998; Hull 2008). It has been suggested that the psychoactive properties of the resin may have contributed to religiosity in ancient Judea and other places (Dannaway 2010). The resin was used throughout the ancient world to dress wounds, treat inflammation, for oral health, and as a perfume and deodorizer (Groom 1981; Hameed 1983; Price et al. 2016; Acıduman et al. 2017). Frankincense was also used, along with myrrh, in ancient Egypt to embalm bodies (Groom 1981). Whether the source of Egyptian frankincense was *B. papyrifera* or *B. sacra* (or both) is still debated; however there is some evidence that *B. sacra* was used (Archier and Vieillescazes 2000; Hamm et al. 2005).

Boswellia frereana Birdw. is endemic to Somalia, distributed from the mid-Somaliland coast to the tip of Cape Guardafui (Thulin and Warfa 1987). The trees inhabit primarily lowland coastal areas, although they are occasionally found as high as 1000 m (Thulin 1999). The oil is primarily composed of α-thujene, α-pinene, sabinene, *p*-cymene, and dimers of phellandrene (Basar 2005; Hamm et al. 2005; Niebler and Buettner 2015). The resin has traditionally been sold as chewing gum or decoration to Arab states; due to the large tears of resin the trees produce, it is considered the best variety of frankincense in Arabia (Thulin 1999).

Boswellia papyrifera Hochst. grows primarily in Ethiopia, Eritrea, and Sudan. *B. papyrifera* is notable for the particularly high levels of incensole and incensole acetate, psychoactive components, which occur in the resin (Hamm et al. 2005; Niebler and Buettner 2015). The oil is dominated by octyl acetate and to a much lesser degree octanol (Dekebo et al. 1999; Camarda et al. 2007; Bekana et al. 2014). The species is likely one of those traded to ancient Egypt by the Land of Punt, given the likely location of Punt in the Eritrea-Somalia corridor (Kitchen 1971; Phillips 1997). Traditionally the leaves and roots have been used medically to treat lymphadenopathy, while the bark is chewed to settle the stomach (Fichtl and Addi 1994; Gebrehiwot et al. 2003). Resin is burned to keep away mosquitoes, as well as chewed to quench thirst (Gebremedhin 1997).

Boswellia serrata Roxb. grows primarily in India where it is extensively tapped and propagated (Gupta et al. 2017). *Boswellia serrata* is the primary source of commercially extracted boswellic acid, and while it is commonly distilled into essential oil, the oil is typically considered less desirable than *B. sacra* due to its high levels of α-thujene and estragole (methyl chavicol), with lower levels of α-pinene, sabinene, myrcene, *p*-cymene, limonene, β-bourbonene, methyl eugenol, and kessane (Camarda et al. 2007; Singh et al. 2007; Gupta et al. 2017). It is used extensively in Ayurvedic medicine for arthritis, asthma, Crohn's Disease, and a variety of inflammatory ailments (Frawley and Lad 2001).

Other species have been investigated to a lesser degree, partially due to more limited commercial interest. *Boswellia neglecta* S. Moore inhabits arid areas across Somalia, Ethiopia, Kenya, Tanzania, and Uganda (Eslamieh 2017). The resin oil typically contains a mixture of α-pinene (20–50%), α-thujene (10–20%), and terpinen-4-ol (5–30%) (Başer et al. 2003; Basar 2005; Van Vuuren et al. 2010; Bekana et al. 2014). *Boswellia rivae* Engl. is found in the same areas, and its resin oil is composed of limonene (10–30%), δ-3-carene (5–15%), *p*-cymene (5–20%), and α-pinene (5–30%) (Başer et al. 2003; Basar 2005; Camarda et al. 2007; Schillaci et al. 2008; Bekana et al. 2014). There has been only limited work on the remaining species, including *Boswellia ameero*, *B. bullata*, *B. dalzielii*, *B. dioscoridis*, *B. elongata*, *B. nana*, *B. ovalifoliolata*, *B. pirottae*, *B. popoviana*, and *B. socotrana* (Başer et al. 2003; Kubmarawa et al. 2006; Ali et al. 2008; Mothana et al. 2011; Lebaka et al. 2015; Kohoude et al. 2017; Benelli et al. 2017; Maděra et al. 2017). There are no published studies, to our knowledge, on three species: *Boswellia globosa*, *B. microphylla*, and *B. ogadensis*.

4.2.2.1 Traditional Uses of *Boswellia*

Historical uses of frankincense from a medicinal perspective are varied and numerous. Some of the ailments treated by frankincense included coughing, vomiting, gastrointestinal issues, ulcers, arthritis, and other various inflammatory diseases, just to name a few of the traditional remedies of frankincense (Ammon 2008).

Oral Uses Frankincense resin has been chewed as a gum in many places. In the Arab states, the resin of *Boswellia frereana*, endemic to Somalia/Somaliland, is particularly prized. Somalis also chew the resin locally (Thulin 1999). In Soqotra, the resin from several species is chewed locally for oral hygiene due to its antiseptic qualities (Mies et al. 2000). In Egypt, fumigation with both frankincense and myrrh was believed to treat toothaches (Acıduman et al. 2017). *Boswellia papyrifera* resin is chewed in Ethiopia to quench thirst, while the bark is chewed to settle stomach issues (Gebremedhin 1997; Gebrehiwot et al. 2003).

Treatment of Wounds Frankincense resin has been sprinkled over open wounds, particularly those on the head, to prevent hemorrhaging (Price et al. 2016). Taken

with leek juice, it was thought to have a similar effect internally (Michie and Cooper 1991). It is also used in Kenya to dress wounds (Moussaieff and Mechoulam 2009).

Psychoactive Effects Sources from both the Middle East and Ethiopia mention the resin's calming effect, leading to it being used as a tranquilizer (Getahon 1976; Epstein 1990). The psychoactive properties of the resin were well known: frankincense was key to many religious ceremonies, likely acting as an entheogen and mild narcotic (Dannaway 2010). Ibn Sina (Avicenna, Persia) mentions it as being beneficial for amnesia and amentia (Hameed 1983). Indian Ayurvedic medicine also acknowledges its impact on the nervous system (Frawley and Lad 2001).

Inflammatory Conditions Frankincense is used in Chinese traditional medicine for inflammatory diseases, to control pain and swelling (Shen and Lou 2008), while Ibn Sina discussed its use in inflammation as well (Hameed 1983). Frankincense is used extensively for inflammatory conditions in the Ayurvedic system, including for Crohn's disease, arthritis, and asthma (Frawley and Lad 2001).

Assorted Other Medicinal Uses *Boswellia* have been used for a variety of other ailments as well. *Boswellia dalzielii* bark in West Africa is used for rheumatism, septic sores, venereal diseases, and gastrointestinal issues (Burkill 2000; Evans 2009). Frankincense is used as a cancer/antitumoral therapy in Chinese traditional medicine (Shen and Lou 2008), for leprosy (Tucker 1986), as well as to reduce fever in Ethiopia (Fichtl and Addi 1994). In Egypt, frankincense was highly prized as a fumigant and deodorizer. It was used in embalming bodies, as was its sister resin, myrrh (Pickenhagen 2017).

4.2.2.2 Ecology of *Boswellia*

The 19 species of *Boswellia* are broadly distributed across the lower elevation (0–1500 m) frost-free tropics of Africa, Arabia, and the Indian subcontinent. Most species inhabit arid environments, but a small number *(B. dalzielii*, *B. serrata*, *B. ovalifoliolata*) require larger amounts of water and are found in the humid tropics; however, many arid tropical specialists inhabit areas with significant levels of mist or oceanic fog, which likely provides a large degree of their total water intake (Somalia/Somaliland, Oman, Soqotra) (Thulin and Warfa 1987; Attorre et al. 2011; Eslamieh 2017).

While many *Boswellia* are capable of growing on a variety of substrates, a subset of the genus is specifically lithophilic, dwelling primarily on rocks and most often cliffs. The most specialized cliff-dwellers are found in Soqotra (*B. nana*, *B. bullatta*, *B. dioscordis*, *B. popoviana*). Soqotra represents a particularly interesting case, as at least seven species of *Boswellia* have evolved in a relatively small area, four of which specialize on cliffs and three of which are found in flat areas (Mies et al. 2000; Miller and Morris 2004). The reasons for this divergence in specialization are

not well understood. Other species, such as *B. sacra* and *B. frereana* in Somalia and *B. papyrifera* in Ethiopia/Eritrea, may grow on either soil or on rock, flat, or cliff areas (Thulin and Warfa 1987; Thulin 1999; Eshete et al. 2005). *Boswellia sacra* in Somalia and *B. frereana* especially seem to specialize on jagged rocks, germinating in small crevices filled with water and detritus and then forming swollen, disk-shaped base to "sucker" themselves onto the rock (Thulin and Warfa 1987). *Boswellia* trees are often reported as growing on limestone, while this may certainly be true, in Somalia/Somaliland and Oman, they seem to prefer a layer of volcanic rock overlying the limestone (DeCarlo, pers. obs.).

Members of the genus rarely exceed 10–12 m in height, even under ideal conditions, although the growth form is highly dependent on the environment, and some species occasionally reach up to 20 m (Thulin and Warfa 1987; Lemenih and Kassa 2011; Eslamieh 2017). *Boswellia sacra*, for instance, is highly variable morphologically throughout Somalia and Arabia; a Somali coastal population was observed to have distinct trunks, swollen bases, barely undulating leaves, and panicle inflorescences. By contrast, a population in the interior featured a non-swollen base from which the trees branched directly, distinctly undulating leaves and racemes. This has led to multiple species being described, but the presence of varying intermediates between extremes has led to classification as a single species (Thulin and Warfa 1987). *Boswellia* also hybridize readily, leading to further confusion about species distinctions (Eslamieh 2017).

Like the majority of the Burseraceae, *Boswellia* trees have resin canals in their inner bark that contain a complex terpenoid gum-oleoresin known commercially as frankincense. The gum-oleoresin protects the trees from both disease and boring insects, which are a major cause of mortality (Langenheim 2003; Eshete 2011; Tolera et al. 2013).

The trees are deciduous; some species flower without leaves, while others flower while in leaf (Thulin and Warfa 1987; Mies et al. 2000; Lemenih and Kassa 2011). The variation in floral coloration across species suggests possible variation in pollination syndrome. However, this has been poorly studied. *Boswellia papyrifera*, *B. sacra*, *B. serrata*, and *B. ovalifoliolata* appear to be pollinated primarily by bees and to a lesser extent by wasps, ants, flies, and butterflies (Sunnichan et al. 2005; Lippi et al. 2011; Raju et al. 2012). *Boswellia ovalifoliolata* may also be facultatively ornithophilous, and *B. ameero* on Soqotra Island seems to specifically attract sunbirds (Mies et al. 2000; Raju et al. 2012). *Boswellia* trees are anemochorous, producing large sets of winged seeds. Fruits are non-fleshy, sometimes dehiscent pods with three to five seeds (Thulin and Warfa 1987; Raju et al. 2012; Eslamieh 2017).

Many *Boswellia* populations that have been assessed in recent years show some degree of threat and/or decline (Farah 2008; Attorre et al. 2011; Alaamri 2012; Groenendijk et al. 2012). Declines are caused by a variety of factors, frequently including uncontrolled ungulate grazing particularly by livestock such as goats which kills seedlings and in several cases has completely blocked natural regeneration of the trees (Attorre et al. 2011; Groenendijk et al. 2012). High levels of tapping for resin deplete tree resources resulting in less reproductive effort;

fewer fruits, flowers, and seeds; reduced germination success; and overall reduction in foliage production, annual carbon gain, and carbon stock (Rijkers et al. 2006; Mengistu 2011; Eshete et al. 2012; Mengistu et al. 2012). Tapping also opens wounds and depletes the amount of resin available to fight off microbial and arthropod attack, possibly increasing mortality from natural enemies. Fire, cutting for wood, and land clearing for other activities further increase adult mortality (Groenendijk et al. 2012). Climate change may also pose a significant threat, though this has not been well-investigated.

Due to the anthropogenic nature of the threats, cliff-dwelling *Boswellia* seem to do better than species in flat areas (Attorre et al. 2011), with less grazing and a higher level of regeneration. In species in which some individuals are located on cliffs and others in flat areas, cliff-dwelling populations may act as genetic reserves, with limited tapping and grazing, and higher regeneration (Attorre et al. 2011; Eshete et al. 2012; DeCarlo, pers. obs.).

4.2.2.3 Chemical Ecology of *Boswellia*

Plants produce secondary metabolic chemical compounds for a variety of reasons: defense against pathogens, discouragement of herbivory, signals to conspecifics, etc. (Pichersky and Raguso 2018). The specific chemicals involved are determined both by evolutionary history (biosynthetic pathways available) and the use of the compound. In other words, the chemicals should be adaptive to the specific needs, such as local pathogens.

Boswellia chemical ecology is not well understood. All *Boswellia* produce a wide variety of terpenes, approximately 340 so far identified, most isolated from the trees' resin (Mertens et al. 2009). Most species show some combination of α-thujene, α-pinene, myrcene, sabinene, *p*-cymene, limonene, δ-3-carene, and β-caryophyllene, in quantities that vary both inter- and intraspecifically. By contrast, *B. papyrifera* shows a unique chemotype, dominated by octyl acetate (Başer et al. 2003; Hamm et al. 2005; Camarda et al. 2007; Bekana et al. 2014; Niebler and Buettner 2016). As *B. papyrifera* is sympatric with several other *Boswellia* species, the differential chemistry is curious – although it shows similar antimicrobial properties as other *Boswellia* resins (Camarda et al. 2007; Mertens et al. 2009).

There is considerable intraspecific variation in chemotypes as well, perhaps most apparent in *Boswellia sacra*. *Boswellia sacra* shows several distinct chemotypes, even within similar geographic areas (Gollis mountains, Somalia/Somaliland, for instance). Chemotypes include α-pinene dominant, α-thujene dominant, limonene dominant, and (*E*)-β-ocimene dominant (Al-Harrasi and Al-Saidi 2008; Mertens et al. 2009; DeCarlo, pers. obs.). An octyl acetate chemotype has been reported as coming from *B. sacra* in Ethiopia; however this is geographically and chemically more likely a misidentification of *B. papyrifera* (Basar 2005; Marongiu et al. 2006; Eslamieh 2017). In addition, a very unusual methoxydecane chemotype has been recently reported from Somalia, in which the oil is composed of two-thirds methoxydecane (Satyal and Pappas 2016). The appearance of this new chemotype

may be related to hybridization or long-term stress due to harvesting and/or environmental conditions.

The reason for the diversity of phytochemicals has been a topic of debate for many years. Although efforts have been made to elucidate the function of individual compounds, this is difficult considering the coexisting diversity (Berenbaum and Zangerl 2008; Pichersky and Raguso 2018). A possible explanation is that functional effects arise out of the interactive effects of the diverse compounds (Moussaieff and Mechoulam 2009). Another explanation is that the majority of the compounds are vestiges of an evolutionary arms race between plants and herbivores, and it is the most recently evolved compounds that are adaptive (Ehrlich and Raven 1964; Becerra et al. 2009; Pichersky and Raguso 2018). This argument may have some merit considering that although *Boswellia* resin may have hundreds of compounds, typically only a few appear in large amounts.

While a small number of compounds may be efficacious for any one purpose, maintaining chemical diversity may be adaptive by providing ready resources to deal with an array of natural enemies (Firn and Jones 2003; Richards et al. 2015). This is supported by the fact that *B. serrata*, *B. sacra*, and *B. rivae* contain most of the same compounds, although in different levels, and the three oils deal best with different microbial pathogens. For instance, *B. serrata* and *B. carteri* were far more effective against *Pseudomonas aeruginosa* than *B. rivae*, but the latter was far more effective against *Escherichia coli* than the former (Camarda et al. 2007). Thus, the varying chemotypes may represent adaptation to local threats. This variation manifests on both broad and fine spatial scales: Even trees adjacent to each other may present unique chemotypes (DeCarlo, pers. obs.), suggesting that adaptation is both general and in response to contact with specific pathogens.

4.2.3 The Genus Commiphora

The genus *Commiphora* comprises between 150 and 200 species of resiniferous shrubs or trees characterized by peeling bark, soft wood, small leaves, production of aromatic gum-oleoresin, and a tendency toward pachycauly (Thulin 1999; Mahr 2012). *Commiphora* species are broadly distributed across tropical and subtropical areas of sub-Saharan Africa, Madagascar, Arabia, across to Iran, Pakistan, and India. A single species, *C. leptophloeos*, is found in southeastern Brazil (Mahr 2012). *Commiphora* produce aromatic gum-oleoresins, the most common of which are known as myrrh and opopanax (Tucker 1986; Thulin and Claeson 1991). The resin has been used religiously and medicinally throughout the ancient world including ancient Greece, Egypt, China, and in the Middle East (Hanuš et al. 2005; Pickenhagen 2017). The resin functions as protection for the trees by sealing wounds and aiding in defense against invasive insects, disease, herbivory, and infection (Langenheim 2003; Pichersky and Raguso 2018). It has been traditionally utilized to treat gastrointestinal diseases, inflammatory disease, fractures, obesity, blood stagnation, and as an analgesic (Shen et al. 2012). Today it is a valuable commodity

for its use in aromatherapy and as a perfume additive (Shen et al. 2012). The essential oil compositions and biological activities of the *Commiphora* species are summarized in Table 4.3.

Commiphora myrrha (T. Nees) Engl./*Commiphora molmol* (Engl.) Engl. ex Tschirch predominantly grow in the dry forests of Africa, India, and the Arabian Peninsula (Eslamieh 2016). The resin oil chemistry is variable but often contains furanoeudesma-1,3-diene (940%), lindestrene (6–15%), curzerene (15–40%), and sometimes 2-acetoxyfuranodiene (6–10%) (Başer et al. 2003; Morteza-Semnani and Saeedi 2003; Marongiu et al. 2005; Hanuš et al. 2008; Nikolić et al. 2016). The resin has traditionally been used for controlling inflammation and pain, as well as treatment of blood stagnation, dermatological care, and treatment of trauma (Shen and Lou 2008; Shen et al. 2012). The resin has been shown to have antimicrobial, antiviral, anti-inflammatory, and analgesic effects (Hammer et al. 1999; Ali 2007; Shen and Lou 2008; Shen et al. 2012; Adam and Selim 2013).

Commiphora mukul (Hook. ex Stocks) Engl. [syn. *Commiphora wightii* (Arn.) Bhandari] grows predominantly in India and Pakistan (Eslamieh 2016). The oil contains curzerene, furanoeudesma-1,3-diene, lindestrene, and curzerenone (Saeed and Sabir 2004). It is predominantly used in Indian and Arabian traditional medicine for its anti-inflammatory, anticoagulant, and antibacterial properties, as well as for atherosclerosis (Sarup et al. 2015; Ur Rehman et al. 2017). It has also been noted to treat bone fractures, arthritis, cardiovascular disease, and lipid disorders (Shen et al. 2012).

Commiphora guidotii Chiov., a source of opopanax, is endemic to Ethiopia and Somalia (Gebrehiwot et al. 2015) where it grows on rocky slopes between 70 and 800 m (Thulin and Claeson 1991). The essential oil contains α-santalene (15–20%), (*E*)-β-ocimene (6–30%), and (*Z*)-α-bisabolene (20–30%) as major constituents (Craveiro et al. 1983; Başer et al. 2003; Gebrehiwot et al. 2015; Yeo et al. 2016). In Somalian traditional medicine, it is mostly used to treat stomach ailments, wounds, and diarrhea (Shen et al. 2012). In Ethiopia, the resin is sometimes fed to cattle to improve dairy production (Gebrehiwot et al. 2015).

Commiphora africana (A. Rich.) Engl. is widely distributed across sub-Saharan Africa. The oil consists of bisabolone, β-sesquiphellandrene, curcumenes, and α-oxobisabolene (Ayédoun et al. 1998; Avlessi et al. 2005). In many African countries, the plant is used for cancer treatment, malaria, and inflammatory disease (Compaoré et al. 2016). It is also commonly used in Nigeria for removing tapeworms from the body and Uganda for treating wounds (Shen et al. 2012).

4.2.3.1 Traditional Uses of *Commiphora*

Commiphora exudates have been used for their therapeutic, religious, and medicinal values throughout the ancient world, including Rome, Greece, China, Babylon, and India for at least 3000 years (Shen et al. 2012; Pickenhagen 2017). *Commiphora* products are also used locally where they occur for a variety of medicinal purposes. Myrrh was a highly valuable commodity in the ancient world, where it was used

Table 4.3 Chemical compositions and biological activities of *Commiphora* essential oils

Commiphora species	Collection site	Essential oil	Major components (>5%)	Bioactivity of EO	Reference
C. africana (A. Rich.) Engl.	Abomey-Calavi, Benin	Leaf (HD)	(*Z*)-γ-Bisabolene (10.0%), α-oxobisabolene (61.6%)	–	Ayédoun et al. (1998)
	Bohicon, Benin	Leaf (HD)	*ar*-Curcumene (8.2%), γ-curcumene (6.7%), β-bisabolene (5.2%), β-sesquiphellandrene (19.1%), (6*S*,7*R*)-bisabolone (38.4%)	–	Avlessi et al. (2005)
C. erythraea Engl.	Commercial (Agarsu Liben Cooperative)	Oleoresin (HD)	1(10),4-Furanodien-6-one (21.5%), 1,10(15)-furanogermacradien-6-one (14.3%), 3*R*-methoxy-4*S*-furanogermacra-1*E*,10(15)-dien-6-one (7.4%)	–	Marcotullio et al. (2009)
	Commercial (Agarsu Liben Cooperative)	Oleoresin (HD)	Camphene (8.2%), β-elemene (8.2%), α-gurjunene (6.0%), 1(10),4-furanodien-6-one (9.0%)	Antifungal (*Alternaria solani*, MIC 3000 μg mL^{-1}; *Fusarium culmorum*, MIC 5500 μg mL^{-1}; *Phytophtora cryptogea*, MIC 5500 μg mL^{-1})	Fraternale et al. (2011)
	Commercial (Agarsu Liben Cooperative)	Oleoresin (SD)	β-Elemene (5.4%), 1(10),4-furanodien-6-one (20.6%), 1,10(15)-furanogermacradien-6-one (10.4%)	–	Fraternale et al. (2011)
C. gileadensis (L.) C. Chr. [syn. *C. opobalsamum* (L.) Engl.]	Makkah, Saudi Arabia	Aerial parts (HD)	Terpinen-4-ol (8.5%), δ-cadinene (5.0%), α-calacorene (9.4%)	Cytotoxic (SK-Mel, IC_{50} 97 μg mL^{-1}; KB, IC_{50} 70 μg mL^{-1}; BT-549, IC_{50} 48 μg mL^{-1}; SK-OV3, IC_{50} 82 μg mL^{-1})	Al-Massarany et al. (2007)
	Ein Gedi Botanical Garden, Israel	Aerial parts (HD)	α-Pinene (7.2%), sabinene (21.1%), β-caryophyllene (20.1%), germacrene D (19.6%), terpinen-4-ol (5.3%)	Cytotoxic (BS-24-1 mouse lymphoma cells, MoFir human B lymphocytes)	Amiel et al. (2012)

	Almog, Dead Sea, Israel	Aerial parts (HD)	α-Pinene (18.4%), sabinene (29.1%), β-pinene (8.2%), *p*-cymene (6.4%), γ-terpinene (5.9%), terpinen-4-ol (6.9%)	–	Dudai et al. (2017)
	Ein Gedi Botanical Garden, Israel	Fruit (HD)	α-Pinene (15.9%), sabinene (35.9%), β-pinene (18.0%), *p*-cymene (5.3%), limonene (6.2%), terpinen-4-ol (5.3%)	–	Dudai et al. (2017)
C. gileadensis (L.) C. Chr. [syn. *C. opobalsamum* (L.) Engl.]	Ein Gedi Botanical Garden, Israel	Leaf (HD)	α-Pinene (11.2%), sabinene (15.8%), β-pinene (5.8%), *p*-cymene (8.4%), γ-terpinene (5.8%), terpinen-4-ol (18.5%)	–	Dudai et al. (2017)
	Ein Gedi Botanical Garden, Israel	Stem (HD)	α-Pinene (15.4%), sabinene (28.5%), β-pinene (7.5%), γ-terpinene (8.1%), terpinen-4-ol (11.6%)	–	Dudai et al. (2017)
C. guidottii Chiov.	South-eastern Somalia	Oleoresin (SD)	α-Santalene (major), β-santalene, *epi*-β-santalene, β-bergamotene, (*E*)-β-farnesene, α-bisabolene (major), β-bisabolene, furanodiene (major) (percentages not reported)	–	Craveiro et al. (1983)
	Bulagere, Ogaden region, Ethiopia	Oleoresin (HD)	(*E*)-β-Ocimene (33.0%), α-santalene (15.8%), β-*trans*-bergamotene (6.6%), α-*cis*-bisabolene (22.2%)	–	Başer et al. (2003)

(continued)

Table 4.3 (continued)

Commiphora species	Collection site	Essential oil	Major components (>5%)	Bioactivity of EO	Reference
	Ogaden region, eastern Ethiopia	Oleoresin (SD)	(*E*)-β-Ocimene (6.7%), α-santalene (19.5%), α-*trans*-bergamotene (9.3%), curcerene (11.4%), furanoeudesma-1,3-diene (18.6%), isofuranodiene (6.8%)	Antibacterial (*Bacillus pubilus*, MIC 50 μg mL^{-1}; *Bacillus subtilis*, MIC 100 μg mL^{-1}; *Escherichia coli*, MIC 25 μg mL^{-1}; *Salmonella typhi*, MIC 10 μg mL^{-1}; *Shigella boydii*, MIC 50 μg mL^{-1}; *Shigella dysenteriae*, MIC 50 μg mL^{-1}; *Shigella flexneri*, MIC 50 μg mL^{-1}; *Shigella soneii*, MIC 50 μg mL^{-1}; *Staphylococcus aureus*, MIC 25 μg mL^{-1}; *Vibrio cholerae*, MIC 10 μg mL^{-1}). Antifungal (*Aspergillus niger*, MIC 1000 μg mL^{-1}; *Candida albicans*, MIC 400 μg mL^{-1}; *Penicillium funiculosum*, MIC 1000 μg mL^{-1}; *Penicillium notatum*, MIC 1000 μg mL^{-1})	Gebrehiwot et al. (2015)

C. guidottii Chiov.	Commercial (Sigma-Aldrich)	Oleoresin (SD)	(*E*)-β-Ocimene (11.5%), α-santalene (21.9%), trans-α-bergamotene (9.0%), (*Z*)-α-bisabolene (27%), β-bisabolene (5.1%)	β-Bisabolene cytotoxic (human breast tumor cell lines, MCF7, IC_{50} 66.9 μg mL^{-1}; MCF10A, IC_{50} 114 μg mL^{-1}; MDA-MB-231, IC_{50} 98.4 μg mL^{-1}; SKBR3, IC_{50} 70.6 μg mL^{-1}; BT474, IC_{50} 74.3 μg mL^{-1}); cytotoxicity due to induction of apoptosis	Yeo et al. (2016)
C. habessinica (O. Berg) Engl.	Hujarriyah district, Taiz province, Yemen	Oleoresin (HD)	β-Elemene (32.1%), α-selinene (18.9%), cadina-1,4-diene (7.5%)	–	Awadh Ali et al. (2009)
C. holtziana ssp. *holtziana* Engl.	Marsabit district, northern Kenya	Oleoresin (hexane extract)	δ-Elemene (16.7%), β-bourbonene (20.8%), calarene (5.7%), (+)-germacrene D (11.6%)	Acarine (*Rhipicephalus microplus*, *Dermanyssus gallinae*) repellent	Birkett et al. (2008)
C. kua Vollesen	Isolo district, Kenya	Oleoresin (SD)	α-Thujene (22.4%), α-pinene (44.3%), sabinene (5.2%), β-pinene (10.0%), *p*-cymene (28.7%), limonene (5.4%)	–	Manguro et al. (1996)
	Soqotra Island, Yemen	Oleoresin (HD)	δ-Cadinene (17.0%), γ-cadinene (22.5%), α-cadinol (33.0%)	Antifungal, disk diffusion assay (*Cladosporium cucumerinum*)	Awadh Ali et al. (2008)
	Soqotra Island, Yemen	Oleoresin (MeOH extract)	δ-Cadinene (10.0%), α-cadinol (35.2%), α-eudesmol (12.3%)	–	Maděra et al. (2017)

(continued)

Table 4.3 (continued)

Commiphora species	Collection site	Essential oil	Major components (>5%)	Bioactivity of EO	Reference
C. leptophloeos (Mart.) J.B. Gillett	Catimbau National Park, Pernambuco state, Brazil	Leaf (HD)	α-Phellandrene (26.3%), β-phellandrene (12.9%), β-caryophyllene (18.0%), α-humulene (5.5%), germacrene D (6.0%)	Oviposition deterrent (*Aedes aegypti*, 59% reduction at 25 μg mL^{-1}); larvicidal (*Aedes aegypti*, LC_{50} 99.4 μg mL^{-1}); activity due to β-caryophyllene and α-humulene	Da Silva et al. (2015)
C. molmol (Engl.) Engl. ex Tschirch (syn. *C. myrrha var. molmol* Engl.)	Shiraz, Fars province, Iran	Oleoresin (HD)	β-Elemene (8.4%), curzerene (40.1%), furanoeudesma-1,3-diene (15.0%), acetoxyfuranodiene (6.5%)	–	Morteza-Semnani and Saeedi (2003)
	Commercial (market in Baghdad, Iraq)	Oleoresin (HD)	–	Antifungal, disk diffusion assay (*Aspergillus niger*, *Aspergillus flavus*)	Ali (2007)
C. molmol (Engl.) Engl. ex Tschirch (syn. *C. myrrha* var. *molmol* Engl.)	Commercial (El-Captain Company, Egypt	Oleoresin (HD)	Limonene (12.3%), benzyl alcohol (5.6%), carvone (21.1%)	Larvicidal (*Culex pipiens*, LC_{50} 0.99 μL mL^{-1})	Habeeb et al. (2009)
	Commercial (market in Al Jouf, Saudi Arabia)	Oleoresin (HD)	–	Antibacterial (*Bacillus cereus*, MIC 250 μg mL^{-1}; *Bacillus subtilis*, MIC 250 μg mL^{-1}; *Staphylococcus aureus*, MIC 100 μg mL^{-1}; *Escherichia coli*, MIC 250 μg mL^{-1}; *Klebsiella pneumoniae*, MIC 50 μg mL^{-1})	Adam and Selim (2013)

	Commercial (Tamar Ltd., Israel)	Oleoresin (EtOH extract)	Isofuranogermacrene (6.7%), furanoeudesma-1.3-diene (9.0%), 2-acetoxyfuranodiene (9.8%)	–	Hanuš et al. (2008)
	Commercial (Pamir Ltd., Israel)	Oleoresin (EtOH extract)	Isofuranogermacrene (17.9%), furanoeudesma-1.3-diene (20.6%), lindestrene (6.2%), 2-methoxyfuranodiene (7.3%), 2-acetoxyfuranodiene (8.8%)	–	Hanuš et al. (2008)
C. myrrha (T. Nees) Engl.	Commercial (Améo, Zija International)	Oleoresin (HD)	α-Pinene (6.8%), neryl acetate (6.3%), curzerene (16.1%), furanoeudesma-1,3-diene (18.1%), lindestrene (6.9%)	–	Setzer (unpublished)
	Somaliland	Oleoresin (HD)	β-Elemene (20.2%), curzerene (23.7%), furanoeudesma-1,3-diene (24.6%), lindestrene (6.7%)	–	Satyal (unpublished)
	Commercial (Sunspirit Oils Pty. Ltd., Australia)	Oleoresin (SD)	–	Antibacterial (*Enterococcus faecalis*, MIC 2500 μg mL^{-1}; *Staphylococcus aureus*, MIC 5000 μg mL^{-1})	Hammer et al. (1999)
	Bulagere, Ogaden region, Ethiopia	Oleoresin (HD)	β-Elemene (8.7%), furanodiene (19.7%), furanoeudesma-1,3-diene (34.0%), lindestrene (12.0%)	–	Başer et al. (2003)
	Ethiopia	Oleoresin (HD, SD)	Curzerene (17.5%, 14.7%), furanoeudesma-1,3-diene (38.6%, 33.5%), lindestrene (14.4%, 13.1%)	–	Marongiu et al. (2005)

(continued)

Table 4.3 (continued)

Commiphora species	Collection site	Essential oil	Major components (>5%)	Bioactivity of EO	Reference
C. myrrha (T. Nees) Engl.	Commercial (Harraz Herbs Co., Cairo, Egypt)	Oleoresin (HD)	Analysis in doubt	Antimicrobial (*Bacillus circulans*, MIC 600 μg mL^{-1}; *Bacillus subtilis*, MIC 200 μg mL^{-1}; *Escherichia coli*, MIC 100 μg mL^{-1}; *Pseudomonas aeruginosa*, MIC 200 μg mL^{-1}; *Streptococcus faecalis*, MIC 100 μg mL^{-1}; *Saccharomyces cerevisiae*, MIC 100 μg mL^{-1})	Mohamed et al. (2014)
	Commercial (Sensient Essential Oils, Germany)	Oleoresin (HD)	Curzerene (34.7%), furanoeudesma-1,3-diene (32.8%), lindestrene (10.2%)	Weakly antifungal (*Candida albicans*, MIC 2500 μg mL^{-1})	Nikolić et al. (2016)
C. ornifolia J.B. Gillett	Soqotra Island, Yemen	Bark (HD)	*endo*-Fenchol (15.5%), camphor (27.3%), caryophyllene oxide (6.5%), thunbergol (6.4%)	Antibacterial, broth dilution assay (*Bacillus subtilis*, MIC 400 μg mL^{-1}; *Staphylococcus aureus*, MIC 810 μg mL^{-1})	Mothana et al. (2010)
	Soqotra Island, Yemen	Oleoresin (MeOH extract)	α-Thujene (14.5%), terpinen-4-ol (10.6%), β-caryophyllene (8.2%), α-humulene (24.8%)	–	Maděra et al. (2017)
C. parvifolia Engl.	Soqotra Island, Yemen	Bark (HD)	Camphor (9.1%), caryophyllene oxide (14.2%), β-eudesmol (7.7%), bulnesol (5.7%), palmitic acid (18.4%), phytol (5.8%)	–	Mothana et al. (2010)

	Soqotra Island, Yemen	Oleoresin (MeOH extract)	Limonene (10.9%), α-ylangene (7.8%), α-copaene (9.2%), phytol (15.7%)	–	Maděra et al. (2017)
C. planifrons Engl.	Soqotra Island, Yemen	Oleoresin (MeOH extract)	δ-3-Carene (9.3%), *p*-cymene (13.5%), *cis*-verbenol (8.8%), terpinen-4-ol (20.4%), α-eudesmol (7.0%)	–	Maděra et al. (2017)
C. pyracanthoides Engl.	Ethiopia	Oleoresin (HD)	Analysis in doubt	Cytotoxic (MCF-7, IC_{50} 19.8 μg mL^{-1}; HepG2, IC_{50} 39.2 μg mL^{-1}; HeLa, IC_{50} 34.3 μg mL^{-1}; HS-1, IC_{50} 22.7 μg mL^{-1}; A459, IC_{50} 41.4 μg mL^{-1})	Chen et al. (2013)
C. socotrana (Balf. f.) Engl.	Soqotra Island, Yemen	Oleoresin (MeOH extract)	*p*-Cymene (6.7%), limonene (5.5%), viridiflorol (8.8%), α-eudesmol (35.4%)	–	Maděra et al. (2017)
C. tenuis Vollesen	Filtu, Sidamo region, Ethiopia	Oleoresin (SD)	α-Thujene (8.9%), α-pinene (60.8%), sabinene (6.3%), β-pinene (8.8%), limonene (5.5%)	Antibacterial, broth dilution assay (*Staphylococcus aureus*, MIC 500 μg mL^{-1}; MRSA, MIC 500 μg mL^{-1})	Asres et al. (1998)

by wealthy families as an odorant and cosmetic (Groom 1981; Pickenhagen 2017). Myrrh, along with frankincense, was traded from the ancient Land of Punt, likely located in the Horn of Africa, to the Egyptian Empire from 2500 BC to 600 BC (Kitchen 1971; Phillips 1997). After 600 BC the primary trade routes shifted to Arabia (Hull 2008). In modern times, synthetic sources have replaced myrrh in some aromatic applications; however the resin is still used extensively in traditional medicine, especially in China (Northrup et al. 2005; Shen and Lou 2008). The most predominant pharmacological uses are as follows: anti-infection; anti-skin inflammation; painkiller; anti-sore; treat worm infestation, coughs, and wounds; and as a traditional cancer therapy (Lemenih and Teketay 2003; Singh et al. 2003; Reddy et al. 2009; Annu et al. 2010; Shen et al. 2012; Gebrehiwot et al. 2015).

Oral Uses *Commiphora* resin can be used in the treatment of infections such as oral ulcers (El Ashry et al. 2003). It also has long been used to treat symptoms of skin fungal infections, mouth ulcers, and gum diseases in traditional medicine in Iran (Mahboubi and Kashani 2016) and India (El Ashry et al. 2003). In some traditional Arab medicinal practices, the resinous exudates are used as a mouthwash (Ageel et al. 1987). In India, it is used to treat mouth ulcers, gingivitis, and skin infections (Karnick 1995; Frawley and Lad 2001; Lemenih and Teketay 2003).

Treatment of Wounds Myrrh can be applied to wounds and lesions due to its antiseptic properties (El Ashry et al. 2003; Walsh et al. 2010; Gebrehiwot et al. 2015). In India, a paste of the resin is applied to cracks in the feet (Reddy et al. 2009). *Commiphora guidottii* resin is applied topically to wounds in Somalia (Thulin and Claeson 1991), while *C. erythraea*, *C. kua*, and *C. habessinica* are used by the Borana people of southern Ethiopia for burns, wounds on cattle, and killing cattle ticks (Gemedo-Dalle et al. 2005). *Commiphora holtziana* is likewise used for ectoparasites in Kenya (Birkett et al. 2008).

Antitumoral Uses *Commiphora* resin has long been used in Arabian traditional medicine to treat tumors of the liver, stomach, breast, and head (Ageel et al. 1987; El Ashry et al. 2003; Amin and Mousa 2007; Evans 2009).

Treatment of Infections A decoction of the roots of *C. marlothii* is drunk daily to treat sexually transmitted infections in Zimbabwe (Chigora et al. 2007). *Commiphora africana* is used as a treatment for elephantiasis in Ethiopia (Tadesse and Demissew 1992). *Commiphora* resin is used in Arab traditional practice as an antiseptic and general antimicrobial, as well as to address stomach and bronchial complaints (Ageel et al. 1987; Brown 2001; Evans 2009; Iwu 2014).

Inflammatory Conditions Myrrh and frankincense are often prescribed together in traditional Chinese medicine for the treatment of inflammatory diseases and blood stagnation (Chen et al. 2013). This is due to their capability of breaking up

congealed blood and promoting blood circulation (Shen et al. 2012). In India, the leaves of *C. caudata* are used to reduce inflammation and pain (Annu et al. 2010), while the resin is used throughout Eastern Africa and Arabia for inflammation and rheumatism (Iwu 2014). The traditional uses of myrrh to treat inflammation are supported by in vivo anti-inflammatory screening of myrrh resin extracts in mice (Su et al. 2011).

Assorted Other Uses *Commiphora* resin and oil have been and are currently used for a myriad of other uses. In India, it is used to alleviate pain from bone fractures, cardiovascular disease, stomach aches, and the common cold (Shen et al. 2012). In Iran, myrrh is also used to protect women who are in labor against infection (Mahboubi and Kashani 2016). In the ancient cultures surrounding the Fertile Crescent, myrrh was primarily used for ointment, perfumes, and the embalming of Egyptian mummies (Northrup et al. 2005). *Commiphora* are also used to treat diarrhea and stomach ailments in Somalia (Thulin and Claeson 1991) and by Bapedi healers in South Africa (Semenya and Maroyi 2012).

4.2.4 The Genus Aucoumea

Aucoumea is a monotypic genus represented only by *A. klaineana* Pierre (Gabon mahogany), an important timber tree (Thulin et al. 2008). The oleoresin of this species is rich in tirucallane and oleanane triterpenoids (Tessier et al. 1982; Liang et al. 1988b). The essential oils derived from the oleoresin of *A. klaineana* are dominated by monoterpene hydrocarbons and show only weak antibacterial activity (see Table 4.4).

4.3 Tribe Canarieae

4.3.1 The Genus Canarium

The word *Canarium* is derived from the Malay word "Kanari." There are 77 species of the *Canarium* genus, mostly found in tropical Asia and the Pacific, but two species are found in tropical Africa (Mabberley 2008). Members of the *Canarium* genus represent medium to large trees up to 40–50 m in height or, rarely, shrubs (Mogana and Wiart 2011), and they are important sources of timber, food, oils, and traditional medicines (Thomson and Evans 2006).

Canarium schweinfurthii Engl. ranges in equatorial forest regions in tropical Africa and is used in various traditional medicinal practices (Tcheghebe et al. 2016). The leaves are used as stimulant, against malarial fever, postpartum pain, constipation, and diarrhea (Tcheghebe et al. 2016). Traditionally, the stem bark decoction is

Table 4.4 Chemical compositions and biological activities of *Aucoumea klaineana* essential oils

Aucoumea species	Collection site	Essential oil	Major components (>5%)	Bioactivity of essential oil	Reference
A. klaineana Pierre	Libreville, Gabon	Oleoresin (HD)	α-Pinene (20.6%), α-phellandrene (11.2%), *p*-cymene (30.2%), limonene (5.4%), α-terpineol (5.2%)	–	Liang et al. (1988a)
	Sebang Herbarium, Libreville, Gabon	Oleoresin (HD)	δ-3-Carene (72.3%), terpinolene (6.3%)	–	Koudou et al. (2009)
	Lolodorf, Cameroon	Oleoresin (HD)	α-Pinene (29.3%), α-phellandrene (30.9%), *p*-cymene (9.2%), 1,8-cineole (9.0%)	–	Dongmo et al. (2010)
	Mekuê forest, Mebane Endama, Oyem, Gabon	Oleoresin (HD)	–	Antibacterial, disk diffusion assay (*Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, MIC 10,000 μg mL^{-1})	Obame et al. (2014)
	Lolodorf, Cameroon	Oleoresin (HD)	α-Pinene (29.3%), α-phellandrene (30.9%), *p*-cymene (9.2%), 1,8-cineole (9.0%)	Not antifungal (*Aspergillus* spp.)	Ambindei et al. (2014)
	N'Toum, Estuaire province, Gabon	Oleoresin (headspace)	α-Pinene (5.9%), δ-3-carene (8.6%), α-phellandrene (63.4%), limonene (6.1%), β-phellandrene (5.9%), *p*-cymene (5.4%)	–	Medzegue et al. (2013)
	Cocobeach, Estuaire province, Gabon	Oleoresin (headspace)	α-Pinene (5.8%), δ-3-carene (40.1%), α-phellandrene (12.9%), limonene (16.5%), *p*-cymene (10.3%)	–	Medzegue et al. (2013)
	Bokoué, Estuaire province, Gabon	Oleoresin (headspace)	α-Pinene (5.3%), δ-3-carene (8.1%), limonene (24.1%), *p*-cymene (42.5%), α-terpineol (7.2%)	–	Medzegue et al. (2013)
	Bokoué, Estuaire province, Gabon	Oleoresin (headspace)	α-Pinene (5.4%), δ-3-carene (12.8%), α-phellandrene (19.6%), limonene (31.9%), *p*-cymene (9.9%), α-terpineol (11.9%)	–	Medzegue et al. (2013)

used as a remedy for roundworms, colic, stomach pains, postpartum pains, dysentery, and gonorrhea (Tcheghebe et al. 2016). Sore throat is treated from a drink made from burnt seed of *C. schweinfurthii* (Tcheghebe et al. 2016). Decoctions of the tree bark are used in the Ivory Coast against cough, to treat chest pain in Sierra Leone, venereal diseases in Cameroon, and remedies for abscesses and dysentery in Nigeria (Dongmo et al. 2010). In the Congo and the Central African Republic, the plant is used as a stimulant, emollient, and as a treatment for rheumatism (Bouquet 1969). Scientifically, extracts from the tree have demonstrated several biological activities, including antimalarial, antineoplastic, antioxidant, antimicrobial, antidiabetic, analgesic, nephroprotective, anthelmintic, and termiticidal activities (Tcheghebe et al. 2016). The oleoresin produced by *C. schweinfurthii* has an odor reminiscent of lavender, and it is used as incense in Uganda (Nagawa et al. 2015). The essential oil (EO) from the oleoresin has shown significant analgesic effects in mouse models of pain (acetic acid-induced writhing and hot plate test) (Koudou et al. 2005). The resin oil was tested for anti-termitic activity against *Macrotermes bellicosus* and was found to be remarkably active, and its major components were also tested to confirm its anti-termitic property (Nagawa et al. 2015). The resin oil has also shown antifungal activity against several *Aspergillus* species (Ambindei et al. 2014).

A popular commercial essential oil, "elemi," obtained from the oleoresin of *Canarium luzonicum* (Blume) A. Gray (Villanueva et al. 1993), is used as an expectorant in addition to treatment of stomach disorders (Rajagopal 2014). Traditionally the dried powdered oleoresin from *Canarium strictum* Roxb., known in India as black dammer resin, is used to treat skin diseases, hernia, syphilis, asthma, rheumatism, and fevers. The resin has shown anti-inflammatory activities (Ragunathan and Senthamarai 2013). *Canarium bengalenese* Roxb. is locally called "tram hong" in Vietnam, and its bark and leaves are used externally in rheumatic swellings (Thang et al. 2004). The chemical compositions and biological activities of *Canarium* essential oils are summarized in Table 4.5.

4.3.2 The Genus Dacryodes

The genus *Dacryodes* comprises about 70 species of evergreen, perennial trees distributed in America, South and Central Africa, and Southeast Asia (Onana 2008). Members of *Dacryodes* species have been used in folk medicine for their antioxidant, antibacterial, antiplasmodial, and anticarcinogenic properties for treating malaria, anemia, headache, fever, and skin diseases (Ajibesin et al. 2008; Kong et al. 2011; Dike et al. 2012; Mvitu-Muaka et al. 2012; Fonkeng et al. 2015). The stem bark secretes an aromatic oleoresin when injured. The edible fruits contain large amounts of vitamins, amino acids, lipids, and proteins (Tee et al. 2017). The essential oil compositions and biological activities of *Dacryodes* species are summarized in Table 4.6.

Table 4.5 Chemical compositions and biological activities of *Canarium* essential oils

Canarium species	Collection site	Essential oil	Major components (> 5%)	Bioactivity of essential oil	Reference
C. album Leenh.	Ha Giang, Vietnam	Oleoresin (HD)	β-Pinene (33.3%), α-terpinene (14.1%), terpinen-4-ol (11.9%)	–	Giang et al. (2006)
C. bengalense Roxb.	Thanh Nho village, Nghe An province, Vietnam	Leaf (HD)	Sabinene (15.9%), γ-terpinene, β-caryophyllene (17.5%), "*epi*-bicyclosesquiterpene"[a] (10.4%), γ-elemene (7.3%)	–	Thang et al. (2004)
C. luzonicum (Blume) A. Gray	Alabat Island, Quezon province, Philippines	Oleoresin (HD)	Sabinene (5.7%), α-phellandrene (17.6%), limonene (56.0%), elemol (6.3%)	–	Villanueva et al. (1993)
	Commercial (Sensient Essential Oils, Germany)	Oleoresin (HD)	α-Phellandrene (6.0%), limonene (45.6%), elemol (21.7%)	Weakly antifungal (*Candida albicans*, MIC 2500 μg mL^{-1})	Nikolić et al. (2016)
	Commercial (Bontoux, France)	Oleoresin (HD)	α-Phellandrene (13%), limonene (56%), elemol (11%)	–	Satyal (unpublished)
C. parvum Leenh.	Bến En National Park, Thanh Hóa province, Vietnam	Bark (HD)	α-Pinene (6.1%), α-phellandrene (6.2%), limonene (6.3%), (*E*)-β-ocimene (7.7%), α-copaene (20.5%), β-caryophyllene (30.5%), α-humulene (5.3%)	–	Thang et al. (2014)
	Bến En National Park, Thanh Hóa province, Vietnam	Leaf (HD)	(*Z*)-β-Ocimene (11.9%), (*E*)-β-ocimene (12.9%), *allo*-ocimene (6.8%), β-caryophyllene (18.7%), α-humulene (8.4%), germacrene D (8.8%)	–	Thang et al. (2014)
	Bến En National Park, Thanh Hóa province, Vietnam	Oleoresin (HD)	α-Copaene (9.8%), β-elemene (8.6%), α-humulene (8.1%), germacrene D (23.2%), α-amorphene (14.9%), valerenol (5.4%)	–	Thang et al. (2014)
C. pimela K.D. Koenig	Rongxian County, Yulin, Guangxi, China	Leaf (HD)	α-Pinene (9.2%), eremophila-1(10),11-diene (11.7%), α-selinene (10.3%), γ-muurolene (9.7%), cadina-1,4-diene (7.5%)	–	Li et al. (2015)

C. schweinfurthii Engl.	Boukoko, Central African Republic	Oleoresin (HD)	*n*-Octanol (9.5%), octyl acetate (60.0%), (*E*)-nerolidol (14.0%)	Antinociceptive, mouse (acetic acid-induced writhing, ED_{50} 1.6 mL kg^{-1}; hot plate ED_{50} 1.4 mL kg^{-1})	Koudou et al. (2005)
C. schweinfurthii Engl.	Boukoko, Central African Republic	Oleoresin (HD)	–	Antibacterial, broth dilution assay (*Listeria innocua*, *Staphylococcus aureus*, *Staphylococcus camorum*, MIC 2500 μg mL^{-1}), antifungal (*Candida albicans*, MIC 2500 μg mL^{-1})	Obame et al. (2007b)
	Lolodorf, Cameroon	Oleoresin (HD)	*p*-Cymene (9.8%), limonene (42.7%), α-terpineol (34.4%)	Inhibitor of 5-lipoxygenase (IC_{50} 62.6 μg mL^{-1})	Dongmo et al. (2010)
	Mbouda, Cameroon	Oleoresin (HD)	*p*-Cymene (9.8%), limonene (42.7%), α-terpineol (34.4%)	–	Dongmo et al. (2010)
	Côte d'Ivoire	Root (HD)	δ-2-Carene (14.5%), limonene (20.0%), terpinolene (42.6%)	–	Affouet et al. (2012)
	Gabon	Oleoresin (HD)	α-Pinene (10.7%), sabinene (19.2%), limonene (52.1%)	–	Engonga et al. (2012)
	Lolodorf, Cameroon	Oleoresin (HD)	*p*-Cymene (9.8%), limonene (42.7%), α-terpineol (34.4%)	Weakly antifungal, disk diffusion assay (*Aspergillus flavus*, MIC 1800 μg mL^{-1}; *Aspergillus niger*, MIC 2800 μg mL^{-1}; *Aspergillus fumigatus*, MIC 1300 μg mL^{-1})	Ambindei et al. (2014)

(continued)

Table 4.5 (continued)

Canarium species	Collection site	Essential oil	Major components (> 5%)	Bioactivity of essential oil	Reference
	Sango Bay, Uganda	Oleoresin (HD)	α-Thujene (14.0%), α-phellandrene (17.9%), γ-terpinene (34.5%), *p*-cymene (8.5%), β-phellandrene (12.9%)	Termiticidal (*Macrotermes bellicosus*, LC_{50} 1.12 mg g^{-1} after 48 h)	Nagawa et al. (2015)
C. strictum Roxb.	Rayriath garden Thrissur, Kerala state, India	Oleoresin (HD)	–	Anti-inflammatory, mouse paw edema test (79% reduction with 10 mg kg^{-1} after 3 h)	Ragunathan and Senthamarai (2013)
C. tramdenum C.D. Dai & Yakovlev	Pù Mát National Park, Nghệ An province, Vietnam	Bark (HD)	α-Pinene (12.3%), α-phellandrene (21.7%), limonene (25.7%), β-caryophyllene (10.9%), γ-elemene (7.9%)	–	Thang et al. (2014)
	Pù Mát National Park, Nghệ An province, Vietnam	Leaf (HD)	α-Pinene (9.4%), α-phellandrene (15.9%), limonene (11.8%), β-caryophyllene (16.8%), γ-elemene (13.1%), phytol (8.6%)	–	Thang et al. (2014)
C. tramdenum C.D. Dai & Yakovlev	Pù Mát National Park, Nghệ An province, Vietnam	Oleoresin (HD)	δ-Elemene (14.6%), β-bourbonene (6.8%), γ-elemene (6.8%), germacrene D (6.5%), guaiol (6.1%), bulnesol (16.0%)	–	Thang et al. (2014)
C. zeylanicum (Retz.) Blume	Royal Botanical Gardens, Peradeniya, Sri Lanka	Oleoresin (HD)	α-Pinene, α-phellandrene, β-phellandrene, limonene, α-terpineol, carvone (percentages not reported)	–	Bandaranayake (1980)

[a]*epi*-Bicyclosesquiterpene is not found in the *Dictionary of Natural Products* (Dictionary of Natural Products 2017) nor the Adams database (Adams 2007)

Table 4.6 Chemical compositions and biological activities of *Dacryodes* essential oils

Dacryodes species	Collection site	Essential oil	Major components (> 5%)	Bioactivity of essential oil	Reference
D. buettneri (Engl.) H.J. Lam	Sebang Herbarium, Libreville, Gabon	Oleoresin (HD)	α-Pinene (13.2%), β-pinene (42.0%), *p*-cymene (19.0%), terpinen-4-ol (27.3%)	Antibacterial, broth microdilution assay (*Shigella dysenteriae*, MIC 2500 μg mL^{-1})	Obame et al. (2007a)
	Gabon	Fruit (HD)	α-Pinene (29.2%), β-pinene (7.7%), limonene (24.3%), α-copaene (5.2%), germacrene D (5.4%)	–	Cravo et al. (1992)
D. edulis (G. Don) H.J. Lam	University of Ibadan, Nigeria	Fruit (HD)	α-Pinene (8.8%), myrcene (45.3%), α-terpineol + germacrene D (12.5%)	–	Onocha et al. (1999)
	University of Ibadan, Nigeria	Leaf (HD)	β-Caryophyllene (26.4%), germacrene D (7.5%), palmitic acid (12.7%)	–	Onocha et al. (1999)
	University of Ibadan, Nigeria	Bark (HD)	α-Thujene + α-pinene (25.2%), limonene (12.5%), γ-terpinene (8.6%), terpinen-4-ol (25.6%)	–	Onocha et al. (1999)
	University of Ibadan, Nigeria	Root (HD)	α-Pinene (7.3%), β-pinene (8.7%), α-phellandrene (26.5%), limonene (10.2%), β-phellandrene (6.6%), *p*-cymene (6.6%)	–	Onocha et al. (1999)
	Ngaoundere, Cameroon	Fruit (headspace SPME)	α-Pinene (60.3%), β-pinene (8.2%), myrcene (15.0%)	–	Jirovetz et al. (2003)
	Ngaoundere, Cameroon	Fruit (HD)	α-Pinene (22.3%), β-pinene (13.7%), α-phellandrene (10.8%), limonene (7.2%), (2*E*,4*E*)-decadienal (6.7%)	–	Jirovetz et al. (2003)

(continued)

Table 4.6 (continued)

Dacryodes species	Collection site	Essential oil	Major components (> 5%)	Bioactivity of essential oil	Reference
	Ngaoundere, Cameroon	Seed (HD)	α-Pinene (21.5%), β-pinene (19.7%), α-phellandrene (12.1%), limonene (27.5%)	–	Jirovetz et al. (2003)
	Sebang Herbarium, Libreville, Gabon	Oleoresin (HD)	α-Pinene (17.5%), sabinene (21.8%), *p*-cymene (11.3%), limonene (5.7%), γ-terpinene (5.8%), terpinen-4-ol (19.8%)	Weakly antibacterial (MIC ≥1%)	Obame et al. (2008)
	Etoug-Ebe, Yaoundé, Cameroon	Leaf (HD)	*trans*-Carveol (11.8%), elemol (29.2%), spathulenol (6.3%), caryophyllene oxide (5.1%), ishwarone (15.3%)	Weakly antibacterial (*Bacillus cereus*, *Salmonella typhi*, *Staphylococcus aureus*, *Shigella* sp., *Escherichia coli*, MIC 18.8 mg mL^{-1})	Riwom et al. (2015)
D. edulis (G. Don) H.J. Lam	Etoug-Ebe, Yaoundé, Cameroon	Bark (HD)	α-Thujene (14.9%), β-phellandrene (8.7%), *p*-cymene (35.1%), *trans*-carveol (22.6%), β-elemene (5.2%)	Weakly antibacterial (*Bacillus cereus*, *Salmonella typhi*, *Staphylococcus aureus*, *Shigella* sp., *Escherichia coli*, MIC 50 mg mL^{-1})	Riwom et al. (2015)
	Etoug-Ebe, Yaoundé, Cameroon	Oleoresin (HD)	α-Thujene (28.6%), α-phellandrene (27.1%), β-phellandrene (10.2%), *p*-cymene (30.3%)	Weakly antibacterial (*Bacillus cereus*, *Salmonella typhi*, *Staphylococcus aureus*, *Shigella* sp., *Escherichia coli*, MIC 200 mg mL^{-1})	Riwom et al. (2015)
D. igaganga Aubrév. & Pellegr.	Gabon	Fruit (HD)	Limonene (6.9%), α-copaene (15.5%), β-elemene (6.5%), β-caryophyllene (8.3%), α-humulene (13.8%), germacrene D (8.1%)	–	Cravo et al. (1992)

Dacryodes buettneri (Engl.) H.J. Lam, "ozigo," "assia," is widely distributed in the equatorial forest region from Gabon to Equatorial Guinea. *D. buettneri* is traditionally used to treat jaundice, fever, malaria, constipation, microbial infections, and diarrhea. The resin of *D. buettneri* is used as an antimicrobial agent and astringent (Obame et al. 2007a). The antibacterial and antioxidant properties of the essential oil are attributed to its major components (Table 4.6).

Dacryodes edulis (G. Don) H.J. Lam (syn. *Pachylobus edulis* G. Don, *Pachylobus saphu* Engl.) "safou," "African pear," is native to the humid tropical forests of Nigeria, Cameroon, Gabon, and Ghana. Due to its antioxidant, antimicrobial, antidiabetic, and anticarcinogenic activities, various parts of the plant are used to treat several diseases (Agbor et al. 2007; Atawodi et al. 2009; Atawodi 2011; Mvitu-Muaka et al. 2012; Zofou et al. 2013; Erukainure et al. 2017). The essential oils extracted from various parts of *D. edulis* are dominated by mono- and sesquiterpenes (Onocha et al. 1999). The bark decoction is orally taken to treat leprosy, headaches, fever, and malaria and topically when mixed with palm oil to relieve pain and stiffness and treat parasitic skin diseases (Tee et al. 2014). It is also used as a gargle or mouthwash. The resin is used topically to treat parasitic skin diseases and wounds (Ajibesin et al. 2008; Obame et al. 2008). In Nigeria, the resin is burned as lamp oil for lighting and to avoid evil spirits (Omonhinmin 2012). The leaves are used as antiemetic when chewed with kola nut, while the leaf sap is used as an eardrop. In southwest Cameroon, the leaves are used as plasters to treat snakebites. A decoction of the leaves is taken orally to manage hypertension or used to prepare vapor baths for treating fever and headache (Omonhinmin 2012). The fruit is usually consumed raw, boiled, or roasted (Jirovetz et al. 2003). In some African nations, the fruit extract is used to treat wounds, parasitic skin diseases, sickle cell anemia, dysentery, and fever (Kalenda et al. 2002; Mpiana et al. 2007). Recently, the hexane extract of *D. edulis* fruit was proven to have antidiabetic and hypolipidemic activities (Okolo et al. 2016).

Dacryodes hexandra (Ham.) Griseb. (syn. *D. excelsa* Vahl), "tabonuco," is abundant in the West Indies and the subtropical wet forests of Puerto Rico. Because of being sticky, the oleoresin of *D. hexandra* is used as a polish to varnish wood or other materials (More 1899; Tee et al. 2014).

Dacryodes klaineana (Pierre) H.J. Lam (syn. *Aucoumea klaineana* Pierre), "eben ekpo," "African cherry fruit," is found in the humid tropical forests of West and Central Africa. Boiled roots of *D. klaineana* are used to treat skin diseases (Ajibesin et al. 2008; Tee et al. 2014). The oleoresin contains tirucallane triterpenes and a monoterpene-rich volatile oil. It has been used as an immunostimulant, for treating sores, and as incense (Liang et al. 1988a, b).

Dacryodes rostrata (Blume) H.J. Lam, "kembayau," "kedondong kerut," "pinanasan," and "palaspas," is found in Indochina, Thailand, Peninsular Malaysia, Sumatra, Borneo, and the Philippines. The fruit is very nutritious (rich in vitamins, minerals, amino acids, lipids, and proteins) and rich in antioxidants (Kong et al. 2011). *D. rostrata* fruits are often consumed boiled or preserved in salt or soy sauce

to be used as appetizers. Due to its high nutritional value, consuming *D. rostrata* fruit can prevent malnutrition (Hoe and Siong 1999; Kong et al. 2011; Tee et al. 2014). The fruit oil proved to be hepatoprotective and reversed lipid peroxidation in paracetamol-induced toxicity (Tee et al. 2017).

4.3.3 The Genus Santiria

The genus *Santiria* consists of about 24 species of tall resiniferous trees distributed in the Old World tropics (Mabberley 2008). *Santiria trimera* (Oliv.) Aubrév. (syn. *Pachylobus trimerus* (Oliv.) Guillaumin), "Krio," is a very large dioecious tree found in the tropical rainforests of West Africa (from Sierra Leone to Nigeria, and extending to Zaïre) (Bikanga et al. 2010). *S. trimeria* is one of the important medicinal species in this area for its antiseptic properties. The tree bark has a balsamic odor and yields an oleoresin. In Gabon, the bark is used traditionally for wound healing and for treating infectious diseases, while in São Tomé and Príncipe islands, it is used for the treatment of pulmonary problems including tuberculosis and venereal diseases (Martins et al. 2003; Bikanga et al. 2010). The powdered bark is used to treat yaws and to treat children's whooping cough when mixed with palm oil and salt. The bark is also employed as a purgative and vermifuge. A decoction of the bark is used in vapor baths to treat fever and eczema. *S. trimera* bark extract contains more than 60% terpenes with antimicrobial properties and is considered a good source of lanostane derivatives including 20(*R*),24(*E*)-6β-acetoxy-3-oxo-9β-lanosta-7,24-dien-26-oic acid and 6β-acetoxy-3,23-dioxo-9β-20β-lanost-7,24-dien-26-oic acid (da Silva et al. 1990). The bark essential oils are rich in monoterpenoids. A sample from Fraternidade, São Tomé and Príncipe, was dominated by α-pinene (66.6%) and β-pinene (20.0%) (Martins et al. 2003), while a bark essential oil from Franceville, Gabon, showed α-pinene (51.5%), β-pinene (5.8%), terpinen-4-ol (8.5%), and α-terpineol (16.2%) (Bikanga et al. 2010). The leaf essential oil from Gabon was dominated by the sesquiterpene hydrocarbons β-caryophyllene (14.9%) and α-humulene (34.6%), along with α-pinene (9.4%) and humulene epoxide II (5.6%) (Bikanga et al. 2010). Both leaf essential oil and bark oil possess weak antimicrobial effects with the bark oil being more active (Martins et al. 2003; Bikanga et al. 2010). In addition to its medicinal uses, the wood of *S. trimeria* is used in Gabon for carving and for the production of personal items, musical instruments, and toys.

4.3.4 The Genus Trattinnickia

There are around 18 species of *Trattinnickia* found in Central America and northern South America (Daly 1999; Mabberley 2008; Daly and Melo 2017). Like other members of the family, *Trattinnickia* produces oleoresins rich in tirucallane, ursane,

and oleanane triterpenoids (Lima et al. 2004). The Tembé people of the Amazon use the resins (breu) from *Protium* and *Trattinnickia* to make ceremonial smoke or as medicine for treatment of skin infections and parasites and to relieve nasal congestion (Plowden et al. 2002). The resin of *T. aspera* (Standl.) Swart is apparently used by white-nosed coatis (*Nasua narica*) in Panama to rub into their own fur and/or that of conspecifics (Gompper and Hoylman 1993).

Very little research has been published on the essential oil compositions or biological activities of *Trattinnickia*. The hydrodistilled essential oil from the oleoresin of *T. rhoifolia* Willd., collected from the Adolfo Ducke Biological Reserve, Amazonas, Brazil, has been analyzed. The major components in the resin oil were the monoterpenoids α-pinene (23–25%), α-phellandrene (4.3–8.1%), α-terpinene (4.3–5.8%), *p*-cymene (40–49%), β-phellandrene (7.6–8.7%), *trans*-dihydro-α-terpineol (4.2–6.4%), and α-terpineol (1.7–5.4%) (Ramos et al. 2003). The essential oils from *T. rhoifolia* branches, on the other hand, were dominated by sesquiterpenoids, α-cubebene (12.4%), α-copaene (16.4%), β-caryophyllene (29.6%), *cis*-calamenene (5.3%), δ-cadinene (15.1%), and 1-*epi*-cubenol (5.1%) (de Carvalho et al. 2009).

4.4 Tribe Protieae

4.4.1 The Genus Protium

The genus *Protium* is the largest genus of Burseraceae in the Neotropics, with about 150 species (Mabberley 2008). Some *Protium* species are found in Madagascar and Malaysia (Mabberley 2008). The important characteristic feature of *Protium* species, like their Old World counterparts *Boswellia* and *Commiphora*, is the abundance of aromatic resinous exudates from wounds in the bark. The resins of *Protium* species are known as "copal" in Spanish (Stacey et al. 2006) and "breu" in Portuguese (Siani et al. 2017). *Protium* oleoresins have been characterized based on their age and color as well as volatile and nonvolatile chemical constituents (Siani et al. 2012; da Silva et al. 2013; Siani et al. 2017). Throughout their ranges, *Protium* species have been used by native peoples to treat various diseases and conditions, including wounds, skin infections, toothache, headache, pain, rheumatism, and coughs and colds (Morton 1981; Schultes and Raffauf 1990; Rüdiger et al. 2007; Lago et al. 2016). For example, native people of the Unini River communities in the Amazon forest biome burn the oleoresin of *P. amazonicum* (Cuatrec.) Daly and inhale the smoke to relieve headache and anxiety, while the oleoresin of *P. decandrum* (Aubl.) Marchand is used to treat skin problems such as boils and wounds (Santos et al. 2012; Lago et al. 2016). The Yucatan Mayas used the resin of *P. copal* Engl. as a styptic on infections, wounds, and sores (Morton 1981; Duke et al. 2009). The resins of *Protium* species are also used as varnishes and calking and burned as incense (Morton 1981; Duke et al. 2009).

Protium heptaphyllum (Aubl.) Marchand is native to the warm regions of northern Colombia, northern Venezuela, Brazil, Guyana, Frensh Guiana, Suriname, and Paraguay (Morton 1981; Missouri Botanical Garden 2017). In Venezuela, the resin of *P. heptaphyllum* is applied on tumors and ringworm; the resin is placed behind the ears to relieve headache, toothache, and rheumatism; and the resin is placed in the cavity of an aching tooth (Morton 1981). In Colombia, the resin is used to treat pimples, ulcers, swellings, syphilis, and headache (Morton 1981). The Chocó people of western Colombia use the resin as calking material as well as for extracting botfly maggots (Duke 1970). The Kubeo people of northwestern Amazonia use the resin to clear nasal passages due to heavy colds (Schultes and Raffauf 1990). People of the Usina São José community of the Atlantic Forest of Pernambuco, Brazil, use *P. heptaphyllum* to treat toothache and headache (Gazzaneo et al. 2005).

There has been much work on the volatile chemistry of *P. heptaphyllum*. However, there seems to be much variation in essential oil composition depending on geographical location (see Table 4.7). Although *P. heptaphyllum* fruit essential oils are dominated by monoterpenoids, the fruit oil from Crato, Ceara state, Brazil, was rich in α-pinene (71.2%) with a lesser quantity of limonene (5.2%) (Bandeira et al. 2001), while that from Timon, Maranhão state, Brazil, was dominated by limonene (92.7%) but only 0.2% α-pinene (Citó et al. 2006). In complete contrast, the fruit oil from Tamandaré Beach, Pernambuco state, Brazil, showed α-terpinene (47.6%) and α-terpinyl acetate (5.0%) as the major components but only 1.1% α-pinene and no detectable limonene (Pontes et al. 2007a). The leaf essential oils of *P. heptaphyllum* are generally dominated by sesquiterpene hydrocarbons, including β-caryophyllene, α-copaene, and germacrane sesquiterpenoids (Table 4.7).

Table 4.7 Chemical compositions and biological activities of *Protium* essential oils

Protium species	Collection site	Essential oil	Major components (>5%)	Bioactivity of essential oil	Reference
P. altsonii Sandwith	Erepecuru River, Oriximiná, Brazil	Aged oleoresin (HD), "black breu"	*p*-Cymene (16.3%), γ-gurjunene (5.2%), γ-cadinene (9.5%)	–	da Silva et al. (2016)
	Adolfo Ducke Forest Reserve, Amazonas, Brazil	Oleoresin (HD)	α-Pinene (11.0%), *p*-cymene (31.5%), *p*-menthene (13.1%), *trans*-dihydro-α-terpineol (25.8%)	–	Zoghbi et al. (2005)
P. amazonicum (Cuatrec.) Daly	Quito, Ecuador	Fresh oleoresin (HD)	(−)-δ-3-Carene (47.9%), limonene (5.1%), α-terpineol (5.5%)	Antifungal (*Cryptococcus neoformans*, MIC 156 μg mL^{-1}; *Candida albicans*, MIC 313 μg mL^{-1})	Satyal et al. (2017)

(continued)

Table 4.7 (continued)

Protium species	Collection site	Essential oil	Major components (>5%)	Bioactivity of essential oil	Reference
P. apiculatum Swart	Porto Alegre Farm, Amazonas state, Brazil	Oleoresin (hexane extract)	*p*-Menthane (12.2%), *p*-cymene (36.3%)	–	Silva et al. (2009)
P. bahianum Daly	Guadalupe, Pernambuco, Brazil	Fruit (HD)	α-Pinene (34.0%), β-pinene (10.3%), γ-terpinene (7.3%), dillapiole (10.6%)	Acaricidal (*Tetranychus urticae*, LC_{50} 9.07 mL L^{-1} of air after 24 h)	Pontes et al. (2010)
	Guadalupe Biological Reserve, Pernambuco, Brazil	Leaf (HD)	α-Pinene (7.8%), β-cubebene (16.5%), aromadendrene (20.3%), *cis*-β-guaiene (9.9%), α-cadinene (10.1%)	Acaricidal (*Tetranychus urticae*, LC_{50} 3.54 mL L^{-1} of air after 24 h)	Pontes et al. (2010)
	Guadalupe Biological Reserve, Pernambuco, Brazil	Fresh oleoresin (HD)	Tricyclene (11.4%), β-pinene (6.6%), α-phellandrene (14.0%), *p*-cymene (18.3%), β-phellandrene (9.1%), terpinen-4-ol (7.4%)	Acaricidal (*Tetranychus urticae*, LC_{50} 9.08 μL L^{-1} of air after 48 h)	Pontes et al. (2007b)
	Guadalupe Biological Reserve, Pernambuco, Brazil	Aged oleoresin (HD)	(*E*)-β-Santalol acetate (83.1%)	Acaricidal (*Tetranychus urticae*, LC_{50} 7.45 μL L^{-1} of air after 72 h)	Pontes et al. (2007b)
P. colombianum Cuatrec.	Cocorná, Colombia	Fruit (HD)	α-Pinene (2.3–9.5%), sabinene (51.8–70.6%), α-terpinene (2.3–5.0%), γ-terpinene (4.2–7.4%), terpinen-4-ol (6.0–13.4%)	–	Carvajal et al. (2016)
	San Luis, Colombia	Fruit (HD)	α-Thujene (9.4–20.4%), α-pinene (17.5–25.5%), sabinene (7.7–15.0%), β-pinene (4.0–5.0%), limonene (21.5–32.7%), *p*-mentha-2,4(8)-diene (trace-7.6%)	Antifungal (*Fusarium oxysporum*, MIC 625 μg mL^{-1})	Carvajal et al. (2016)

(continued)

Table 4.7 (continued)

Protium species	Collection site	Essential oil	Major components (>5%)	Bioactivity of essential oil	Reference
P. crassipetalum Cuatrec.	Adolpho Ducke Forest Reserve, Amazonas, Brazil	Leaf (HD)	α-Copaene (19.6%), β-caryophyllene (16.4%), spathulenol (13.9%), τ-cadinol (5.5%)	–	de Carvalho et al. (2013)
	Adolpho Ducke Forest Reserve, Amazonas, Brazil	Stem (HD)	α-Copaene (15.2%), β-caryophyllene (10.1%), *trans*-α-bergamotene (6.2%), (*E*)-β-farnesene (9.2%), *ar*-curcumene (10.2%), β-bisabolene (5.3%), δ-cadinene (6.3%), khusimone (7.9%)	–	de Carvalho et al. (2013)
P. decandrum (Aubl.) Marchand	Museu Paraense Emílio Goeldi, Belém, Pará, Brazil	Aerial parts (HD)	α-Pinene (78.6%), β-pinene (5.1%), limonene (7.3%)	–	Zoghbi et al. (2005)
	Adolfo Ducke Forest Reserve, Amazonas, Brazil	Leaf (HD)	Terpinen-4-ol (33.0%), β-caryophyllene (22.8%)	–	de Carvalho et al. (2010)
	Adolfo Ducke Forest Reserve, Amazonas, Brazil	Stem (HD)	Terpinen-4-ol (13.2%), *trans*-α-bergamotene (22.0%), caryophyllene oxide (10.5%)	–	de Carvalho et al. (2010)
	Adolfo Ducke Forest Reserve, Amazonas, Brazil	Aged oleoresin (HD)	*cis*-α-Bergamotene (6.5%), β-caryophyllene (5.9%), *trans*-α-bergamotene (47.7%), (*E*)-β-farnesene (5.5%), *ar*-curcumene (5.2%)	–	de Carvalho et al. (2010)
	Erepecuru River, Oriximiná, Brazil	Aged oleoresin (HD), "black breu"	δ-3-Carene + *iso*-sylvestrene (40.9%), *p*-cymene (13.4%), limonene + β-phellandrene (20.3%)	–	da Silva et al. (2016)

(continued)

Table 4.7 (continued)

Protium species	Collection site	Essential oil	Major components (>5%)	Bioactivity of essential oil	Reference
	Erepecuru River, Oriximiná, Brazil	Aged oleoresin (HD), "white breu"	α-Pinene (19.0%), α-phellandrene (21.0%), *p*-cymene (32.4%), limonene + β-phellandrene (12.0%)	–	da Silva et al. (2016)
P. elegans Engl.	Adolfo Ducke Forest Reserve, Amazonas, Brazil	Leaf (HD)	β-Caryophyllene (35.9%), α-humulene (12.6%), β-selinene (5.9%), caryophyllene oxide (27.1%)	–	de Carvalho et al. (2009)
	Adolfo Ducke Forest Reserve, Amazonas, Brazil	Stem (HD)	β-Caryophyllene (6.8%), caryophyllene oxide (55.8%)	–	de Carvalho et al. (2009)
P. grandifolium Engl.	Porto Alegre Farm, Amazonas state, Brazil	Oleoresin (hexane extract)	α-Pinene (20.9%), *p*-cymene (55.8%), α-cubebene (14.6%)	–	Silva et al. (2009)
P. hebetatum Daly	Porto Alegre Farm, Amazonas state, Brazil	Oleoresin (hexane extract)	α-Pinene (12.2%), *p*-cymene (14.2%), α-cubebene (14.4%)	–	Silva et al. (2009)
P. heptaphyllum (Aubl.) Marchand	Crato, Ceara, Brazil	Fruit (HD)	α-Pinene (71.2%), β-pinene (8.6%), limonene (5.2%)	–	Bandeira et al. (2001)
	Timon, Maranhão state, Brazil	Fruit (HD)	(Z)-β-Ocimene (5.0%), limonene (92.7%)	–	Citó et al. (2006)
	Tamandaré Beach, Pernambuco, Brazil	Fruit (HD)	α-Terpinene (47.6%), α-terpinyl acetate (5.0%)	Acaricidal (*Tetranychus urticae*, LC_{50} 6.85 μL L^{-1} of air after 72 h)	Pontes et al. (2007a)
	Crato, Ceara, Brazil	Leaf (HD)	Myrcene (18.6%), β-caryophyllene (18.6%), α-humulene (8.0%), bicyclogermacrene (7.3%)	–	Bandeira et al. (2001)

(continued)

Table 4.7 (continued)

Protium species	Collection site	Essential oil	Major components (>5%)	Bioactivity of essential oil	Reference
	Timon, Maranhão state, Brazil	Leaf (HD)	(*E*)-β-Ocimene (15.7%), α-copaene (5.2%), β-caryophyllene (32.1%), viridiflorene (14.6%), germacrene B (16.7%)	–	Citó et al. (2006)
	Manaus, Amazonas, Brazil	Leaf (SD)	Terpinolene (15.5%), β-elemene (22.1%), β-caryophyllene (11.1%), α-humulene (7.2%)	–	Zoghbi et al. (1995)
	Tamandaré Beach, Pernambuco, Brazil	Leaf (HD)	α-Copaene (7.3%), 9-*epi*-(*E*)-caryophyllene (21.4%), *trans*-isolongifolanone (10.3%), 14-hydroxy-9-*epi*-(*E*)-caryophyllene (16.7%)	Acaricidal (*Tetranychus urticae*, LC_{50} 10.0 μL L^{-1} of air after 72 h)	Pontes et al. (2007a)
	Manaus, Amazonas, Brazil	Stem (SD)	Terpinolene (40.3%), β-elemene (9.0%)	–	Zoghbi et al. (1995)
	Valença, Bahia state, Brazil	Aerial parts (SD)	α-Pinene (40.3%), α-phellandrene (10.3%), δ-3-carene (5.8%), *p*-cymene (9.6%), *m*-mentha-1,8-diene (8.9%), *p*-mentha-1,4(8)-diene (12.1%)	Gastroprotection (Wistar rat, ED_{50} 23.6 mg kg^{-1})	Araujo et al. (2011)
	Timon, Maranhão state, Brazil	Oleoresin (HD)	α-Phellandrene (10.0%), (*E*)-β-ocimene (11.8%), *p*-cymene (10.8%), limonene (50.0%), 1,8-cineole (10.9%)	Anti-inflammatory (Wistar rat paw edema, ED_{50} 75.1 mg kg^{-1})	Amaral et al. (2009)
	Guriri, São Mateus, Espírito Santo, Brazil	Oleoresin (HD)	Tricyclene (11.1%), *p*-cymene (26.7%), terpinolene (35.8%), *p*-cymen-8-ol (10.1%)	Antibacterial (*Streptococcus mutans*, MIC 0.13 μg mL^{-1})	Pinto et al. (2015)
	Teresina, PI, Brazil	Oleoresin (HD)	δ-3-Carene (5.1%), *p*-cymene (17.0%), limonene (34.5%), 1,8-cineole (20.6%), α-terpineol (9.8%)	Vasorelaxant (rat upper mesenteric artery ring, IC_{50} 316 μg mL^{-1})	Mobin et al. (2017)

(continued)

Table 4.7 (continued)

Protium species	Collection site	Essential oil	Major components (>5%)	Bioactivity of essential oil	Reference
	Timon, MA, Brazil	Oleoresin (HD)	α-Phellandrene (7.0%), *p*-cymene (26.9%), limonene (28.9%), α-terpineol (18.4%)	–	Mobin et al. (2017)
	Timon, Maranhão state, Brazil	Oleoresin (HD)	α-Phellandrene (10.4%), α-terpinene (13.7%), 1,8-cineole (58.7%), γ-terpineol (7.7%)	Antinociceptive (capsaicin mouse paw assay, 50 mg kg^{-1}; rat tail flick assay, 100 mg kg^{-1})	Rao et al. (2007)
	Porto Alegre Farm, Amazonas state, Brazil	Oleoresin (hexane extract)	α-Pinene (5.6%), *p*-cymene (26.4%), terpinolene (20.3%), α-cubebene (5.6%), apiole (16.2%)	–	Silva et al. (2009)
	Crato, Ceara, Brazil	Fresh oleoresin (HD)	α-Pinene (10.5%), α-phellandrene (16.7%), *p*-cymene (6.0%), limonene (16.9%), terpinolene (28.5%)	–	Bandeira et al. (2001)
	Reserva da Campina, Amazonas, Brazil	Fresh oleoresin (HD)	α-Terpinene (18.0%), *p*-cymene (36.0%), γ-terpinene (12.0%)	–	Siani et al. (1999)
	Restinga of Carapebus, Atlantic Forest, Rio de Janeiro, Brazil	Fresh oleoresin (HD)	α-Pinene (27.0%), sabinene (11.0%), myrcene (35.0%), β-caryophyllene (7.2%)	Cytotoxic (SP2/0 murine plasmocytoma cell line)	Siani et al. (2011)
	Crato, Ceara, Brazil	Fresh oleoresin (HD)	α-Pinene (10.5%), α-phellandrene (16.7%), *p*-cymene (6.0%), limonene (16.9%), terpinolene (28.5%)	Antimicrobial (*Candida albicans*, MIC 1.25 μg mL^{-1}; *Klebsiella pneumoniae*, MIC 5.0 μg mL^{-1}; *Proteus mirabilis*, MIC 10.0 μg mL^{-1}; *Serratia marcescens*, MIC 5.0 μg mL^{-1}; *Staphylococcus aureus*, MIC 1.25 μg mL^{-1})	Bandeira et al. (2006)

(continued)

Table 4.7 (continued)

Protium species	Collection site	Essential oil	Major components (>5%)	Bioactivity of essential oil	Reference
	Restinga of Carapebus, Atlantic Forest, Rio de Janeiro, Brazil	Freshly tapped oleoresin (HD)	α-Pinene (8.7%), α-terpinene (6.6%), *p*-cymene (16.0%), limonene (5.5%), terpinolene (28.0%), *p*-cymen-8-ol (5.6%)	Cytotoxic (Neuro-2a murine neuroblastoma, SP2/0 murine plasmocytoma, J774 murine monocytic macrophage cell lines)	Siani et al. (2011)
	Reserva da Campina, Amazonas, Brazil	Aged oleoresin (HD)	*p*-Cymene (11.0%), terpinolene (15.0%), *p*-cymenene (5.3%), *p*-cymen-8-ol (11.0%), dillapiole (16.0%)	–	Siani et al. (1999)
	Erepecuru River, Oriximiná, Brazil	Aged oleoresin (HD), "black breu"	δ-3-Carene + *iso*-sylvestrene (69.0%), *p*-cymene (6.4%), limonene + β-phellandrene (5.7%)	–	da Silva et al. (2016)
	Erepecuru River, Oriximiná, Brazil	Aged oleoresin (HD), "black breu"	δ-3-Carene + *iso*-sylvestrene (79.5%)	–	da Silva et al. (2016)
	Erepecuru River, Oriximiná, Brazil	Aged oleoresin (HD), "black breu"	δ-3-Carene + *iso*-sylvestrene (56.4%), *p*-cymene (14.0%), limonene + β-phellandrene (6.8%)	–	da Silva et al. (2016)
	Erepecuru River, Oriximiná, Brazil	Aged oleoresin (HD), "black breu"	δ-3-Carene + *iso*-sylvestrene (14.7%), *p*-cymene (33.0%)	–	da Silva et al. (2016)
P. heptaphyllum subs. *heptaphyllum* (Aubl.) Marchand	Cruzeiro do Sul, Acre state, Brazil	Oleoresin (HD)	α-Phellandrene (7.4%), *p*-cymene (39.9%), dihydro-4-carene (11.7%), tetradecane (13.4%)	–	Marques et al. (2010)
P. heptaphyllum subs. *ulei* (Swart) Daly	Adolpho Ducke Forest Reserve, Amazonas, Brazil	Leaf (HD)	α-Copaene (11.8%), β-caryophyllene (16.9%), germacrene D (7.7%), δ-cadinene (5.4%), germacrene B (12.8%)	–	de Carvalho et al. (2013)

(continued)

Table 4.7 (continued)

Protium species	Collection site	Essential oil	Major components (>5%)	Bioactivity of essential oil	Reference
	Cruzeiro do Sul, Acre state, Brazil	Oleoresin (HD)	Limonene (11.9%), terpinolene (42.3%), *p*-cymen-8-ol (13.6%)	–	Marques et al. (2010)
P. icicariba (DC.) Marchand	Carapebus, Rio de Janeiro, Brazil	Leaf (HD)	α-Terpinene (1.9–5.8%), terpinolene (4.4–12%), α-copaene (7.5–12%), γ-elemene (5.6–9.7%), germacrene D (14–23%), bicyclogermacrene (6.6–12%), δ-cadinene (5.6–8.3%), germacrene B (9.3–16%)	–	Siani et al. (2004)
	Carapebus, Rio de Janeiro state, Brazil	Fruit (HD)	α-Terpinene (21–30%), *p*-cymene (2.2–7.9%), γ-terpinene (9.8–12%), terpinolene (33–35%), terpinen-4-ol (3.9–6.1%)	–	Siani et al. (2004)
	Carapebus, Rio de Janeiro, Brazil	Oleoresin (HD)	α-Pinene (5.6–7.7%), *p*-cymene (20–40%), limonene (5.8–8.0%), α-terpinolene (5.8–31%), *p*-cymen-8-ol (10–26%)	–	Siani et al. (2004)
P. neglectum Swart	Maturin, Monagas state, Venezuela	Fresh oleoresin (HD)	*p*-Cymene (5.2%), durenol (15.6%), α-terpineol (6.9%), piperitenone (25.4%), thymol (17.5%), methyl eugenol (9.2%)	Antibacterial, disk diffusion assay (*Bacillus subtilis*, *Staphylococcus aureus*)	Suárez et al. (2007)
P. occultum Daly	Erepecuru River, Oriximiná, Brazil	Aged oleoresin (HD), "white breu"	α-Pinene (8.0%), *p*-cymene (10.4%), limonene + β-phellandrene (41.1%), α-terpineol (30.9%)	–	da Silva et al. (2016)

(continued)

Table 4.7 (continued)

Protium species	Collection site	Essential oil	Major components (>5%)	Bioactivity of essential oil	Reference
P. opacum Swart	Erepecuru River, Oriximiná, Brazil	Aged oleoresin (HD), "black breu"	*p*-Cymene (6.6%), α-*neo*-clovene (5.3%), α-*neo*-callitropsene (7.3%), γ-cadinene (14.4%),	–	da Silva et al. (2016)
P. paniculatum Engl.	Porto Alegre Farm, Amazonas state, Brazil	Oleoresin (hexane extract)	α-Pinene (10.8%), *p*-cymene (6.4%), β-caryophyllene (6.8%)	–	Silva et al. (2009)
P. pilosissimum Engl.	Museu Paraense Emílio Goeldi, Belém, Pará, Brazil	Aerial parts (HD)	α-Pinene (31.7%), α-phellandrene (24.1%), *p*-cymene (31.2%)	–	Zoghbi et al. (2005)
	Adolpho Ducke Forest Reserve, Amazonas, Brazil	Leaf (HD)	α-Copaene (11.6%), β-caryophyllene (12.6%), β-sesquiphellandrene (24.3%), (*E*)-nerolidol (6.9%)	–	de Carvalho et al. (2013)
	Adolpho Ducke Forest Reserve, Amazonas, Brazil	Stem (HD)	Caryophyllene oxide (9.8%), selin-11-en-4α-ol (56.5%)	–	de Carvalho et al. (2013)
P. polybotryum (Turcz.) Engl.	Adolpho Ducke Forest Reserve, Amazonas, Brazil	Stem (HD)	β-Caryophyllene (15.0%), caryophyllene oxide (11.9%), khusimone (35.9%)	–	de Carvalho et al. (2013)
P. spruceanum (Benth.) Engl.	Museu Paraense Emílio Goeldi, Belém, Pará, Brazil	Aerial parts (HD)	Sabinene (56.3%), γ-terpinene (6.3%), terpinen-4-ol (12.2%), β-caryophyllene (10.9%)	–	Zoghbi et al. (2005)
P. strumosum Daly	Adolfo Ducke Forest Reserve, Amazonas, Brazil	Oleoresin (HD)	α-Pinene (6.3%), limonene (75.5%), α-terpineol (7.7%)	–	Zoghbi et al. (2005)
	Porto Alegre Farm, Amazonas state, Brazil	Oleoresin (hexane extract)	*p*-Cymene (26.1%), limonene (14.7%)	–	Silva et al. (2009)

(continued)

Table 4.7 (continued)

Protium species	Collection site	Essential oil	Major components (>5%)	Bioactivity of essential oil	Reference
	Erepecuru River, Oriximiná, Brazil	Aged oleoresin (HD), "white breu"	α-Pinene (57.7%), β-pinene (9.3%), *p*-cymene (9.2%), limonene + β-phellandrene (10.8%)	–	da Silva et al. (2016)
P. subserratum (Engl.) Engl.	Manaus, Amazonas, Brazil	Oleoresin (hexane extract)	α-Pinene (8.5%), α-phellandrene (20.8%), β-phellandrene (56.3%)	–	Zoghbi et al. (1998)
P. unifoliolatum Engl.	Manaus, Amazonas, Brazil	Leaf (SD)	Limonene (24.2%), α-copaene (6.2%), β-caryophyllene (37.5%), α-humulene (9.9%)	–	Zoghbi et al. (1993)

Protium heptaphyllum oleoresin essential oils show very different chemical compositions, depending on geographical location, subspecies, as well as color and age of the resin (Table 4.7). In addition to evaporation of volatile resin components, some of the components can also undergo oxidation upon exposure to atmospheric oxygen (Hausen et al. 1999; Sawamura et al. 2004; Turek and Stintzing 2012).

The chemical compositions along with the many biological activities of *P. heptaphyllum* oleoresin essential oils generally corroborate the traditional medicinal uses of this resin. For example, the presence of monoterpenoids such as limonene, myrcene, α-pinene, *p*-cymene, and 1,8-cineole in *P. heptaphyllum* resin oils likely contributes to the decongestant use of *P. heptaphyllum* resin by the Kubeo people (see above) (Lis-Balchin 2010; Djilani and Dicko 2012; Ferrara 2016). The oleoresin essential oils of *P. heptaphyllum* have also shown antimicrobial (Bandeira et al. 2006; Suárez et al. 2007; Pinto et al. 2015), antinociceptive (Rao et al. 2007), and vasorelaxant (Mobin et al. 2017) properties.

4.5 Summary and Conclusions

This is a comprehensive review of the composition of essential oils of the Burseraceae family and the uses of oils and isolates from the various species found in this aromatic plant family. In addition to including only those oils from botanically authenticated sources of the oils, we have included commercial samples whose

source origins may or may not be correct. Nevertheless, they were included for a completeness of this review. It is hoped that this review will become a reference source for future studies on oils from this fascinating plant family.

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References

Abdel Wahab SM, Aboutabl EA, El-Zalabani SM, Fouad HA, De Pooter HL, El-Fallaha B (1987) The essential oil of olibanum. Planta Med 53(4):382–384

Acıduman A, Tuzcu K, Belen D (2017) One of the earliest accounts of head wounds in Firdaws al-Ḥikma by al-Ṭabar from the 9th century. World Neurosurg 107:834–838

Adam ME, Selim SA (2013) Antimicrobial activity of essential oil and methanol extract from *Commiphora molmol* (Engl.) resin. Int J Curr Microbiol Appl Sci 2(12):1–6

Adams RP (2007) Identification of essential oil components by gas chromatography/mass spectrometry, 4th edn. Allured Publishing, Carol Stream, IL

Adorisio S, Fierabracci A, Gigliarelli G, Muscari I, Cannarile L, Liberati AM, Marcotullio MC, Riccardi C, Curini M, Robles Zepeda RE, Delfino DV (2017) The hexane fraction of *Bursera microphylla* A. Gray induces p21-mediated antiproliferative and proapoptotic effects in human cancer-derived cell lines. Integr Cancer Ther 16(3):426–435

Affouet KM, Tonzibo ZF, Attioua BK, Chalchat JC (2012) Chemical investigations of volatile oils from aromatic plants growing in Côte d'Ivoire: *Harisonia abyssinica* Oliv., *Canarium schwerfurthii* Engl., *Zanthoxylum gilletti* (De wild) Waterm. and *Zanthoxylum zanthoxyloides* Lam. Anal Chem Lett 2(6):367–372

Agbor GA, Kuate D, Oben JE (2007) Medicinal plants can be good sources of antioxidants: case study in Cameroon. Pak J Biol Sci 10(4):537–544

Ageel AM, Tariq M, Mossa JS, Ai-Yahya MA, AI-Said MS (1987) Plants used in Saudi folk medicine. King Saud University Press, Riyadh

Ahmed HH, Abd-Rabou AA, Hassan AZ, Kotob SE (2015) Phytochemical analysis and anticancer investigation of *Boswellia serrata* bioactive constituents in vitro. Asian Pac J Cancer Prev 16(16):7179–7188

Ajibesin KK, Ekpo BA, Bala DN, Essien EE, Adesanya SA (2008) Ethnobotanical survey of Akwa Ibom State of Nigeria. J Ethnopharmacol 115:387–408

Alaamri MMH (2012) Distribution *Boswellia sacra* in Dhofar Mountains, Sultinate of Oman: economic value and environmental role. J Life Sci 6(6):632–636

Al-Harrasi A, Al-Saidi S (2008) Phytochemical analysis of the essential oil from botanically certified oleogum resin of *Boswellia sacra* (Omani luban). Molecules 13:2181–2189

Al-Harrasi A, Ali L, Hussain J, Ur Rehman N, Mehjabeen AM, Al-Rawahi A (2014) Analgesic effects of crude extracts and fractions of Omani frankincense obtained from traditional medicinal plant *Boswellia sacra* on animal models. Asian Pac J Trop Med 7(Suppl 1): S485–S490

Ali BZ (2007) Evaluation of myrrh (*Commiphora molmol*) essential oil against some storage fungi. J Al-Nahrain Univ 10(2):107–111

Ali NAA, Wurster M, Arnold N, Teichert A, Schmidt J, Lindequist U, Wessjohann L (2008) Chemical composition and biological activities of essential oils from the oleogum resins of three endemic Soqotraen *Boswellia* species. Rec Nat Prod 2(1):6–12

Al-Massarany SM, Abbas FA, Demirci B, Baser KHC, Khan SI, Al-Rehaily AJ, Mossa JS, Abourashed EA (2007) Chemical composition and biological evaluation of the essential oil of *Commiphora opobalsamum* L. J Herb Spices Med Plant 13(4):110–121

Al-Saidi S, Rameshkumar KB, Hisham A, Sivakumar N, Al-Kindy S (2012) Composition and antibacterial activity of the essential oils of four commercial grades of Omani luban, the oleo-gum resin of *Boswellia sacra* Flueck. Chem Biodivers 9:615–624

Amaral MPM, Braga FAV, Passos FFB, Almeida FRC, Oliveira RCM, Carvalho AA, Chaves MH, Oliveira FA (2009) Additional evidence for the anti-inflammatory properties of the essential oil of *Protium heptaphyllum* resin in mice and rat. Latin Am J Pharm 28(5):775–782

Ambindei WA, Ngouné LT, Sameza ML, Sonwa ET, Nguimatsia F, Dongmo PMJ (2014) Antifungal activities against some *Aspergillus* species of the essential oils of *Canarium schweinfurthii* and *Aucoumea klaineana* growing in Cameroon. Int J Curr Microbiol Appl Sci 3(5):691–701

Amer A, Mehlhorn H (2006) Repellency effect of forty-one essential oils against *Aedes*, *Anopheles*, and *Culex* mosquitoes. Parasitol Res 99:478–490

Amiel E, Ofir R, Dudai N, Soloway E, Rabinsky T, Rachmilevitch S (2012) β-Caryophyllene, a compound isolated from the biblical balm of gilead (*Commiphora gileadensis*), is a selective apoptosis inducer for tumor cell lines. Evid Based Complement Altern Med 2012:872394

Amin A, Mousa M (2007) Merits of anti-cancer plants from the Arabian Gulf region. Cancer Ther 5:55–66

Ammon HPT (2008) Medical uses of incense (olibanum) in different historical periods. Phytomed 15:541

Ankli A, Sticher O, Heinrich M (1999) Medical ethnobotany of the Yucatec Maya: healers' consensus as a quantitative criterion. Econ Bot 53(2):144–160

Annu W, Latha PG, Shaji J, Anuja GI, Suja SR, Sini S, Shyamal S, Shine VJ, Shikha P, Rajasekharan S (2010) Anti-inflammatory, analgesic and anti-lipid peroxidation studies on leaves of *Commiphora caudata* (Wight & Arn.) Engl. Indian J Nat Prod Resour 1:44–48

Araujo DAOV, Takayama C, De-Faria FM, Socca EAR, Dunder RJ, Manzo LP, Luiz-Ferreira A, Souza-Brito ARM (2011) Gastroprotective effects of essential oil from *Protium heptaphyllum* on experimental gastric ulcer models in rats. Braz J Pharmacogn 21(4):721–729

Archier P, Vieillescazes C (2000) Characterisation of various geographical origin incense based on chemical criteria. Analusis 28:233–237

Arvigo R, Balick M (1993) Rainforest remedies: one hundred healing herbs of belize, 2nd edn. Lotus Press, Twin Lakes, WI

Asres K, Tei A, Moges G, Sporer F, Wink M (1998) Terpenoid composition of the wound-induced bark exudate of *Commiphora tenuis* from Ethiopia. Planta Med 64(5):473–475

Atawodi SE (2011) Nigerian foodstuffs with prostate cancer chemopreventive polyphenols. Infect Agents Cancer 6(Suppl 2):S9

Atawodi SE, Atawodi JC, Idakwo P, Pfundstein B, Haubner R, Wurtele G, Spiegelhalder B, Baartsch H, Owen RW (2009) Evaluation of the polyphenol composition and antioxidant activity of African variety of *Dacryodes edulis* (G.Don) H.J Lam fruit. J Med Food 12(6):1321–1325

Attorre F, Taleb N, De Sanctis M, Farcomeni A, Guillet A, Vitale M (2011) Developing conservation strategies for endemic tree species when faced with time and data constraints: *Boswellia* spp. on Socotra (Yemen). Biodivers Conserv 20(7):1483–1499

Avlessi F, Alitonou GA, Sohounhloue DK, Bessiere JM, Menut C (2005) Aromatic plants of tropical West Africa. Part XV. Chemical and biological evaluation of leaf essential oil of *Commiphora africana* from Benin. J Essent Oil Res 17(5):569–571

Awadh Ali NA, Wurster M, Arnold N, Lindequist U, Wessjohann L (2008) Essential oil composition from oleogum resin of Soqotraen *Commiphora kua*. Rec Nat Prod 2(3):70–75

Awadh Ali NA, Wurster M, Lindequist U (2009) Chemical composition of essential oil from the olegum resin of *Commiphora habessinica* (Berg.) Engl. from Yemen. J Essent Oil Bear Plants 12(2):244–249

Ayédoun MA, Sohounhloué DK, Menut C, Lamaty G, Molangul T, Cassanova J, Tomi F (1998) Aromatic plants of Tropical West Africa. VI. α-Oxobisabolene as main constituent of the leaf essential oil of *Commiphora africana* (A. Rich.) Engl. from Benin. J Essent Oil Res 10(1):105–107

Bakır B, Him A, Özbek H, Düz E, Tütüncü M (2008) Investigation of the anti-inflammatory and analgesic activities of β-caryophyllene. Int J Essent Oil Therap 2:41–44

Bandaranayake WM (1980) Terpenoids of *Canarium zeylanicum*. Phytochemistry 19:255–257

Bandeira PN, Machado MIL, Cavalcanti FS, Lemos TLG (2001) Essential oil composition of leaves, fruits and resin of *Protium heptaphyllum* (Aubl.) March. J Essen Oil Res 13(1):33–34

Bandeira PN, Fonseca AM, Costa SMO, Lins MUDS, Pessoa ODL, Monte FJQ, Nogueira NAP, Lemos TLG (2006) Antimicrobial and antioxidant activities of the essential oil of resin of *Protium heptaphyllum*. Nat Prod Commun 1(2):117–120

Baratta MT, Dorman HJD, Deans SG, Figueiredo AC, Barrosi JG, Ruberto G (1998) Antimicrobial and antioxidant properties of some commercial essential oils. Flavour Fragr J 13:235–244

Basar S (2005) Phytochemical investigations on *Boswellia* species. Dissertation, Universität Hamburg

Basar S, Koch A, König WA (2001) A verticillane-type diterpene from *Boswellia carterii* essential oil. Flavour Fragr J 16(5):315–318

Başer KHC, Demirci B, Dekebo A, Dagne E (2003) Essential oils of some *Boswellia* spp., myrrh and opopanax. Flavour Fragr J 18:153–156

Becerra JX, Noge K (2010) The Mexican roots of the Indian lavender tree. Acta Bot Mex (91):27–36

Becerra JX, Noge K, Venable DL (2009) Macroevolutionary chemical escalation in an ancient plant–herbivore arms race. Proc Natl Acad Sci U S A 106(43):18062–18066

Bekana D, Kebede T, Assefa M, Kassa H (2014) Comparative phytochemical analyses of resins of *Boswellia* species (*B. papyrifera* (Del.) Hochst., *B. neglecta* S. Moore, and *B. rivae* Engl.) from northwestern, southern, and southeastern Ethiopia. ISRN Anal Chem 2014:374678

Ben Yehoshua S, Rachmilevitch S, Amiel E, Ofir R, Dudai N, Soloway E (2015) Revival of the extinct balm of Gilead in Istrael: studying its anti-cancer activity. Acta Hortic (1088):509–514

Benelli G, Rajeswary M, Vijayan P, Senthilmurugan S, Alharbi NS, Kadaikunnan S, Khaled JM, Govindarajan M (2017) *Boswellia ovalifoliolata* (Burseraceae) essential oil as an eco-friendly larvicide? Toxicity against six mosquito vectors of public health importance, non-target mosquito fishes, backswimmers, and water bugs. Environ Sci Pollut Res 2017:1–8

Berenbaum MR, Zangerl AR (2008) Facing the future of plant-insect interaction research: Le retour à la "raison d'être". Plant Physiol 146(3):804–811

Beyra A, León MC, Iglesias E, Ferrándiz D, Herrera R, Volpato G, Godínez D, Guimarais M, Álvarez R (2004) Estudios etnobotánicos sobre plantas medicinales en la provincia de Camagüey (Cuba). An Jardín Bot Madrid 61(2):185–204

Bikanga R, Makani T, Agnaniet H, Obame LC, Abdoul-Latif FM, Lebibi J, Menut C (2010) Chemical composition and biological activities of *Santiria trimera* (Burseraceae) essential oils from Gabon. Nat Prod Commun 5(6):961–964

Birkett MA, Al Abassi S, Kröber T, Chamberlain K, Hooper AM, Guerin PM, Pettersson J, Pickett JA, Slade R, Wadhams LJ (2008) Antiectoparasitic activity of the gum resin, gum haggar, from the East African plant, *Commiphora holtziana*. Phytochemistry 69(8):1710–1715

Bouquet A (1969) Féticheurs et Médecines Traditionnelles du Congo (Brazzaville). Office de la Recherche Scientifique et Technique Outre-Mer, Paris

Brown D (2001) New encyclopedia of herbs & their uses, revised ed. Dorling Kindersley, London

Budowski G, Russo RO (1993) Live fence posts in Costa Rica. J Sustain Agric 3(2):65–87

Burkill HM (2000) Useful plants of West Tropical Africa, vol. 4: Families M - R., 2nd edn. Royal Botanic Gardens, Kew

Butzer KW (1981) Rise and fall of Axum, Ethiopia: a geo-archaeological interpretation. Am Antiq 46(3):471–495

Camarda L, Dayton T, Di Stefano V, Pitonzo R (2007) Chemical composition and antimicrobial activity of some oleogum resin essential oils from *Boswellia* spp. (Burseraceae). Ann Chim 97:837–844

Canales-Martinez M, Rivera-Yañez CR, Salas-Oropeza J, Lopez HR, Jimenez-Estrada M, Rosas-Lopez R, Duran DA, Flores C, Hernandez LB, Rodriguez-Monroy MA (2017) Antimicrobial activity of *Bursera morelensis* Ramírez essential oil. African J Trad Complement Altern Med 14(3):74–82

Carrera-Martinez CA, Rosas-López R, Rodríguez-Monroy MA, Canales-Martínez M, Román-Guerrero A, Jiménez-Alvarado R (2014) Chemical composition and in vivo anti-inflammatory activity of *Bursera morelensis* Ramírez essential oil. J Essent Oil Bear Plants 17(5):758–768

Carvajal D, Alvarez R, Osorio E (2016) Chemical variability of essential oils of *Protium colombianum* from two tropical life zones and their in vitro activity against isolates of *Fusarium*. J Pest Sci 89:241–248

Case RJ, Tucker AO, Maciarello MJ, Wheeler KA (2003) Chemistry and ethnobotany of commercial incense copals, copal blanco, copal oro, and copal negro, of North America. Econ Bot 57(2):189–202

Chen Y, Zhou C, Ge Z, Liu Y, Liu Y, Feng W, Li SEN, Chen G, Wei T (2013) Composition and potential anticancer activities of essential oils obtained from myrrh and frankincense. Oncol Lett 6:1140–1146

Chigora P, Masocha R, Mutenheri F (2007) The role of indigenous medicinal knowledge (IMK) in the treatment of ailments in rural Zimbabwe: the case of Mutirikwi communal lands. J Sustain Develop Africa 9(2):26–43

Choi IH, Park JY, Shin SC, Kim J, Park IK (2007) Nematicidal activity of medicinal plant essential oils against the pinewood nematode (*Bursaphelenchus xylophilus*). Appl Entomol Zool 42(3):397–401

Citó AMGL, Costa FB, Lopes JAD, Oliveira VMM, Chaves MH (2006) Identificação de constituintes voláteis de frutos e folhas de *Protium heptaphyllum* Aubl (March). Rev Bras Plant Med 8(4):4–7

Compaoré M, Meda RNT, Bakasso S, Vlase L, Kiendrebeogo M (2016) Antioxidative, anti-inflammatory potentials and phytochemical profile of *Commiphora africana* (A. Rich.) Engl. (Burseraceae) and *Loeseneriella africana* (Willd.) (Celastraceae) stem leaves extracts. Asian Pac J Trop Biomed 6(8):665–670

Craveiro A, Corsano S, Proietti G, Strappaghetti G (1983) Constituents of essential oil of *Commiphora guidotti*. Planta Med 48:97–98

Cravo L, Périneau F, Delmas M, Bessiere JM (1992) Volatiles of the fruit of *Dacryodes buettneri* H. J. Lam and *D. igaganga* Aubrev. et Pellegr. (Burseraceae). J Essent Oil Res 4(1):95–96

da Silva MFGF, RHP F, Gray AI, Lechat JR, Waterman PG (1990) Lanost-7-en triterpenes from stem bark of *Santiria trimera*. Phytochemistry 29(5):1629–1632

da Silva ER, Oliveira DR, Leitão SG, Assis IM, Veiga-Junior VF, Lourenço MC, Alviano DS, Alviano CS, Bizzo HR (2013) Essential oils of *Protium* spp. samples from Amazonian popular markets: chemical composition, physicochemical parameters and antimicrobial activity. J Essent Oil Res 25(3):171–178

Da Silva RCS, Milet-Pinheiro P, Da Silva PCB, Da Silva AG, Da Silva MV, Do Amaral Ferraz Navarro DM, Da Silva NH (2015) (*E*)-Caryophyllene and α-humulene: *Aedes aegypti* oviposition deterrents elucidated by gas chromatography-electrophysiological assay of *Commiphora leptophloeos* leaf oil. PLoS One 10(12):1–14

da Silva ER, de Oliveira DR, Melo MFF, Bizzo HR, Leitão SG (2016) Report on the Malungo expedition to the Erepecuru river, Oriximiná, Brazil. Part I: is there a difference between black and white breu? Rev Bras Farmacogn 26:647–656

Daly DC (1999) Notes on *Trattinnickia*, including a synopsis in eastern Brazil's Atlantic forest complex. Studies in Neotropical Burseraceae IX. Kew Bull 54(1):129–137

Daly DC, Melo MF (2017) Four new species of *Trattinnickia* from South America. Studies in Neotropical Burseraceae XXII. Brittonia 69(3):376–386

Daly DC, Harley MM, Martínez-Habibe MC, Weeks A (2010) Burseraceae. In: Kubitzki K (ed) The families and general of vascular plants, vol. X. Flowering plants. Eudicots. Springer, Heidelberg, pp 76–104

Dannaway FR (2010) Strange fires, weird smokes and psychoactive combustibles: entheogens and incense in ancient traditions. J Psychoactive Drugs 42(4):485–497

de Carvalho LE, Pinto DS, Lima MP, Marques MOM, Facanali R (2009) The chemistry of essential oils of *Crepidospermum rhoifolium*, *Trattinnickia rhoifolia* and *Protium elegans* of the Amazon region. J Essent Oil Bear Plants 12(1):92–96

de Carvalho LE, Pinto DS, Magalhães LAM, Lima MP, Marques MOM, Facanali R (2010) Chemical constituents of essential oil of *Protium decandrum* (Burseraceae) from western Amazon. J Essent Oil Bear Plants 13:181–184

de Carvalho LE, Magalhães LAM, Lima MP, Marques MOM, Facanali R (2013) Essential oils of *Protium* of the Adolpho Ducke Forest Preserve: *Protium crassipetalum*, *P. heptaphyllum* subs. *ulei*, *P. pilosissimum* and *P. polybotryum*. J Essent Oil Bear Plants 16(4):551–554

de Rapper S, Van Vuuren SF, Kamatou GPP, Viljoen AM, Dagne E (2012) The additive and synergistic antimicrobial effects of select frankincense and myrrh oils – a combination from the pharaonic pharmacopoeia. Lett Appl Microbiol 54(4):352–358

Dekebo A, Zewdu M, Dagne E (1999) Volatile oils of frankincense from *Boswellia papyrifera*. Bull Chem Soc Ethiopia 13(1):93–96

De-Nova JA, Medina R, Montero JC, Weeks A, Rosell JA, Olson ME, Eguiarte LE, Magallón S (2011) Insights into the historical construction of species-rich Mesoamerican seasonally dry tropical forests: the diversification of *Bursera* (Burseraceae, Sapindales). New Phytol 193:276–287

Dictionary of Natural Products (2017) Dictionary of natural products on DVD. CRC Press, Boca Raton, FL

Dike IP, Obembe OO, Adebiyi FE (2012) Ethnobotanical survey for potential anti-malarial plants in south-western Nigeria. J Ethnopharmacol 144(3):618–626

Djilani A, Dicko A (2012) The therapeutic benefits of essential oils. In: Bouayed J (ed) Nutrition, well-being and health. InTech, Rijeka, Croatia, pp 155–178

Dongmo PMJ, Tchoumbougnang F, Ndongson B, Agwanande W, Sandjon B, Zollo PHA, Menut C (2010) Chemical characterization, antiradical, antioxidant and anti-inflammatory potential of the essential oils of *Canarium schweinfurthii* and *Aucoumea klaineana* (Burseraceae) growing in Cameroon. Agric Biol J North Am 1(4):606–611

Dounchaly AR, Jaimand K, Mozaffari S (2016) Comparison of essential oils compositions of *Boswellia carteri* Birdwood as a food and non-food in different distillation from Iranian market. J Med Plants By Prod 1:39–44

Dozmorov MG, Yang Q, Wu W, Wren J, Suhail MM, Woolley CL, Young DG, Fung K, Lin H (2014) Differential effects of selective frankincense (ru xiang) essential oil versus non-selective sandalwood (tan xiang) essential oil on cultured bladder cancer cells: a microarray and bioinformatics study. Chin Med 9:18

Dudai N, Shachter A, Satyal P, Setzer WN (2017) Chemical composition and monoterpenoid enantiomeric distribution of the essential oils from apharsemon (*Commiphora gileadensis*). Medicines 4(3):66

Duke JA (1970) Ethnobotanical observations on the Chocó Indians. Econ Bot 24(3):344–366

Duke JA, Bogenschutz-Godwin MJ, Ottesen AR (2009) Duke's handbook of medicinal plants of Latin America. CRC Press, Boca Raton, FL

Ehrlich PR, Raven PH (1964) Butterflies and plants: a study in coevolution. Evolution 18(4):586–608

El Ashry ESH, Rashed N, Salama OM, Saleh A (2003) Components, therapeutic value and uses of myrrh. Pharmazie 58(3):163–168

Eldridge J (1975) Bush medicine in the Exumas and Long Island, Bahamas: a field study. Econ Bot 29(4):307–332

Engonga PE, Abdoul-Latif FM, Obame LC, Mewono L (2012) Volatile constituents of *Canarium schweinfurthii* Engl. essential oil from Gabon. Int J Agri Sci 2(3):200–203

Epstein I (1990) The Babylonian Talmud, 30 volumes. Bloch Publishing Company, New York
Erukainure OL, Mopuri R, Oyebode OA, Koorbanally NA, Islam MS (2017) *Dacryodes edulis* enhances antioxidant activities, suppresses DNA fragmentation in oxidative pancreatic and hepatic injuries; and inhibits carbohydrate digestive enzymes linked to type 2 diabetes. Biomed Pharmacother 96:37–47
Eshete A (2011) The frankincense tree of ethiopia: ecology, productivity and population dynamics. Dissertation, Wageningen University
Eshete A, Teketay D, Hulten H (2005) The socio-economic importance and status of populations of *Boswellia papyrifera* (Del.) Hochst. in northern Ethiopia: the case of North Gonder Zone. Forests Trees Livelihoods 15:55–74
Eshete A, Sterck FJ, Bongers F (2012) Frankincense production is determined by tree size and tapping frequency and intensity. Forest Ecol Manag 274:136–142
Eslamieh J (2016) The genus *Commiphora*; preservation through horticulture. A Book's Mind, Fort Collins, CO
Eslamieh J (2017) Cultivation of *Boswellia*, 2nd edn. A Book's Mind, Fort Collins, CO
Espinosa D, Llorente J, Morrone JJ (2006) Historical biogeographical patterns of the species of *Bursera* (Burseraceae) and their taxonomic implications. J Biogeogr 33:1945–1958
Evans WC (2009) Trease and evans pharmacognosy, 16th edn. Saunders Ltd, Edinburgh
Evans PH, Becerra JX (2006) Non-terpenoid essential oils from *Bursera chemapodicta*. Flavour Fragr J 21:616–618
Farah MH (2008) Non-Timber Forest Production (NTFP) extraction in arid environments: land-use change, frankincense production and the sustainability of *Boswellia sacra* in Dhofar (Oman). Dissertation, University of Arizona
Ferrara L (2016) Phytotherapy as a preventive and adjuvant for the rhinitis. IOSR J Pharm 6(1):6–11
Fichtl R, Addi A (1994) Honeybee flora of ethiopia: some 500 common herbs, shrubs and trees. Margraf Publishers GmbH, Weikersheim
Firn RD, Jones CG (2003) Natural products – a simple model to explain chemical diversity. Nat Prod Rep 20(4):382–391
Fonkeng LS, Mouokeu RS, Tume C, Njateng GSS, Kamcthueng MO, Ndonkou NJ, Kuiate JR (2015) Anti-*Staphylococcus aureus* activity of methanol extracts of 12 plants used in Cameroonian folk medicine. BMC Res Notes 8:710
Fraternale D, Sosa S, Ricci D, Genovese S, Messina F, Tomasini S, Montanari F, Marcotullio MC (2011) Anti-inflammatory, antioxidant and antifungal furanosesquiterpenoids isolated from *Commiphora erythraea* (Ehrenb.) Engl. resin. Fitoterapia 82(4):654–661
Frawley D, Lad V (2001) The yoga of herbs: an ayurvedic guide to herbal medicine, 2nd edn. Lotus Press, Twin Lakes, WI
Gazzaneo LRS, de Lucena RFP, de Albuquerque UP (2005) Knowledge and use of medicinal plants by local specialists in an region of Atlantic Forest in the state of Pernambuco (Northeastern Brazil). J Ethnobiol Ethnomed 8:9
Gebrehiwot K, Muys B, Haile M, Mitloehner R (2003) Introducing *Boswellia papyrifera* (Del.) Hochst and its non-timber forest product, frankincense. Int Forest Rev 5(4):348–353
Gebrehiwot M, Asres K, Bisrat D, Mazumber A, Lindemann P, Bucar F (2015) Evaluation of the wound healing property of *Commiphora guidottii* Chiov. ex. Guid. BMC Complemen Altern Med 15:282
Gebremedhin T (1997) *Boswellia papyrifera* (Del.) Hochst. from Western Tigray: opportunities, constrains and seed germination responses. Dissertation, Swedish University of Agricultural Sciences
Gemedo-Dalle T, Maass BL, Isselstein J (2005) Plant biodiversity and ethnobotany of Borana pastoralists in southern Oromia, Ethiopia. Econ Bot 59(1):43–65
Gertsch J, Leonti M, Raduner S, Racz I, Chen JZ, Xie XQ, Altmann KH, Karsak M, Zimmer A (2008) Beta-caryophyllene is a dietary cannabinoid. Proc Natl Acad Sci U S A 105(26):9099–9104
Getahon A (1976) Some common medicinal and poisonous plants used in ethiopian folkmedicine. Addis Abeba University, Addis Abebe

Ghelardini C, Galeotti N, Mannelli LDC, Mazzanti G, Bartolini A (2001) Local anaesthetic activity of β-caryophyllene. Farmaco 56:387–389
Giang PM, Konig WA, Son PT (2006) Chemical composition of the resin essential oil of *Canarium album* from Vietnam. Chem Nat Comp 42(5):523–524
Gigliarelli G, Becerra JX, Curini M, Marcotullio MC (2015) Chemical composition and biological activities of fragrant Mexican copal (*Bursera* spp.). Molecules 20:22383–22394
Gompper ME, Hoylman AM (1993) Grooming with *Trattinnickia* resin: possible pharmaceutical plant use by coatis in Panama. J Trop Ecol 9(4):533–540
Groenendijk P, Eshete A, Sterck FJ, Zuidema PA, Bongers F (2012) Limitations to sustainable frankincense production: blocked regeneration, high adult mortality and declining populations. J Appl Ecol 49:164–173
Groom N (1981) Frankincense and myrrh: a study of the Arabian incense trade. Longman Group, London
Gupta M, Rout PK, Misra LN, Gupta P, Singh N, Darokar MP, Saikia D, Singh SC, Bhakuni RS (2017) Chemical composition and bioactivity of *Boswellia serrata* Roxb. essential oil in relation to geographical variation. Plant Biosyst 151(4):623–629
Habeeb SM, El-Namaky AH, Salama MA (2009) Efficiency of *Allium cepa* and *Commiphora molmol* as a larvicidal agent against fourth stage larvae of *Culex pipiens* (Diptera: Culicidae). Am Euras J Agric Environ Sci 5(2):196–203
Hakkim FL, Al-Buloshi M, Al-Sabahi J (2015) Frankincense derived heavy terpene cocktail boosting breast cancer cell (MDA-MB-231) death in vitro. Asian Pac J Trop Biomed 5(10):824–828
Hameed HA (1983) Avicenna's tract on cardiac drugs and essays on Arab cardiotherapy. Hamdard Foundation, Karachi
Hamm S, Bleton J, Connan J, Tchapla A (2005) A chemical investigation by headspace SPME and GC–MS of volatile and semi-volatile terpenes in various olibanum samples. Phytochemistry 66:1499–1514
Hammer KA, Carson CF, Riley TV (1999) Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol 86:985–990
Hanuš LO, Řezanka T, Dembitsky VM, Moussaieff A (2005) Myrrh – *Commiphora* chemistry. Biomed Pap 149(1):3–28
Hanuš LO, Rosenthal D, Řezanka T, Dembitsky VM, Moussaief A (2008) Fast and easy GC/MS identification of myrrh resins. Pharmaceut Chem J 42(12):719–720
Hausen BM, Reichling J, Harkenthal M (1999) Degradation products of monoterpenes are the sensitizing agents in tea tree oil. Am J Cont Dermat 10(2):68–77
Higgs L (1978) Bush medicine in the Bahamas. Nassau Guardian, Nassau
Hoe VB, Siong KH (1999) The nutritional value of indigenous fruits and vegetables in Sarawak. Asia Pac J Clin Nutr 8(1):24–31
Hull BZ (2008) Frankincense, myrrh, and spices: the oldest global supply chain? J Macromark 28(3):275–288
İşcan G, Kırımer N, Demirci F, Demirci B, Noma Y, Başer KHC (2012) Biotransformation of (−)-(*R*)-α-phellandrene: antimicrobial activity of its major metabolite. Chem Biodivers 9:1525–1532
Iwu MM (2014) Handbook of African medicinal plants, 2nd edn. CRC Press, Boca Raton, FL
Jirovetz L, Buchbauer G, Geissler M, Ngassoum MB, Parmentier M (2003) Pulp aroma compounds of untreated, boiled and roasted African pear [*Dacryodes edulis* (G. Don) H.J. Lam] fruits from Cameroon by HS-SPME analysis coupled with GC/FID and GC/MS. Eur Food Res Technol 218:40–43
Junor GO, Porter RBR, Facey PC, Yee TH (2007) Investigation of essential oil extracts from four native Jamaican species of *Bursera* for antibacterial activity. West Indian Med J 56:22–25
Junor GAO, Porter RBR, Yee TH (2008a) The leaves, bark and fruits of *Bursera simaruba* (L.) Sarg. from Jamaica. J Essent Oil Res 20(5):426–429
Junor GAO, Porter RBR, Yee TH, Williams LAD (2008b) Chemical composition and insecticidal activity of the essential oils from *Bursera hollickii* (Britton) Found in Jamaica. J Essent Oil Res 20(6):560–566

Junor GAO, Porter RBR, Yee TH (2010a) Chemical composition of essential oils from the aerial parts of Jamaican *Bursera lunanii* Spreng. J Essent Oil Res 22(6):602–606

Junor GAO, Porter RBR, Yee TH, Waugh T (2010b) The volatile constituents from the leaves, bark and fruits of *Bursera aromatica* (Proctor) found in Jamaica. J Essent Oil Res 22(1):19–23

Kalenda DT, Missang CE, Kinkela TT, Krebs HC, Renard CMGC (2002) New developments in the chemical characterisation of the fruit of *Dacryodes edulis* (G. Don) H.J. Lam. Forests Trees Livelihoods 12(1–2):119–123

Karnick CR (1995) Pharmacopoeial standards of herbal plants. Sri Satguru Publications, Delhi

Kasali AA, Adio AM, Oyedeji AO, Eshilokun AO, Adefenwa M (2002) Volatile constituents of *Boswellia serrata* Roxb. (Burseraceae) bark. Flavour Fragr J 17:462–464

Katsuyama S, Mizoguchi H, Kuwahata H, Komatsu T, Nagaoka K, Nakamura H, Bagetta G, Sakurada T, Sakurada S (2013) Involvement of peripheral cannabinoid and opioid receptors in β-caryophyllene-induced antinociception. Eur J Pain 17(5):664–675

Khalid SA (1983) Chemistry of the Burseraceae. In: Waterman PG, Grundon MF (eds) Chemistry and chemical taxonomy of the Rutales. Academic Press, New York, pp 281–299

Kitchen KA (1971) Punt and how to get there. Orientalia 40(2):184–207

Kohoude MJ, Gbaguidi F, Agbani P, Ayedoun MA, Cazaux S, Bouajila J (2017) Chemical composition and biological activities of extracts and essential oil of *Boswellia dalzielii* leaves. Pharm Biol 55(1):33–42

Kong KW, Chew LY, Prasad KN, Lau CY, Ismail A, Sun J, Hosseinpoursarmadi B (2011) Nutritional constituents and antioxidant properties of indigenous kembayau (*Dacryodes rostrata* (Blume) H. J. Lam) fruits. Food Res Int 44(7):2332–2338

Koudou J, Abena AA, Ngaissona P, Bessière JM (2005) Chemical composition and pharmacological activity of essential oil of *Canarium schweinfurthii*. Fitoterapia 76:700–703

Koudou J, Obame LC, Kumulungui BS, Edou P, Figueredo G, Chalchat JC, Traore AS (2009) Volatile constituents and antioxidant activity of *Aucoumea klaineana* Pierre essential oil. African J Pharm Pharmacol 3(6):323–326

Kubmarawa D, Ogunwande IA, Okorie DA, Olawore NO, Kasali AA (2006) Constituents of the essential oils of *Boswellia dalzielii* Hutch. from Nigeria. J Essent Oil Res 18(2):119–120

Lago JHG, Tezoto J, Yazbek PB, Cassas F, Santos JFL, Rodrigues E (2016) Exudates used as medicine by the "caboclos river-dwellers" of the Unini River, AM, Brazil – classification based in their chemical composition. Rev Bras Farmacogn 26:379–384

Langenheim JH (2003) Plant resins: chemistry, evolution, ecology, and ethnobotany. Timber Press, Portland, OR

Lebaka PAR, Ratnam KV, Lepakshi BM, Bhumi NR, Lebaka VR (2015) Chemical profile, antioxidant and antimicrobial activity of essential oils from *Boswellia ovalifoliolata* Bal. et. Henry. Int J Pharmaceut Clin Res 7(1):96–101

Lemenih M, Kassa H (2011) Management guide for sustainable production of frankincense. Center for International Forestry Research, Bogor

Lemenih M, Teketay D (2003) Frankincense and myrrh resources of Ethiopia: II. Medicinal and industrial uses. Ethiop J Sci 26(2):161–172

Li ZF, Li K, Li FY, Chen H, Zhao HH, Du YY, Li QP (2015) [GC-MS analysis and antioxidant activity of volatile oil from leaves of *Canarium pimela* Leenh. in Rongxian county]. J South Agric 46(2):317–321

Liang GY, Bates CD, Gray AI, Waterman PG (1988a) The volatile oil of the oleo resin of *Aucoumea klaineana* collected in Gabon. Planta Med 54(4):368–369

Liang GY, Gray AI, Waterman PG (1988b) Tirucallane and oleanane triterpenes from the resin of *Aucoumea klaineana*. Phytochemistry 27(7):2283–2286

Lima MP, Braga PAC, Macedo ML, da Silva MFGF, Ferreira AG, Fernandes JB, Vieira PC (2004) Phytochemistry of *Trattinnickia burserifolia*, *T. rhoifolia*, and *Dacryodes hopkinsii*: chemosystematic implications. J Braz Chem Soc 15(3):385–394

Lippi MM, Giuliani C, Gonnelli T, Bini LM (2011) Floral color changes in *Boswellia sacra* Flueck. (Burseraceae): a dialogue between plant and pollinator. Flora 206(9):821–826

Lis-Balchin M (2010) Aromatherapy with essential oils. In: Başer KHC, Buchbauer G (eds) Handbook of essential oils. CRC Press, Boca Raton, FL, pp 549–583
Lis-Balchin M, Hart S (1997) A preliminary study of the effect of essential oils on skeletal and smooth muscle in vitro. J Ethnopharmacol 58:183–187
Lugo AE, Wadsworth FH (1990) *Dacryodes excelsa* Vahl Tabonuco. In: Burns RM, Honkala BH (eds) Silvics of North America, vol. 2, Hardwoods. United States Department of Agriculture, Washington, DC, pp 284–287
Mabberley DJ (2008) Mabberley's plant-book, 3rd edn. Cambridge University Press, Cambridge
Maděra P, Paschová Z, Ansorgová A, Vrškový B, Lvončík S, Habrová H (2017) Volatile compounds in oleo-gum resin of Socotran species of Burseraceae. Acta Univ Agric Silvic Mendel Brun 65(1):73–90
Mahboubi M, Kashani LMT (2016) The anti-dermatophyte activity of *Commiphora molmol*. Pharm Biol 54(4):720–725
Mahr D (2012) *Commiphora*: an introduction to the genus. Cactus Succulent J 84(3):140–154
Manguro LOA, Mukonyi KM, Githiomi JK (1996) Bisabolenes and furanosesquiterpenoids of Kenyan *Commiphora kua* resin. Planta Med 62:84–85
Manzano Santana P, Miranda M, Gutiérrez Y, Garcia G, Orellana T, Orellana A (2009) Efecto antiinflamatorio y composición química del aceite de ramas de *Bursera graveolens* Triana & Planch. (palo santo) de Ecuador. Rev Cub Plant Med 14(3):45–53
Marcotullio MC, Santi C, Mwankie GNOM, Curini M (2009) Chemical composition of the essential oil of *Commiphora erythraea*. Nat Prod Commun 4(12):1751–1754
Marongiu B, Piras A, Porcedda S, Scorciapino A (2005) Chemical composition of the essential oil and supercritical CO_2 extract of *Commiphora myrrha* (Nees) Engl. and of *Acorus calamus*. J Agric Food Chem 53:7939–7943
Marongiu B, Piras A, Porcedda S, Tuveri E (2006) Extraction of *Santalum album* and *Boswellia carterii* Birdw. volatile oil by supercritical carbon dioxide: influence of some process parameters. Flavour Fragr J 21:718–724
Marques DD, Sartori RA, Lemos TLG, Machado LL, de Souza JSN, Monte FJQ (2010) Chemical composition of the essential oils from two subspecies of *Protium heptaphyllum*. Acta Amazon 40(1):227–230
Martins AP, Salgueiro LR, Gonçalves MJ, Proença da Cunha A, Vila R, Cañigueral S (2003) Essential oil composition and antimicrobial activity of *Santiria trimera* bark. Planta Med 69(1):77–79
Mathe C, Culioli G, Archier P, Vieillescazes C (2004) Characterization of archaeological frankincense by gas chromatography–mass spectrometry. J Chromatogr A 1023:277–285
Medzegue MJ, Stokes A, Gardrat C, Grelier S (2013) Analysis of volatile compounds in *Aucoumea klaineana* oleoresin by static headspace/gas chromatography/mass spectrometry. J Nat Prod (India) 6:81–89
Mengistu T (2011) Physiological ecology of the frankincense tree. Dissertation, Wageningen University
Mengistu T, Sterck FJ, Anten NPR, Bongers F (2012) Frankincense tapping reduced photosynthetic carbon gain in *Boswellia papyrifera* (Burseraceae) trees. For Ecol Manag 278:1–8
Mertens M, Buettner A, Kirchhoff E (2009) The volatile constituents of frankincense – a review. Flavour Fragr J 24:279–300
Michie CA, Cooper E (1991) Frankincense and myrrh as remedies in children. J Roy Soc Med 84(10):602–605
Mies BA, Lavranos JJ, James GJ (2000) Frankincense on Soqotra Island (*Boswellia*, Burseraceae; Yemen). Cactus Succulent J 72(5):265–278
Mikhaeil BR, Maatooq GT, Badria FA, Amer MMA (2003) Chemistry and immunomodulatory activity of frankincense oil. Z Naturforsch C 58(3–4):230–238
Miller A (2015) *Boswellia* sp. A., The IUCN Red List of Threatened Species 2015: e.T44811A69307248
Miller AG, Morris M (2004) Ethnoflora of the Soqotra Archipelago. Royal Botanic Gardens, Edinburgh

Missouri Botanical Garden (2017). Tropicos.org. www.tropicos.org. Accessed 13 November 2017
Miyazawa M, Watanabe H, Umemoto K, Kameoka H (1998) Inhibition of acetylcholinesterase activity by essential oils of *Mentha* species. J Agric Food Chem 46:3431–3434
Mobin M, de Lima SG, Almeida LTG, Silva Filho JC, Rocha MS, Oliveira AP, Mendes MB, Carvalho FAA, Melhem MSC, Costa JGM (2017) Gas chromatography-triple quadrupole mass spectrometry analysis and vasorelaxant effect of essential oil from *Protium heptaphyllum* (Aubl.) March. Biomed Res Int 2017:1928171
Mogana R, Wiart C (2011) *Canariu*m L.: a phytochemical and pharmacological review. J Phar Res 4(8):2482–2489
Mohamed AA, Ali SI, El-Baz FK, Hegazy AK, Kord MA (2014) Chemical composition of essential oil and in vitro antioxidant and antimicrobial activities of crude extracts of *Commiphora myrrha* resin. Ind Crop Prod 57:10–16
Monzote L, Hill GM, Cuellar A, Scull R, Setzer WN (2012) Chemical composition and anti-proliferative properties of *Bursera graveolens* essential oil. Nat Prod Commun 7(11):1531–1534
More A (1899) Chemical examination of the oleo-resin of *Dacryodes hexandra*. J Chem Soc Trans 75:718–722
Moreno J, Aparicio R, Velasco J, Rojas LB, Usubillaga A, Lue-Merú M (2010a) Chemical composition and antibacterial activity of the essential oil from fruits of *Bursera tomentosa*. Nat Prod Commun 5(2):311–313
Moreno J, Rojas LB, Aparicio R, Marco LM, Usubillaga A (2010b) Chemical composition of the essential oil from the bark *Bursera tomentosa* (Jacq) Tr & Planch. Bol Latinoamer Caribe Plant Med Arom 9(6):491–494
Morteza-Semnani K, Saeedi M (2003) Constituents of the essential oil of *Commiphora myrrha* (Nees) Engl. var. *molmol*. J Essen Oil Res 15(1):50–51
Morton JF (1981) Atlas of medicinal plants of middle America, vol. I. Charles C. Thomas, Springfield, IL
Mothana RA, Al-Rehaily AJ, Schultze W (2010) Chemical analysis and biological activity of the essential oils of two endemic Soqotri *Commiphora* species. Molecules 15(2):689–698
Mothana RAA, Hasson SS, Schultze W, Mowitz A, Lindequist U (2011) Phytochemical composition and in vitro antimicrobial and antioxidant activities of essential oils of three endemic Soqotraen *Boswellia* species. Food Chem 126(3):1149–1154
Moussaieff A, Mechoulam R (2009) *Boswellia* resin: from religious ceremonies to medical uses; a review of in-vitro, in-vivo and clinical trials. J Pharm Pharmacol 61:1281–1293
Mpiana PT, Tshibangu DST, Shetonde OM, Ngbolua KN (2007) In vitro antidrepanocytary activity (anti-sickle cell anemia) of some Congolese plants. Phytomedicine 14:192–195
Murthy KSR, Reddy MC, Rani SS, Pullaiah T (2016) Bioactive principles and biological properties of essential oils of Burseraceae: a review. J Pharmacogn Phytochem 5(2):247–258
Mvitu-Muaka M, Longo-Mbenza B, Mokondjimobe E, Gombet T, Kibokela ND, Tulomba MD, Wayiza MS (2012) Intake of *Gnetum africanum* and *Dacryodes edulis*, imbalance of oxidant/antioxidant status and prevalence of diabetic retinopathy in Central Africans. PLoS One 7(12):e49411
Nagawa C, Böhmdorfer S, Rosenau T (2015) Chemical composition and anti-termitic activity of essential oil from *Canarium schweinfurthii* Engl. Ind Crop Prod 71:75–79
Ni X, Suhail MM, Yang Q, Cao A, Fung KM, Postier RG, Woolley C, Young G, Zhang J, Lin HK (2012) Frankincense essential oil prepared from hydrodistillation of *Boswellia sacra* gum resins induces human pancreatic cancer cell death in cultures and in a xenograft murine model. BMC Complement Altern Med 12:253
Niebler J, Buettner A (2015) Identification of odorants in frankincense (*Boswellia sacra* Flueck.) by aroma extract dilution analysis and two-dimensional gas chromatography – mass spectrometry/olfactometry. Phytochemistry 109:66–75
Niebler J, Buettner A (2016) Frankincense revisited, part 1: comparative analysis of volatiles in commercially relevant *Boswellia* species. Chem Biodivers 13(5):613–629

Niebler J, Zhuravlova K, Minceva M, Buettner A, Straße G (2016) Fragrant sesquiterpene ketones as trace constituents in frankincense volatile oil of *Boswellia sacra*. J Nat Prod 79(4):1160–1164

Nikolić M, Smiljković M, Marković T, Ćirić A, Glamočlija J, Marković D, Soković M (2016) Sensitivity of clinical isolates of *Candida* to essential oils from Burseraceae family. EXCLI J 15:280–289

Noge K, Becerra JX (2009) Germacrene D, a common sesquiterpene in the genus *Bursera* (Burseraceae). Molecules 14:5289–5297

Noge K, Shimizu N, Becerra JX (2010) (*R*)-(−)-Linalyl acetate and (*S*)-(−)-germacrene D from the leaves of Mexican *Bursera linanoe*. Nat Prod Commun 5(3):351–354

Noge K, Venable DL, Becerra JX (2011) 2-Phenylethanol in the leaves of *Bursera velutina* Bullock (Burseraceae). Acta Bot Mex (97):9–16

Noguera B, Díaz E, García MV, San Feliciano A, López-Perez JL, Israel A (2004) Anti-inflammatory activity of leaf extract and fractions of *Bursera simaruba* (L.) Sarg (Burseraceae). J Ethnopharmacol 92:129–133

Nordberg A, Svensson AL (1998) Cholinesterase inhibitors in the treatment of Alzheimer's disease: a comparison of tolerability and pharmacology. Drug Saf 19(6):465–480

Northrup CC, Bentley JH, Eckes AE, Manning P, Pomeranz K, Topik S (2005) Encyclopedia of world trade: from ancient times to the present. Routledge, New York

Obame LC, Koudou J, Chalchat JC, Bassolé I, Edou P, Ouattara AS, Traore AS (2007a) Volatile components, antioxidant and antibacterial activities of *Dacryodes buettneri* H. J. Lam. essential oil from Gabon. Sci Res Essays 2(11):491–495

Obame LC, Koudou J, Kumulungui BS, Bassolé IHN, Edou P, Ouattara AS, Traoré AS (2007b) Antioxidant and antimicrobial activities of *Canarium schweinfurthii* Engl. essential oil from Centrafrican Republic. African J Biotechnol 6(20):2319–2323

Obame LC, Edou P, Bassolé IHN, Koudou J, Agnaniet H, Eba F, Traore AS (2008) Chemical composition, antioxidant and antimicrobial properties of the essential oil of *Dacryodes edulis* (G. Don) H. J. Lam from Gabon. African J Microbiol Res 2:148–152

Obame LCE, Bongui JB, Andzi BT, Ondo JP, Edou EP, Koudou J (2014) Antifungal and antibacterial activities of *Aucoumea klaineana* Pierre essential oil From Gabon. Vedic Res Int Phytomed 2(1):17–21

Okolo CA, Ejere VC, Chukwuka O, Ezeigbo II, Nwibo DD, Okorie AN (2016) Hexane extract of *Dacryodes edulis* fruits possesses anti-diabetic and hypolipidaemic potentials in alloxan diabetes of rats. African J Trad Complemen Altern Med 13(4):132–144

Omonhinmin AC (2012) Ethnobotany of *Dacryodes edulis* (G. Don) H.J. Lam in southern Nigeria 1: practices and applications among the Yoruba speaking people. Ethnobot Res Appl 10:175–184

Onana JM (2008) A synoptic revision of *Dacryodes* (Burseraceae) in Africa, with a new species from Central Africa. Kew Bull 63(3):385–400

Onocha PA, Ekundayo O, Oyelola O, Laakso I (1999) Essential oils of *Dacryodes edulis* (G. Don) H. J. Lam (African pear). Flavour Fragr J 14:135–139

Paula-Freire LIG, Andersen ML, Gama VS, Molska GR, Carlini ELA (2014) The oral administration of *trans*-caryophyllene attenuates acute and chronic pain in mice. Phytomedicine 21(3):356–362

Peters CM, Purata SE, Chibnik M, Brosi BJ, Lopez AM, Ambrosio M (2003) The life and times of *Bursera glabrifolia* (H.B.K.) Engl. in Mexico: a parable for ethnobotany. Econ Bot 57(4):431–441

Phillips J (1997) Punt and Aksum: Egypt and the Horn of Africa. J African Hist 38:423–457

Pichersky E, Raguso RA (2018) Why do plants produce so many terpenoid compounds? New Phytol 220(3):692–702. https://doi.org/10.1111/nph.14178

Pickenhagen W (2017) History of odor and odorants. In: Buettner A (ed) Springer handbook of odor. Springer-Verlag, Berlin, pp 5–6

Pinto FE, Heringer OA, Silva MA, Uggere T, Ribeiro JS, Lenz D, Campos F, Lessa R, Endringer DC (2015) Stability and disinfecting proprieties of the toothbrush rinse of the essential oil of *Protium heptaphyllum*. African J Pharm Pharmacol 9(6):173–181

Plowden C, Uhl C, Oliveira FA (2002) Breu resin harvest by Tembé Indians and its dependence on a bark-boring beetle. In: Stepp JR, Wyndham FS, Zarger RK (eds) Ethnobiology and biocultural diversity. International Society of Ethnobiology, Athens, GA, pp 365–380

Pontes WJT, de Oliveira JCG, da Cámara CAG, Lopes ACHR, Gondim Junior MGC, de Oliveira JV, Barros R, Schwartz MOE (2007a) Chemical composition and acaricidal activity of the leaf and fruit essential oils of *Protium heptaphyllum* (Aubl.) Marchand (Burseraceae). Acta Amazon 37(1):103–110

Pontes WJT, de Oliveira JCS, da Camara CAG, Lopes ACHR, Gondim Júnior MGC, de Oliveira JV, Schwartz MOE (2007b) Composition and acaricidal activity of the resin's essential oil of *Protium bahianum* Daly against two spotted spider mite (*Tetranychus urticae*). J Essent Oil Res 19:379–383

Pontes WJT, Silva JMO, da Camara CAG, Gondim-Júnior MGC, Oliveira JV, Schwartz MOE (2010) Chemical composition and acaricidal activity of the essential oils from fruits and leaves of *Protium bahianum* Daly. J Essent Oil Res 22(3):279–283

Prakash B, Mishra PK, Kedia A, Dubey NK (2014) Antifungal, antiaflatoxin and antioxidant potential of chemically characterized *Boswellia carterii* Birdw essential oil and its in vivo practical applicability in preservation of *Piper nigrum* L. fruits. LWT Food Sci Technol 56(2):240–247

Price C, Forshaw R, Chamberlain A, Nicholson P (2016) Mummies, magic and medicine in Ancient Egypt: multidisciplinary essays for Rosalie David. Manchester University Press, Manchester

Queiroga CL, Duarte MCT, Ribeiro BB, Melillo P, de Magalhães PM (2007) Linalool production from the leaves of *Bursera aloexylon* and its antimicrobial activity. Fitoterapia 78:327–328

Ragunathan M, Senthamarai R (2013) Anti-inflammatory activity of essential oil of *Canarium strictum* Roxb. Iranian J Pharmaceut Sci 9(2):13–21

Rajagopal MS (2014) Canarium patentinervium Miq. (Burseraceae Kunth.): a phytochemical and pharmacological study. Dissertation, University of Nottingham (Malaysia Campus)

Raju AJS, Lakshmi PV, Ramana KV, Chandra PH (2012) Entomophily, ornithophily and anemochory in the self-incompatible *Boswellia ovalifoliolata* Bal. & Henry (Burseraceae), an endemic and endangered medicinally important tree species. J Threat Taxa 4(7):2673–2684

Ramos MFS, Guimarães AC, Siani AC (2003) Volatile monoterpenes from the oleoresin of *Trattinnickia rhoifolia*. Biochem Systemat Ecol 31:309–311

Rao VS, Maia JL, Oliveira FA, Lemos TLG, Chaves MH, Santos FA (2007) Composition and antinociceptive activity of the essential oil from *Protium heptaphyllum* resin. Nat Prod Commun 2(12):1199–1202

Reddy CS, Sudhakar KN, Murthy EN, Raju VS (2009) Traditional medicinal plants in Seshachalam Hills, Andhra Pradesh, India. J Med Plants Res 3(5):408–412

Rey-Valeirón C, Guzmán L, Saa LR, López-Vargas J, Valarezo E (2017) Acaricidal activity of essential oils of *Bursera graveolens* (Kunth) Triana & Planch and *Schinus molle* L. on unengorged larvae of cattle tick *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae). J Essent Oil Res 29(4):344–350

Richards LA, Dyer LA, Forister ML, Smilanich AM, Dodson CD, Leonard MD, Jeffrey CS (2015) Phytochemical diversity drives plant–insect community diversity. Proc Natl Acad Sci U S A 112(35):10973–10978

Rijkers T, Ogmazghi W, Wessel M, Bongers F (2006) The effect of tapping for frankincense on sexual reproduction in *Boswellia papyrifera*. J Appl Ecol 43:1188–1195

Riwom SH, Ndoye Foe FMC, Nyegue MA, Ambe Wanki R, Voundi Olugu S, Etoa FX (2015) Chemical composition and in vitro antibacterial activity of the essential oils of the leaves, resin and stem-barks of *Dacryodes edulis* (G. Don) H. J Lam growing in Cameroon on diarrhea associated strains. J Appl Pharmaceut Sci 5(10):6–11

Rosales-Ovares KM, Cicció-Alberti JF (2002) Aceite volátil de los frutos de *Bursera simaruba* (L.) Sarg. (Burseraceae) de Costa Rica. Ing Ciencia Quím 20(2):60–61

Roy NK, Deka A, Bordoloi D, Mishra S, Kumar AP, Sethi G, Kunnumakkara AB (2016) The potential role of boswellic acids in cancer prevention and treatment. Cancer Lett 377:74–86

Rüdiger AL, Siani AC, Viega Junior VF (2007) The chemistry and pharmacology of the South America genus *Protium* Burm. f. (Burseracea). Pharmacogn Rev 1(1):93–104

Sadhasivam S, Palanivel S, Ghosh S (2016) Synergistic antimicrobial activity of *Boswellia serrata* Roxb. ex Colebr. (Burseraceae) essential oil with various azoles against pathogens associated with skin, scalp and nail infections. Lett Appl Microbiol 63:495–501

Saeed MA, Sabir AW (2004) Antibacterial activities of some constituents from oleo-gum-resin of *Commiphora mukul*. Fitoterapia 75:204–208

Santos JFL, Pagani E, Ramos J, Rodrigues E (2012) Observations on the therapeutic practices of riverine communities of the Unini River, AM, Brazil. J Ethnopharmacol 142:503–515

Sarup P, Bala S, Kamboj S (2015) Pharmacology and phytochemistry of oleo-gum resin of *Commiphora wightii* (guggulu). Scientifica 2015:138039

Satyal P, Pappas RS (2016) First reporting on the chemistry and biological activity of a novel *Boswellia* chemotype: the methoxy alkane frankincense. Global J Sci Front Res B Chem 16(2):1–9

Satyal P, Powers CN, Parducci R, Mcfeeters RL, Setzer WN (2017) Chemical composition, enantiomeric distribution, and antifungal activity of the oleoresin essential oil of *Protium amazonicum* from Ecuador. Medicines 4(4):70

Sawamura M, Son US, Choi HS, Kim MSL, Phi NTL, Fears M, Kumagai C (2004) Compositional changes in commercial lemon essential oil for aromatherapy. Int J Aromather 14(1):27–63

Schillaci D, Arizza V, Dayton T, Camarda L, Di Stefano V (2008) In vitro anti-biofilm activity of *Boswellia* spp. oleogum resin essential oils. Lett Appl Microbiol 47(5):433–438

Schultes RE, Raffauf RF (1990) The healing forest: medicinal and toxic plants of Northwest Amazonia. Dioscorides Press, Portland, OR

Semenya SS, Maroyi A (2012) Medicinal plants used by the Bapedi traditional healers to treat diarrhoea in the Limpopo Province, South Africa. J Ethnopharmacol 144(2):395–401

Setzer WN (2014) Leaf and bark essential oil compositions of *Bursera simaruba* from Monteverde, Costa Rica. Am J EssentOils Nat Prod 1(3):34–36

Sharma ML, Chowdhury AR, Raina RM (1996) Essential oil from the leaves of *Bursera serrata* Wall. J Non Timber For Prod 3:86–88

Shen T, Lou HX (2008) Bioactive constituents of myrrh and frankincense, two simultaneously prescribed gum resins in Chinese traditional medicine. Chem Biodivers 5:540–553

Shen T, Li GH, Wang XN, Lou H (2012) The genus *Commiphora*: a review of its traditional uses, phytochemistry and pharmacology. J Ethnopharmacol 142(2):319–330

Siani AC, Ramos MFS, Guimarães AC, Susunaga GS, Zoghbi MGB (1999) Volatile constituents from oleoresin of *Protium heptaphyllum* (Aubl.) March. J Essent Oil Res 11:72–74

Siani AC, Garrido IS, Monteiro SS, Carvalho ES, Ramos MFS (2004) *Protium icicariba* as a source of volatile essences. Biochem Systemat Ecol 32:477–489

Siani AC, Ramos MFS, Monteiro SS, Ribeiro-dos-Santos R, Soares ROA (2011) Essential oils of the oleoresins from *Protium heptaphyllum* growing in the Brazilian southeastern and their cytotoxicity to neoplastic cell lines. J Essent Oil Bear Plants 14(3):373–378

Siani AC, Nakamura MJ, Tappin MRR, Monteiro SS, Guimarães AC, Ramos MFS (2012) Chemical composition of South American Burseraceae non-volatile oleoresins and preliminary solubility assessment of their commercial blend. Phytochem Anal 23:529–539

Siani AC, Moraes R, Veiga Junior VF (2017) Toward establishing the productive chain for triterpene-based Amazonian oleoresins as valuable non-timber forest products. Open J Forestry 7:188–208

Silva JRDA, Zoghbi MGB, Pinto AC, Godoy RLO, Amaral ACF (2009) Analysis of the hexane extracts from seven oleoresins of *Protium* species. J Essent Oil Res 21(4):305–308

Singh BB, Mishra LC, Vinjamury SP, Aquilina N (2003) The effectiveness of *Commiphora mukul* for osteoarthritis of the knee: an outcomes study. Altern Ther Health Med 9(3):74–79

Singh B, Kumar R, Bhandari S, Pathania S, Lal B (2007) Volatile constituents of natural *Boswellia serrata* oleo-gum-resin and commercial samples. Flavour Fragr J 22:145–147

Stacey RJ, Cartwright CR, McEwan C (2006) Chemical characterization of ancient Mesoamerican "copal" resins: preliminary results. Archaeometry 48(2):323–340

Su S, Wang T, Duan JA, Zhou W, Hua YQ, Tang YP, Yu L, Qian DW (2011) Anti-inflammatory and analgesic activity of different extracts of *Commiphora myrrha*. J Ethnopharmacol 134:251–258

Suárez AI, Compagnone RS, Acosta D, Vásquez L, Diaz B, Canelón DJ (2007) Chemical composition and antimicrobial activity of the essential oil from oleoresin of *Protium neglectum* S. J Essent Oil Bear Plants 10(1):70–75

Suhail MM, Wu W, Cao A, Mondalek FG, Fung KM, Shih PT, Fang YT, Woolley C, Young G, Lin HK (2011) *Boswellia sacra* essential oil induces tumor cell-specific apoptosis and suppresses tumor aggressiveness in cultured human breast cancer cells. BMC Complement Altern Med 11:129

Sultana A, Ur Rahman K, Padmaja AR, Ur Rahman S (2013) *Boswellia serrata* Roxb. A traditional herb with versatile pharmacological activity: a review. Int J Pharmaceut Sci Res 4(6):2106–2117

Sunnichan VG, Mohan Ram HY, Shivanna KR (2005) Reproductive biology of *Boswellia serrata*, the source of salai guggul, an important gum-resin. Bot J Linnean Soc 147:73–82

Sylvestre M, Longtin APA, Legault J (2007) Volatile leaf constituents and anticancer activity of *Bursera simaruba* (L.) Sarg. essential oil. Nat Prod Commun 2(12):1273–1276

Tadesse M, Demissew S (1992) Medicinal Ethiopian plants: inventory, identification, and classification. In: Botany 2000: East and Central Africa. Plants used in Africal traditional medicine as practiced in Ethiopia and Uganda. NAPRECA, Addis Ababa University, Addis Ababa, pp 1–19

Tcheghebe OT, Seukep AJ, Tatong FN (2016) A review on traditional uses, phytochemical composition and pharmacological profile of *Canarium schweinfurthii* Eng. Nature Sci 14(11):17–22

Tee LH, Yang B, Nagendra KP, Ramanan RN, Sun J, Chan ES, Tey BT, Azlan A, Ismail A, Lau CY, Jiang Y (2014) Nutritional compositions and bioactivities of *Dacryodes* species: a review. Food Chem 165:247–255

Tee LH, Yang B, Tey BT, Chan ES, Azlan A, Ismail A, Sun J, Lau CY, Palanisamy UD, Ramanan RN, Prasad KN (2017) Valorization of *Dacryodes rostrata* fruit through the characterization of its oil. Food Chem 235:257–264

Tessier AM, Delaveau P, Piffault N (1982) Oléo-résine d'*Aucoumea klaineana*. Planta Med 44(4):215–217

Thang TD, Luu HV, Dung NX (2004) Chemical composition of the leaf oil of *Canarium bengalense* Roxb. from Vietnam. J Essent Oil Bear Plants 7(1):43–48

Thang TD, Dai DN, Luong NX, Ogunwande IA (2014) Constituents of essential oils from the leaves, stem barks and resins of *Canarium parvum* Leen., and *Canarium tramdenanum* Dai et Yakovl. (Burseracea) grown in Vietnam. Nat Prod Res 28(7):461–466

Thomson LAJ, Evans B (2006) *Canarium indicum* var. *indicum* and *C. harveyi* (canarium nut). In: Elevitch CR (ed) Species profiles for pacific island agroforestry. Permanent Agriculture Resources, Hōlualoa, HI

Thulin M (1999) Flora of Somalia: volume 2, Angiospermae (Tiliaceae-Apiaceae). Royal Botanic Gardens, Kew

Thulin M, Claeson P (1991) The botanical origin of scented myrrh (blssabol or habak hadi). Econ Bot 45(4):487–494

Thulin M, Warfa AM (1987) The frankincense trees (*Boswellia* spp., Burseraceae) of northern Somalia and southern Arabia. Kew Bull 42(3):487–500

Thulin M, Beier BA, Razafimandimbison SG, Banks HI (2008) *Ambilobea*, a new genus from Madagascar, the position of *Aucoumea*, and comments on the tribal classification of the frankincense and myrrh family (Burseraceae). Nordic J Bot 26:218–229

Tolera M, Menger D, Sass-Klaassen U, Sterck FJ, Copini P, Bongers F (2013) Resin secretory structures of *Boswellia papyrifera* and implications for frankincense yield. Ann Bot 111:61–68

Trevizan LNF, do Nascimento KF, Santos JA, Kassuya CAL, Cardoso CAL, Vieira MC, Moreira FMF, Croda J, Formagio ASN (2016) Anti-inflammatory, antioxidant and anti-*Mycobacterium tuberculosis* activity of viridiflorol: the major constituent of *Allophylus edulis* (A. St.-Hil., A. Juss. & Cambess.) Radlk. J Ethnopharmacol 192:510–515

Tucker AO (1986) Frankincense and myrrh. Econ Bot 40:425–433

Tucker AO, Maciarello MJ, Brown RC, Landrum LR, Lafferty D (2009) Essential oils from the oleo-gum-resins of elephant tree or torote (*Bursera microphylla* A. Gray, Burseraceae) from Arizona. J Essent Oil Res 21(1):57–58

Turek C, Stintzing FC (2012) Impact of different storage conditions on the quality of selected essential oils. Food Res Int 46(1):341–353

Tyldesley J (1998) Hatchepsut: the female pharaoh, revised. Penguin Books, London

Ur Rehman N, Hussain H, Csuk R, Abbas G, Green IR, Al-Harrasi A (2017) A norterpenoid and tripenoids from *Commiphora mukul*: isolation and biological activity. Z Naturforsch B 72(1):11–15

Van Vuuren SF, Kamatou GPP, Viljoen AM (2010) Volatile composition and antimicrobial activity of twenty commercial frankincense essential oil samples. S African J Bot 76:686–691

Villanueva MA, Torres RC, Başer KHC, Özek T, Kürkçüoğlu M (1993) The composition of Manila elemi oil. Flavour Fragr J 8:35–37

Walsh ME, Reis D, Jones T (2010) Integrating complementary and alternative medicine: use of myrrh in wound management. J Vasc Nurs 28(3):102

Weeks A, Daly DC, Simpson BB (2005) The phylogenetic history and biogeography of the frankincense and myrrh family (Burseraceae) based on nuclear and chloroplast sequence data. Mol Phylogen Evol 35:85–101

Woolley CL, Suhail MM, Smith BL, Boren KE, Taylor LC, Schreuder MF, Chai JK, Casabianca H, Haq S, Lin HK, Al-Shahri AA, Al-Hatmi S, Young DG (2012) Chemical differentiation of *Boswellia sacra* and *Boswellia carterii* essential oils by gas chromatography and chiral gas chromatography-mass spectrometry. J Chromatogr A 1261:158–163

Xie J, Wang S, Sun B, Zheng F (2008) Preparative separation and purification of β-caryophyllene from leaf oil of *Vitex negundo* L. var. *heterophylla* (Franch.) Rehd. by high speed countercurrent chromatography. J Liq Chromatogr Relat Technol 31:2621–2631

Yang SA, Jeon SK, Lee EJ, Shim CH, Lee IS (2010) Comparative study of the chemical composition and antioxidant activity of six essential oils and their components. Nat Prod Res 24(2):140–151

Yeo SK, Ali AY, Hayward OA, Turnham D, Jackson T, Bowen ID, Clarkson R (2016) β-Bisabolene, a sesquiterpene from the essential oil extract of opoponax (*Commiphora guidottii*) exhibits cytotoxicity in breast cancer cell lines. Phytother Res 30(3):418–425

Young DG, Chao S, Casabianca H, Bertrand MC, Minga D (2007) Essential oil of *Bursera graveolens* (Kunth) Triana et Planch from Ecuador. J Essent Oil Res 19:525–526

Yukawa C, Iwabuchi H, Kamikawa T, Komemushi S, Sawabe A (2004) Terpenoids of the volatile oil of *Bursera graveolens*. Flavour Fragr J 19:565–570

Yukawa C, Imayoshi Y, Iwabuchi H, Komemushi S, Sawabe A (2006) Chemical composition of three extracts of *Bursera graveolens*. Flavour Fragr J 21:234–238

Zofou D, Tematio EL, Ntie-Kang F, Tene M, Ngemenya MN, Tane P, Titanji VPK (2013) New antimalarial hits from *Dacryodes edulis* (Burseraceae) - Part I: isolation, in vitro activity, in silico "drug-likeness" and pharmacokinetic profiles. PLoS One 8(11):e79544

Zoghbi MGB, da Cunha EVL, Filho WW (1993) Essential oil of *Protium unifoliolatum* (Burseraceae). Acta Amazon 23(1):15–16

Zoghbi MGB, Maia JGS, Luz AIR (1995) Volatile constituents from leaves and stems of *Protium heptaphyllum* (Aubl.) March. J Essent Oil Res 7(5):541–543

Zoghbi MGB, Andrade EHA, Santos AS, Luz AIR, Maia JGS (1998) Volatile constituents of the resins from *Protium subserratum* (Engl.) Kuntz. J Essent Oil Res 10(3):325–326

Zoghbi MGB, Andrade EHA, Lima MP, Silva TMD, Daly DC (2005) The essential oils of five species of *Protium* growing in the north of Brazil. J Essent Oil Bear Plants 8(3):312–317

Zúñiga B, Guevara-Fefer P, Herrera J, Contreras JL, Velasco L, Pérez FJ, Esquivel B (2005) Chemical composition and anti-inflammatory activity of the volatile fractions from the bark of eight Mexican *Bursera* species. Planta Med 71:825–828

Part II
Uses of Essential Oils in Various Industries

Chapter 5
Essential Oil of Betel Leaf (*Piper betle* L.): A Novel Addition to the World Food Sector

Proshanta Guha and Sujosh Nandi

5.1 Introduction

Essential oils are also called volatile oils or ethereal oils (Guenther 1948), but the term is a misnomer. This is because it apprises some substances which are neither essential nor oil in relation to food sector, wherein an oil is supposed to be a mixture of mixed fatty acids. That apart, unlike essential fatty acids, the essential oils are not required for maintaining good health or sound mind. They are not indispensable for the producer organisms as well because the organisms can survive and complete their life cycle without any such oils. Though not strictly necessary, rather optional, the essential oils can help the producer organisms in various ways such as protection from competitors, pathogens, insects, etc. It can also help the plants in various other ways such as reduction in transpiration losses, communication with other plants and microbes, and attraction of the pollinating agents like insects, birds, etc., when present in the plants. In the true sense, the word *essential* relates to essence that means concentrated scent, and the word has its roots in the Latin word *essentia-ae* which means perfumes (Bevilacqua et al. 2018)**.** Therefore, any original volatile substance which has any distinct odour or scent can be termed as essential substance.

Further, the second part of the term *essential oil* is the *oil* for which no proper definition exists though in food sectors it represents merely a mixture of mixed fatty acids. However, oil can ordinarily be identified by some of its principal properties such as liquid at room temperature, lighter than water, slippery to touch, ability to produce a greasy mark on white paper, inflammability, immiscibility in water, saponification, hydrophobicity, solubility in organic solvents, lipophilicity, etc. For example, edible oils (olive oil, coconut oil, mustard oil, sesame oil, etc.), but inclusion

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of fuel oils (kerosene oil; petroleum, petra+oleum meaning rock oil; diesel oil; etc.), mineral oil (liquid paraffins), lubricant oil (motor oils), etc., in the domain of oils could make it very confusing, but still, the meaning of oil in common sense is well understood in the realm of food production, processing, and consumption.

In the above context, the essential oils are called oils perhaps due to the fact that they possess some of the above-mentioned empirical properties. Therefore, the simplest definition of essential oil could be reduced to merely a *pure scented oil* or *volatile scented oil of direct biological origin*. However, some authors have defined essential oil as *any oily and aromatic volatile liquid that can be harvested from any part of the plant* (Burt 2004; Speranza and Corbo 2010; Böhme et al. 2014). The International Organization for Standardization (ISO), on the other hand, defined essential oil as—"product obtained from natural raw material, either by distillation with water and steam, or from the epicarp of citrus fruits by mechanical processing, or by dry distillation" (quoted by Sadgrove and Jones 2015).

There are about 17,500 aromatic plants (Regnault-Roger et al. 2012) belonging to approximately 60 families (Raut and Karuppayil 2014) producing about 3000 different essential oils, but only 300 essential oils are commercially available for different uses such as perfumes, dentistry, agriculture, food preservation, housekeeping, natural remedies, aromatherapy, and so on (Van de Braak and Leijten 1999; Speranza and Corbo 2010). These oils consist mainly of hydrocarbons including some functional compounds. The number of such compounds may be as high as 300 in a single essential oil (Sandra and Bicchi 1987) though mostly restricted to 20–30 detectable compounds with the help of latest scientific technologies like LC-MS, GC-MS, GC × GC-MS, DART-MS, NMR, and so on.

It is a common belief that essential oils are secondary metabolites of the plants or their specific parts such as flowers (rose), buds (clove), seeds (cardamom), bark (cinnamon), wood (sandal), peels (orange), roots (vetiver), leaf (betel leaf), etc. However, it cannot be conclusively said that the essential oils originate only in the plants. This is because there are many other producers of essential oils like animals and microorganisms. The animal sources include musk deer which belong to the family Moschidae that encompasses seven species including *Moschus leucogaster*, commonly known as white-bellied musk deer or Himalayan musk deer in India (TNEB 1974; Wikipedia 2018a). In these species, some strong odoriferous substance, muscone or 3-methylcyclopentadecanone (Fig. 5.1), is produced in the naval

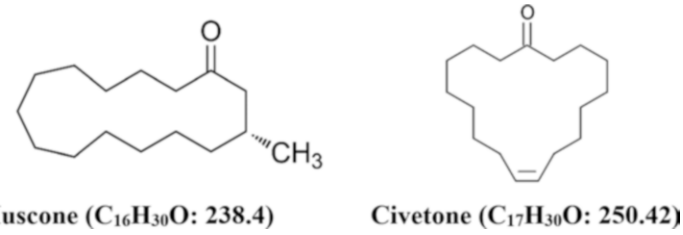

Fig. 5.1 Molecular structure, formula, and weight of muscone and civetone (Rana 2015)

gland of the adult male deer which is supposed to attract its female counterparts. The gland, when fully grown and functional, makes the deer insane probably in search of the source of the highly attractive fragrance, and ultimately, it dies due to accidents by crashing against some trees, hard objects, or in any other ways. The odoriferous substance is so strong that a grain of musk can distinctly provide fragrance to millions of cubic feet of air without any perceptible loss of weight (Kraft 2004). This scent is not only most penetrating but also most persistent among all the known substances which emit odour. In India, some of the rich and wealthy persons, kings, and emperors only could afford this musk for various purposes, such as cosmetics, medicines, and aphrodisiac. Obviously, it was one of the most expensive cosmetic articles in the history. Even in the present day also, it is still in use, though rarely, for religious purposes in the Lord Shiva temples, for instance, Lord Pashupatinath Temple in Nepal. However, use of this substance is coming down to an end since the musk deer is facing extinction. Therefore, they are enlisted as endangered species, and now finding a live specimen in India or Nepal is extremely difficult. Similar is the case with another endangered animal commonly known as civet. This carnivorous mammal looks like a big cat and is mostly found in Asia, Europe, and Africa. There are about 15–20 species of civet which are grouped into 10–12 genera under Viverridae family (Rafferty 2012). Both the male and female civets produce an oil-like substance in their perineal gland (pouches below the tail) with a strong musky odour which is highly valued as fragrance and a stabilizing agent for perfumes. When pure, the odour is strong and putrid, but once diluted it becomes pleasant with sweet odour. The African civet (*Civettictis civetta*) is reared in Africa for obtaining civet oil which contains about 2.5–3.4% civetone or 9-cycloheptadecen-1-one (Fig. 5.1) as the major odoriferous ingredient. The molecular structure, formula, and weight of muscone and civetone are given in Fig. 5.1.

Apart from the animals, some microorganisms are also capable of producing attractive odoriferous substances from which scent is manufactured. It may be a matter of debate if such scented materials may be termed essential oil or not, but they also originate in the lower plants (Barnett 2015). Such odoriferous substance is extracted in Kannauj situated in the Uttar Pradesh province of India, where it is termed as *Mitti Attar* meaning earthy fragrant material. Here, *Mitti* means earth and *Attar* means concentrated odoriferous liquid in Indian language (Hindi). This earthy smell is supposed to be due to a by-product produced by a typical bacteria or actinomyces. The by-product is called geosmin (Wikipedia 2018b, c). This *attar* is stored in a special leather bag called *Kuppi* for retaining its original fragrance.

The essential oils can be classified into various ways such as quality of aroma, time taken for evaporation or persistence of odour, etc. The classification based on the quality of aroma puts the fragrant and flavouring substances into different categories, such as citrus, earthy, floral, herbaceous, camphorous, medicinal, minty, oriental, spicy, woody, etc. On the other hand, the oils, when classified on the basis of time taken for evaporation or persistence, are categorized into three groups, such as top note (1–2 h), middle note (2–4 h), and base note (a few days). However, in the current scenario, the essential oils are designated directly by the name of the source material such as rose oil, sandalwood oil, mint oil, betel leaf oil, etc.

5.2 Origin, Taxonomy, and Nomenclature of Betel Leaf (*Piper betle* L.)

History of betel leaf chewing dates back to the antiquity. However, both the archaeologists and anthropologists could trace it to only 7000 BC (Pradhan et al. 2013) and the Malay Archipelago is generally recognized as the place of origin of the crop (de Candolle 1884; Burkill 1966; Chattopadhyay and Maity 1967).

Betel vine (Fig. 5.2) is a perennial root climber which belongs to the family Piperaceae (Guenther 1952), the black pepper family that includes several herbs, shrubs, small trees, and hanging vines (Ferreres et al. 2014). The family Piperaceae has 10 genera and 2000 species, of which 30 are found in India and 18 in Sri Lanka, and 3 are endemic (Chakraborty and Shah 2011; Gupta and Singh 2016). The taxonomic position of betel vine in the plant kingdom is given below (Pradhan et al. 2013):

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida

Fig. 5.2 Betel leaf (*Tamluk Mitha* variety)

Order: Piperales
Family: Piperaceae
Genus: *Piper*
Species: *betle*
Binomial Name: *Piper betle* L.
Synonyms: *Chavica betle* (L.) Miquel; *Piper pinguispicum* C. DC. and Koord (MHMC 2015); *Piper peepuloides* Wall, *Piper chavya* Ham (Periyanayagam et al. 2011)

The betel vine is also known by various other names in various countries of the world. A list of some of the important vernacular names of the vine in different countries of the World and also in India is given in Tables 5.1 and 5.2, respectively.

In India, betel leaf is known by more than 150 names in various local languages, such as *Nagavalli*, *Nagarvel*, *Saptaseera*, *Sompatra*, *Tamalapaku*, *Tambul*, *Tambuli*, *Vaksha Patra*, *Vetrilai*, V*oojangalata*, and so on in different parts of the country (CSIR 1969; Guha and Jain 1997). However, *Paan* is the most popular name of betel leaf in all parts of India and adjacent countries such as Nepal, Pakistan, and Bangladesh. There are about 100–150 varieties of betel leaf in the world, of which 40 are found in India and 30 in West Bengal (Maity 1989; Samanta 1994; Guha and Jain 1997; Ravindran et al. 2002). However, on the basis of morphology and micro-metrical traits, all these varieties can be grouped into six different categories, namely, *Bangla*, *Desawari*, *Kapoori*, *Khasi*, *Sanchi* (or *Chhaanchi*), and *Mitha* (Rawat et al. 1989).

Table 5.1 Common names of betel leaf in different languages in different countries

S. no.	Language (country)	Name	S. no.	Language (country)	Name
1	Afrikaans (Africa)	Betel blad	12	Khmer (Cambodia)	Sloek phne I
2	Arabic (Saudi Arabia)	Tambol	13	Lao/Laotian (Laos)	Mark
3	Burmese (Myanmar)	Kwm rwat	14	Latin (Malta)	Piperis folium
4	Chamorro (Guam)	Papulu	15	Malay (Indonesia, Malaysia)	Daun sirih
5	Dhivehi (Maldives)	Foah	16	Persian (Iran)	Burg-e-tanbol
6	English (Europe, USA)	Betel leaf	17	Sinhalese (Sri Lanka)	Bulath
7	French (France)	Feuille de betel	18	Spanish (Spain)	Hoja de betel
8	Filipino (Philippines)	Sirang dahon	19	Tetum (East Timor)	Malus, Malu
9	German (Germany)	Betel blatt	20	Thai (Thailand)	Phlu, Bai pluu
10	Javanese (parts Of Indonesia)	Sirih, Suruh, Bodeh	21	Tok Pisin (Papua New Guinea)	Buai
11	Kapampangan (Philippines)	Bulung samat	22	Vietnam (Vietnamese)	Trau

Table 5.2 Common names of betel leaf in different Indian languages[a]

S. No.	Language	Common name
1	Assamese	Paan
2	Bengali	Paan
3	Gujarati	Nagarbael
4	Hindi	Paan
5	Malayalam	Vetta, Vettila
6	Marathi	Nagbael. Vidyache paan
7	Kashmiri	Paan wathur
8	Oriya	Pano
9	Panjabi	Paan
10	Sanskrit	Nagavallari, Nagini, Nagavallika,Tambool, Saptashira, Mukhbhushan, Varnalata
11	Tamil	Vetrilai
12	Telugu	Nagballi, Tamalapaku
13	Urdu	Gillauri
14	Konkani	Kasar
15	Kannada	Veeleyada yele, Tambulika, Tambuladhikara, Tambuladayini, Tambuladyaka

[a]The meaning of the word "Paan" is well understood in almost all the languages in India

Importance and widespreadness of betel leaf can be comprehended from the fact that it has specific synonyms in almost all languages of the world (Table 5.2). This indicates that the betel leaf is known in almost all parts of the world where it is either cultivated or used for different purposes after import. This practically proves that betel leaf is obviously growing more and more popular with passage of time, obviously due to its aromatic, medicinal, stimulant, and other beneficial attributes contributed mostly by the essential oil present in the leaves (Guha 1997; Khanra 1997). That apart, intercontinental migration of the Asian population has also contributed towards dissemination of knowledge and information about the leaves.

5.3 Morphology of Betel Leaf

Betel leaf (Fig. 5.3) is a heart-shaped dorsiventral green leaf. However, the leaves may attain various shades of green with yellow tinge or very dark green with blackish hue. The vine has weak cylindrical stem with green colour which becomes semi-woody when old with earthy or mixed brown or yellow colour. The stem may have many other shades of colour such as green with parallel red lines when young.

From each node of the stem, adventitious roots develop which enable the vine to climb up along the host plants or the inert support (Fig. 5.2). This way, the vines may grow 15–50 feet or more in a year with profuse branching which are removed from time to time for improving quality of the leaves. Some varieties called *Gaach Paan* or "Tree-betel vine" may grow beyond even 50 feet along the support which

Fig. 5.3 Betel vine plants

is mainly a living areca nut tree (*Areca catechu* L.). The leaves are petiolated, glabrous, and alternate. The length of the leaves may range from 5 to 20 cm and width may range from 3 to 15 cm, which may vary beyond these ranges due to edaphoclimatic, management, and genetic factors. The leaves have one main rib (midrib) and 4–8 additional ribs which emerge from the base (petiolar side) and converge towards the apex. The leaf apex is acute or acuminate, and the leaf margin is entire. The stout petiole may attain a length of 10–50% of the leaf blade. The male and female flowers are separate on dioecious plants, and both are spiky, dense, cylindrical, and off-white in colour measuring from 3 to 10 cm in length or more but without any sepals or petals. Fruits are rarely produced under Indian agroclimatic conditions.

5.4 Agrotechnology of Betel Vine

The vine is a tropical shade-loving plant. It requires hot and humid climate. Temperature may range from 15 to 40 °C, relative humidity from 40% to 80%, and rainfall from 2250 to 4750 mm resembling to the ecological conditions of a tropical forest. A well-drained fertile sandy or sandy loam or sandy clay soil with pH range

of 5.6–8.2 is suitable for its cultivation. Normally, the male plants are raised for harvesting the fresh leaves (CSIR 1969; Guha and Jain 1997). However, in low rainfall or hot and dry areas, this crop can be cultivated with assured irrigation and shade net technology. Otherwise also, most of the cultivated varieties except *Gaach Paan* are vegetatively propagated inside a hut-like structure called *Boroj* or *Bareja* or *Bouroj*, which is traditionally constructed with materials like bamboo, banana leaves, straw, jute sticks, dry grasses, and even iron rods in recent times providing a shady and humid environment in side. It is a voracious feeder of nutrients and water and also requires huge investment in terms of pesticides since it is highly susceptible to insect pests and diseases. The nutritional requirement of the crop may be as high as 600, 300, and 250 kg NPK/ha per year, and the crop requires irrigation weekly and fortnightly during summer and winter, respectively, or sooner or later depending upon soil and agroclimatic conditions. The initial cost of cultivation, as was calculated during 2006, was about ₹1–2 lakh/ha at the minimum during the first year which came down to ₹0.5–0.6 lakh/ha during the subsequent years (Guha 2006). The current values of these figures may range from four to six times higher. However, the betel vine is cultivated in small fields, as small as 0.04 ha, which is sufficient for providing proper employment and sufficient income for maintaining a small family of five members in rural India (Jana 1995; SDAMM 1996).

In India, it is cultivated on 40,000–55,000 ha of land with a production worth of ₹7000–10,000 million (Rawat et al. 1989; Guha 2006; Das et al. 2016b) amounting to about 0.20 million tons of betel leaf annually which is the highest known figure in the world. On the other hand, two reports indicate that the crop is also cultivated in Bangladesh with an area production of about 12,660 ha and about 0.06 million tons (Rawat et al. 1989) and 18,247 ha and 0.10 million tons (Islam et al. 2015). However, consolidated global area production data is not available. Therefore, compilation of this requires joint efforts by all concerned governments, scientists, farmers, traders, exporters, and the importers as well, but the scanty data available so far indicate that India exports betel leaves to many Asian, African, Australian, American, and European countries including Afghanistan, Canada, France, Germany, UAE, UK, USA, etc. (Singh et al. 1990; Jana 1996) and earned about $4 million during 2013–2014 (TOI 2014).

5.5 Economic Importance of Betel Leaf

The economic potentiality of the crop can be judged by the data given in the previous section (Agrotechnology of Betel Vine). Further, the potentiality of this crop can also be judged by the fact that about 15–20 million people consume betel leaves on a regular basis in India alone (Jana 1996) besides over two billion consumers in other countries of the world (Jeng et al. 2002). The leaves are also used as a mark of respect and in auspicious occasions in social, cultural, and religious events regularly in India and many other Asian countries (Guha 2006; Sengupta and Banik 2013; Mohanto et al. 2017). Further, it is estimated that about 20 million people derived

their livelihood, directly or indirectly, partly or fully, from production, processing, handling, and marketing of betel leaf in India (Jana 1996). The economic potentiality of the crop can also be adequately judged by the fact that it can be explored for several pharmaceutical, medicinal, and cottage industries (Guha 2012). This is because the plant or its oil is useful in numerous medical conditions and for the treatment of a large number of common, acute, chronic, incurable, and even fatal diseases such as bad breath, cold cough, ring worm, asthma, leukaemia, etc. That apart, the crop can also be explored for many other industries like that in the food sectors which is discussed under the subsection of food product development.

5.6 Biochemical Composition of Betel Leaf

Guha (2006) reported the biochemical composition of the leaves from different sources including his own work as shown in Table 5.3.

However, previously, Gopalan et al. (1984) have reported 12 analytical data out of 21 parameters presented in Table 5.3 which are slightly different but within the range of reported data shown in the above table. In addition, Sarma et al. (2018) reported that the leaves also contained vitamin E in the range of 3.20–3.69 mg/100 g

Table 5.3 Nutritional composition of fresh betel leaf[a]

S. No.	Constituents	Approximate composition
1	Water	85–90%
2	Protein	3–3.5%
3	Fat	0.4–1.0%
4	Minerals	2.3–3.3%
5	Fibre	2.3%
6	Chlorophyll	0.01–0.25%
7	Carbohydrate	0.5–6.10%
8	Nicotinic acid	0.63–0.89 mg/100 g
9	Vitamin C	0.005–0.01%
10	Vitamin A	1.9–2.9 mg/100 g
11	Thiamine	10–70 μg/100 g
12	Riboflavin	1.9–30 μg/100 g
13	Tannin	0.1–1.3%
14	Nitrogen	2.0–7.0%
15	Phosphorus	0.05–0.6%
16	Potassium	1.1–4.6%
17	Calcium	0.2–0.5%
18	Iron	0.005–0.007%
19	Iodine	3.4 μg/100 g
20	Essential oil	0.08–0.2%
21	Energy	44 kcal/100 g

[a]Guha (2006) Source: www.kre.publishers.com

of the dried leaves and Periyanayagam et al. (2012) reported that leaves contained 11.73% (w/w) ash. The above information proves that the leaves are very nutritive and contain substantial amount of vitamins and minerals, and therefore, six leaves with a little bit of slaked lime is said to be comparable to about 300 ml of cow milk particularly for the vitamin and mineral nutrition (Guha 2006). The calcium content of betel leaf gets further elevated when slaked lime is added to it as one of the ingredients of a betel quid before consumption in India and other Asian countries. It is also interesting to note that the leaves contain potassium nitrate ranging from 0.26% to 0.42% on dry weight basis (CSIR 1969). This may be one of the reasons for use of betel leaf extracts in food preparations for augmenting sensory qualities of the native dishes in some countries like India and Bangladesh. This compound is also supposed to be good for the teeth because it relives toothache due to hypersensitivity of the damaged or diseased teeth (caries) to a great extent. This information has been commercially exploited by some toothpaste manufacturing companies who are marketing toothpaste which soothes the sensitive teeth.

5.7 Essential Oil of Betel Leaf

The origin of betel leaf though relates to the antiquity, but essential oil of betel leaf came into major public domain only when Guenther (1952) published some scientific details of the oil. However, commercialization of essential oil of betel leaf started in a massive way in India after the recent advent of the betel leaf oil extractor designed and developed at IIT Kharagpur, India (Guha 2006). Shukla (2015) reported that about 120 million kg of essential oils are produced globally from nearly 300 crops which worth about $4 billion, including 4% production from India amounting to about 21%–22% of the total revenue (Devi et al. 2015). However, no such data relating to essential oil of betel leaf is available in the public domain till today, but still, India holds a great promise for production, utilization, and export of essential oil of betel leaf because the world's largest quantity and finest quality (organoleptically superior) leaves are produced in India, particularly in the East and West Medinipur districts of West Bengal province of India. This promise becomes more obvious when 10–70% of the leaves are wasted every year in India (Rao and Narasimham 1977; Guha and Jain 1997; Guha 2006), amounting to a minimum of ₹900 million every year in monetary terms which could be converted into oil, a measure of generating wealth from waste. In any case, even today, at least 10% of the total production remains unsold or sold at a throwaway price at any point of time, and essential oil may be extracted from these surplus leaves (Guha 2006), be it dried (Hemalatha 2017); fresh, stale, or dechlorophylled; or even partially decayed and rejected for consumption (Guha 2008). That apart, at least 25% of the leaves are rejected during curing or bleaching of the leaves in the cottage industries in India. Moreover, frequently, a large number of the export consignments are destroyed by incineration mainly due to contamination of *Salmonella* spp. for avoiding public health hazards. All these rejected leaves can also be utilized for generating wealth

by extraction of essential oil. Such extracted oil would carry the pertinent fragrance and flavour of the source plants or varieties numbering over 100, but all these oils could be grouped into six different categories as mentioned previously ranging from *Bangla* to *Sanchi*.

5.8 Methods of Extraction of Essential Oil from Betel Leaf

Essential oil from betel leaf may be extracted by the common methods employed for any other essential oil-bearing crops, such as expression, percolation, maceration, enfleurage, solvent extraction, distillation, supercritical fluid extraction, phytonic extraction (using 1,1,1,2-tetrafluoroethane), etc. Among these methods, distillation has become more popular due to several inherent advantages, and as a result, several types of distillation techniques have been attempted, such as hydro-distillation, steam distillation, microwave-assisted hydro-distillation, ultrasound-assisted hydro-distillation, vacuum distillation, and so on. Among these, hydro-distillation has become the most popular method because of its simplicity, easy repair and maintenance, cheapness, and purity of the extracted oil. Mostly, the Clevenger's apparatus is used for extraction of essential oil from betel leaf, but it takes a long time (3–8 h) (Jantan et al. 1994; Arambewela et al. 2005; Arambewela et al. 2006; Guha 2010; Periyanayagam et al. 2011; Saxena et al. 2014; Das et al. 2016a; Preethy et al. 2017). This is mainly because of the near-water density of essential oil of betel leaf (0.958–1.057 g/cc, Gildemeister and Hoffmann 1929) which makes this oil extremely difficult to separate from water present in the receiver tube of the Clevenger's apparatus. In contrast, in cases of other crops, the oils either float above or sink below the water column facilitating separation and collection of essential oils from water. That apart, very rapid emulsifying ability of betel oil with water to form a milky emulsion further makes the process of distillation very difficult. This essential oil does not separate out easily from the milky emulsion, and therefore, redistillation may be required. Thus, it takes a long time for completion of the entire process. Over and above, distillation with the Clevenger's apparatus has several inherent drawbacks with respect to essential oil of betel leaf, such as slow extraction process, poor cooling efficiency, escapement of uncondensed oil vapour, etc. (Guha 1998, 1999; Guha 2003).

Therefore, suitable modifications were made in the Clevenger's apparatus, and the betel leaf oil extractor (modified Clevenger's apparatus) was designed, developed, and patented (Indian Patent number 202600, dated 2.3.2007). The comparative designs of both the above-mentioned apparatuses are shown in Figs. 5.4 and 5.5, respectively, and the material and process flowchart for extraction of essential oil from betel leaf is shown in Fig. 5.6. This extractor saved time and energy to the extent of 43.85% and 29.80%, respectively, besides increasing the oil yield by 16.20% compared to the Clevenger's apparatus. The efficiency of the betel leaf oil extractor could be further enhanced by insulating the heat-radiating portions of the apparatus by using cheap, readily available, and efficient insulating materials such

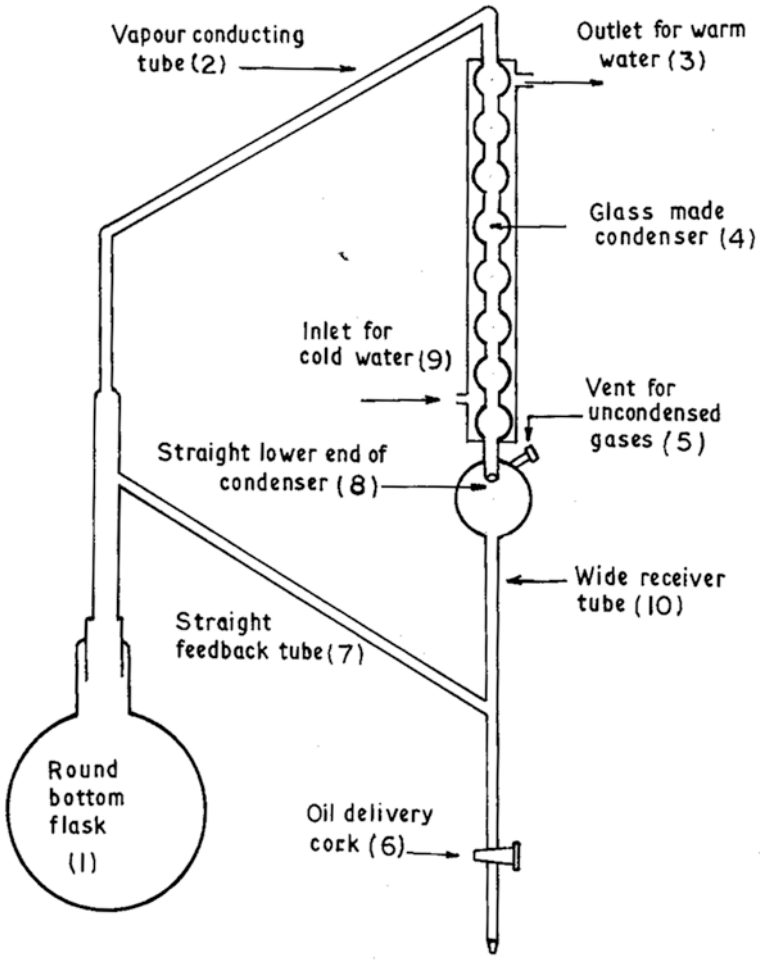

Fig. 5.4 Schematic diagram of Clevenger's apparatus. (Reproduced from Guha 2010)

as asbestos ropes and also by using cold water (15 °C) as cooling agent. Further, the fuel requirement can also be reduced substantially if the by-product (i.e. the de-oiled exhausted leaves) are used as fuel after drying the leaves (Guha 2010). This would further economize the extraction process particularly in the large installations in the rural areas. This extractor consumed about 2.1 kWh of electrical energy in about 2.5 h time for each charge (Guha 2007a, b). Therefore, it is possible to carry out multiple charging in a single day which will also substantially reduce the cost of production. Further, when the density of the betel oil obtained from any particular variety is exactly the same as that of water, then it becomes extremely difficult to separate out the oil from water. In those cases, 15% saline water may be used to separate out betel oil from water with the help of a separating funnel (Guha 2010).

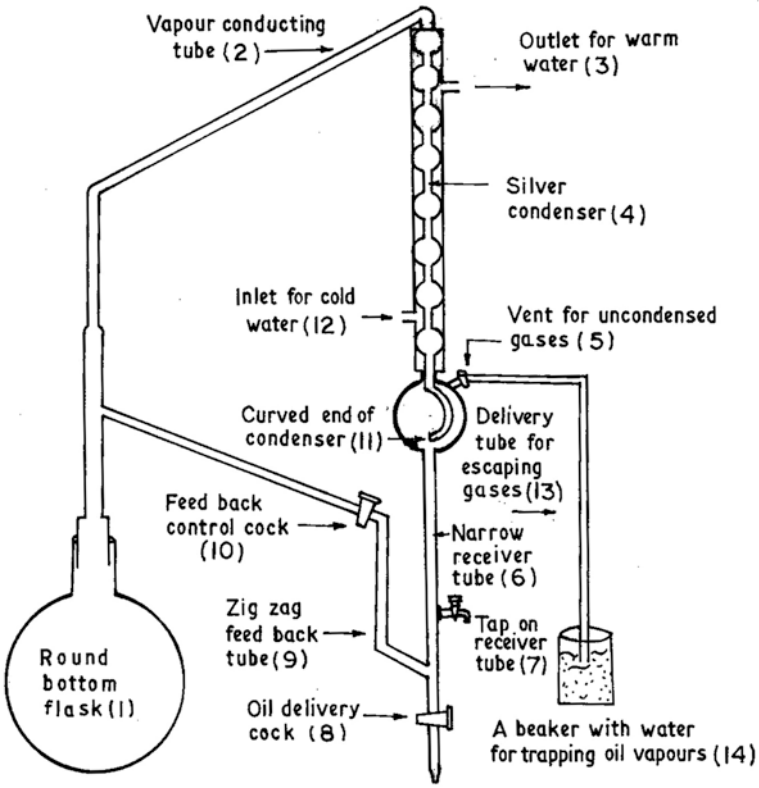

Fig. 5.5 Schematic diagram of Betel leaf oil extractor. (Reproduced from Guha 2010)

However, this extractor was envisaged to be affordable to the betel leaf growers since the cost of fabrication of the extractor was calculated to be ₹10,000 and ₹20,000 for 10 and 20 L sizes, respectively (Guha 2007a). This could be easily maintained by the small farmers and would also be sufficient for processing of surplus leaves in any average-sized Boroj (~0.02 ha) on a daily or weekly basis.

In an attempt to further improve the efficiency of the extraction process and to increase the yield of essential oil, Amaresh et al. (2017) explored extraction of essential oil from betel leaves with the help of microwave (2.45 GHz)-assisted hydro-distillation using modified Clevenger's apparatus (MAHD). In this attempt, the extraction process was completed within about 50 minutes compared to about 3.5 h required by the conventional hydro-distillation method using Clevenger's apparatus (CHD). The power level and leaf-to-water ratio for maximum oil yield was 500 W and 0.33 for MAHD, whereas it was 500 W and 0.2 for CHD. The total power consumed by MAHD was 0.4 kWh, whereas it was 0.7 kWh for CHD. In both the cases, oil yields were about 1.46% on dry weight basis from *Mitha* variety of betel leaf. Thus, there was substantial saving of time (about 76%) and energy (about 43%) by using MAHD compared to CHD. Moreover, there was no difference

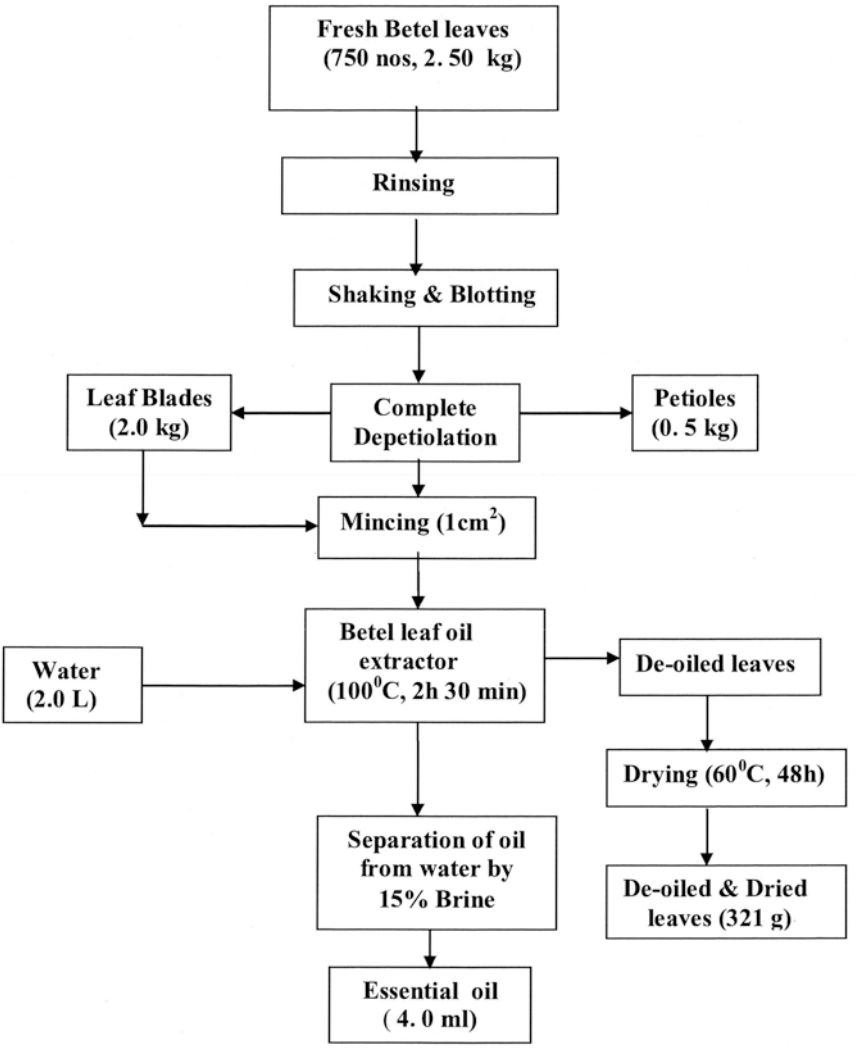

Fig. 5.6 Process and material flow chart for extraction of essential oil from betel leaves of *Ramnagar Mitha* variety with the betel leaf oil extractor. (Reproduced from Guha 2010)

in the quality of the essential oil extracted by both the methods, as in both the cases, 15 compounds were detected by GC-MS analysis and the major compounds identified were 4-allyl-1,2-diacetoxybenzene, caryophyllene, chavibetol, chavicol, and estragole, whereas anethole, camphene, å-cardinol, cubenol, eucalyptol, globulol, linalool, and ô-muurolene were also present in trace amount. The physical properties of the oils extracted by both the methods were also almost the same except that the oil extracted by CHD was yellow in colour, whereas it was colourless to yellow in case of MAHD. Further, the antioxidant activity of both the oils was also almost

the same and was not statistically different. Obviously, microwave-assisted irradiation did not interfere with the radical scavenging activity of the extracted essential oils. This is also confirmed from the fact that the compositions of both the oils were also the same, and these common constituents contributed to the radical scavenging activity in the same way.

In another study, to further improve the efficiency of the extraction process and also to increase the yield of essential oil, Hans (2017) attempted extraction of essential oil from betel leaves by ultrasound-assisted hydro-distillation (UAHD). This method involves application of high-intensity and high-frequency sound waves. Ultrasound is produced by electrical equipment that vibrates with extremely high frequency. Crystals of some materials such as quartz vibrate very fast when electricity passes through it. As the crystal vibrates, it pushes and pulls air around it producing ultrasound waves (>20 kHz). This vibration passes through the medium which is water in this case. As a result, the intermolecular forces are not able to hold the molecular structure leading to breakdown of the bonds among the water molecules, and cavitation process takes place. Thus, bubbles are formed and imploded in an incomprehensively fast manner leading to very high and low pressure points. This damages the plant cell walls and sucks the contents (including oil) into the surrounding medium and also generates heat. Thus, it facilitates much quicker extraction of essential oil. In this study, a Toshniwal ultrasound bath (US bath) of 2 L size with maximum power level of 100 W was used, producing a high-frequency sound waves of 37 ± 3 kHz. About 200 g of de-petiolated betel leaves were placed in the US bath for different duration of 30, 60, 90, and 180 minutes with leaf-to-water ratios of 1:1, 1:2, and 1:3 as a measure of pre-treatment. Subsequently, these leaves were hydro-distilled to obtain essential oil. The highest oil yield of 0.25% (db) was obtained with a pre-treatment of 90 minutes and leaf-to-water ratio of 1:2. On the other hand, the highest oil yield of 0.20% (db) was obtained in all the leaf-to-water ratios with CHD. However, by decreasing the pre-treatment duration from 90 minutes to 60 or 30 minutes, the oil yields were decreased. The subsequent distillation time, however, was reduced to 1.5 h as compared to 4 h required for CHD due to ultrasound pre-treatment. This was due to the fact that the ultrasonication damaged and ruptured the cell structures of the leaves including the epidermal layer which was confirmed by observation with scanning electron microscope. Such damage facilitated enhanced migration of the oil micro-globules from the cells to the outside medium, i.e. water, by the process of cavitation. The CHD consumed 0.67 kWh of electrical energy, while the UAHD consumed 0.46 kWh for completion of the extraction process including pre-treatment and subsequent hydro-distillation. This clearly shows that there was 31% saving of energy in UAHD compared to CHD. However, UAHD can be proved to be much more advantageous compared to CHD if the power level of the US bath can be increased beyond 100 W to a suitably higher level which remains open for further investigation.

It is also possible to employ solvent extraction method for obtaining essential oil from betel leaf. The different solvents tried were hexane, methanol, ethanol, acetone, etc. Hexane when used in combination with Soxhlet apparatus and rotary vacuum evaporator also yielded about the same amount of essential oil as with

CHD, but it contained chlorophyll and all other components that were soluble in organic solvents, such as wax, fat, vitamin, etc. Therefore, the solvent-extracted essential oil had a green colour and was not pure in nature, rather a mixture, though flavour and fragrance were comparable to those extracted by distillation methods. This method may be costlier than CHD due to inclusion of cost of the solvent. Therefore, it is not recommended for commercial purpose unless properly modified.

5.9 Yield of Essential Oil of Betel Leaf

Crop yield is a very wide term and hence, sometimes becomes confusing unless defined properly for a particular objective. Yield of essential oil can be defined in terms of unit oil produced (volume or mass) per ha or oil produced per unit weight of raw material. The most common unit of expression of essential oil yield is percent of volume of oil per unit mass of raw material (% v/w). However, moisture percentage of the raw materials plays an important role in calculating yield; hence, it is expressed as fresh weight basis (wet basis, wb) or dry weight basis (db) and rarely on air dry basis (adb) of the raw materials. When neither the fresh nor the dry weights are available, the moisture content of the raw materials is required to be mentioned along with the oil yield. This is more so because except a few, all the essential oils are very costly, for example, 1 litre of rose oil may cost as high as ₹5 lakh, and that of pure sandal wood oil may cost more than ₹50 lakh depending upon the quality of the products. However, db is the most commonly understood form of expression when it is not mentioned otherwise.

Yield of any crop may take the highest or the lowest value depending upon several factors like genetic factor besides soil, climate, and management factors which interact intricately, sometimes even incomprehensively. Like any other crop, yield, composition, and quality of essential oil-bearing crops also vary due to several factors that may also include age of the plant, age of the leaves or plant parts, time of harvest, method of extraction, process parameters, duration of extraction, nature of solvent used, pre-treatment of the raw materials, duration of storage of the raw materials before extraction, etc. However, yield of essential oil of betel leaf generally varies widely from 0.09% to 0.80% on fresh weight basis (Sankar et al. 1996; Periyanayagam et al. 2011). This depends mainly on the variety of the leaves and the local conditions where the vines are grown like any other crop. The local conditions of East and West Medinipur districts of West Bengal province of India are most suitable for luxuriant growth of betel vines. The world's highest yield and finest quality leaves are found in these areas. From these areas, leaves of five prominent commercial varieties were collected and then oil was extracted by betel leaf oil extractor in about 2.5 h (Guha 2007a). The oil yields were 0.8%, 1.7%, 1.7%, 2.0%, and 2.0% for *Sanchi*, *Sada Bangla*, *Kali Bangla*, *Ramnagar Mitha*, and *Tamluk Mitha* varieties, respectively, on dry weight basis. Sharma et al. (1996) also collected 84 types of betel vines from different parts of India and grew them at National Botanical Research Institute (NBRI), Lucknow, India. After examination of the

quality of the extracted essential oils, these varieties were categorized into five distinguished flavour groups, namely *Mitha*, *Sanchi*, *Bangla*, *Desawari*, and *Kapoori*. The oil yields of the corresponding groups were approximately 0.25%, 0.18%, 0.16%, 0.14%, and 0.11%, respectively, on fresh weight basis. In another attempt, from the same institute, NBRI, Lucknow, Rawat et al. (1989) extracted essential oil from five varieties of betel leaf, namely *Mitha*, *Sanchi*, *Bangla*, *Desawari*, and *Kapoori* and found that the oil yields were 0.85%, 0.19%, 0.16%, 0.12%, and 0.10%, respectively, on fresh weight basis. The yields of both the studies were comparable except that of *Mitha* which may be attributed to the difference in locality wherefrom the original cuttings were collected for growing the vines. Both the varieties though were named as *Mitha*, but their exact identity could have been different, as there are several *Mitha* varieties mentioned in the literature such as *Mitha* cum *Bangla*, *Mitha Calcutta* (Sankar et al. 1996), *Tamulk Mitha* (Guha 2006; Basak and Guha 2015), *Ramnagar Mitha* (Guha 2007a), etc.

Das et al. (2016a) extracted essential oil from eight landraces of betel leaf collected from coastal areas of Odisha (India). The essential oil yields varied from 0.10% to 0.42%. The highest yield was obtained from *Chandrakala* (0.42%) followed by *Godi Bangla* (0.37%), *Balia* (0.35%), *Desibangla* (0.32%), *Maghai* (0.30%), *Dandabalunga* (0.20%), *Nahua* (0.15%), and *Karpada local* (0.15%). Surprisingly, contrary to the above, Rayaguru et al. (2007) reported that *Godi Bangla* variety from Odisha yielded 9.52% essential oil on dry weight basis, which is beyond the range found in the contemporary literature and, therefore, needs confirmation.

Sankar et al. (1996) also collected 13 different cultivars of betel vines from different regions of India and raised them at Guntur, Andhra Pradesh, India. The mature leaves were used for the extraction of essential oil by the Clevenger's apparatus, and the oil yields ranged from 0.09% to 0.51% on fresh weight basis. The minimum oil yield was obtained from *Tellaku of Utukur* variety (0.09%), whereas the maximum oil yield was obtained from *Mitha Calcutta* (0.51%) followed by *Godi Bangla* (0.47%), *Maghi* (0.39%), *Mitha* cum *Bangla* (0.33%), *Gaach Paan* (0.31%), *Kakair* (0.26%), *Karapaku* (0.23%), *Pachaikodi* (0.20%), *Bangla* (0.16%), *Kariele* (0.18%), *Tellaku of Punnur* (0.13%), and *Tellaku of Chennur* (0.11%) varieties, in order.

Preethy et al. (2017) extracted essential oils from five varieties of Indian betel leaves pertaining to Kerala, namely, *Muvattupuzha local*, *Karinadan*, *Puthukodi*, *Nadan*, and *Chelan* and the corresponding oil yields were 0.57%, 0.52%, 0.50%, 0.47%, and 0.45%, respectively, on fresh weight basis. These yields were positively correlated to pungency of the leaves which was established from the fact that *Muvattupuzha local* with 0.57% oil was the most pungent variety, whereas *Chelan* with 0.45% oil was the least pungent variety. In another study, Muhammed (2007) extracted essential oils from three varieties of betel leaves from Kerala, namely, *Nadan*, *Selan*, and *Kuzikkodi*, and obtained oil yields of 1% on fresh weight basis in all the three varieties which was higher than that obtained in the previous study for the first two varieties and the reason behind it remains open for explanation. However, oil yields from these three Keralian varieties were comparatively much higher than those from all other varieties of betel leaf reported so far, except that

reported by Dastane et al. (1958) and Jantan et al. (1994) who obtained 4.20% and 5.10% oil on dry basis, respectively. However, Dastane et al. (op. cit.) reported that the oil yield increased due to curing treatment of the leaves which is explained in the foregoing lines.

The CSIR (1969) reviewed oil yields of different varieties of betel leaves, namely, *Calcutta*, *Gorakhpuri*, *Saugor*, and *Ramtek* (bleached) and found oil yields as 1.20%, 0.70%, 0.70%, and 2.60%, respectively. It may be noted that oil yield increased due to curing (bleaching) of the leaves in *Ramtek* variety. Similar results were also obtained by Dastane et al. (1958) who obtained 4.20% oil yield from the bleached leaves in contrast to 1.23% from the fresh leaves of the same variety. Such increase in oil yield may be explained by the corresponding decrease in other components during bleaching such as nonreducing sugar decreased from 1.30% to 0.29%, starch 3.10% to 1.44%, tannin 2.05% to 1.8%, and ether extract 15.7% to 13.5% which cumulatively decreased the total weight of the leaves and, consequently, increased oil yield proportionately compared to the fresh leaves. However, the phenomenon is not well understood and needs further investigation for elaboration of the results. It is also pertinent to mention here that bleaching of betel leaves is synonymous with curing. It is a process by which the green leaves of some particular variety, e.g. *Bangla* or *Maghi*, are treated with smoke at moderately high temperature (36°–45 °C) and pressure (stacking weight loads of baskets full of betel leaves placed one above the other in rotation). This smoke treatment is given for 30–36 h in a closed chamber and repeated for 4–5 cycles. In absence of light (dark conditions) and presence of heat and smoke (mainly CO and CO_2), the leaves gradually loose chlorophyll and become light yellow or whitish in colour (Sengupta 1996; Guha and Jain 1997) known as cured leaves or bleached leaves or *Banarasi* leaves with greater organoleptic properties (Guha 2009; Sadhukhan and Guha 2011) together with a longer shelf life.

The oil yields from other Indian varieties of betel leaf popular in Tamil Nadu were 0.31% wb (*Vellaikodi*, Sugumaran et al. 2011), 0.80% adb (*Sirugamani-1*, Periyanayagam et al. 2011), and 1.30% adb (*Pachaikodi*, Vasantha-Srinivasan et al. 2017). Similarly, the oil yields of betel leaf varieties popular in Lucknow area of Uttar Pradesh province of India, such as *Bangladesi* and *Desawari*, were 0.12% (wb) and 0.15% (wb), respectively (Saxena et al. 2014). Similarly, essential oil yields of foreign varieties of betel leaves also match with the Indian counterparts, such as the Philippines (1.44%, Caburian and Osi 2010), Sri Lanka (0.84–1.12% db, Arambewela et al. 2005, 3.30% w/w adb, Arambewela et al. 2006), and Nepal (0.10%, Satyal and Setzer 2012), but a report from Malaysia shows a pretty high value (5.10% db, Jantan et al. 1994). However, one of the earliest reports on essential oil of betel leaf was published by Guenther during 1958 who reported that the yields ranged from 0.60% to 1.80%, highest being in the young leaves which are more pungent than the older leaves. Similar finding was also reported by Pradhan et al. (2013) and Bhalerao et al. (2013). It may be true that the younger leaves may contain higher amount of essential oil, but there may be about fivefold (or more) difference between the fresh weights of the young and fully matured leaves, and because of this reason, the older leaves are used for extraction of essential oil from betel leaf on commercial scale.

5.10 Storage of Essential Oil of Betel Leaf

After the process of extraction is complete, the apparatus is allowed to be cooled down, and the oil is taken out from the apparatus into a nonreactive coloured bottle preferably made of glass. Traces of water in the collected oil, if any, are removed by sufficient amount of anhydrous sodium sulphate or magnesium sulphate. The bottle is then sealed with an air tight lid and stored in dark at around 4 °C, and in this way, the oil can be stored for more than 3 years without any appreciable loss of aromatic properties (Jantan et al. 1994; Guha 2007a, 2010; Periyanayagam et al. 2011; Vasantha-Srinivasan et al. 2016; Das et al. 2016a; Preethy et al. 2017). On the contrary, the oil can also be stored at room temperature for more than 3 years, but there would be some losses of volatile components without significant loss of perceptible aroma (Guha 2007a). However, this loss needs to be scientifically investigated and quantified.

5.11 Physical Properties of Essential Oil of Betel Leaf

Essential oil of betel leaf is a slightly viscous, greasy, and slippery liquid at room temperature (Guha 2003). The oil is though colourless immediately after extraction in most of the cases, but it may vary from faint yellow to yellowish brown (Guenther 1952; Guha 2003). Such difference in colour may be attributed to genetic or varietal, environmental, edaphic, managerial, processing, and other factors. However, the colourless oil samples may turn to slightly yellowish after a few hours and dark coffee colour after a few years. It may also turn into dark yellow or orange on exposure to light or heat (Caburian and Osi 2010), but its aromatic properties remain almost unaltered during the period of storage. However, the oil possesses a sharp burning taste (Chakraborty and Shah 2011), burning flavour, and odour reminiscent of creosote and tea (Guenther 1952). On the other hand, Guha (2007a) reported that the oil extracted from *Mitha* variety had a pleasant sweet and spicy fragrance, while the *Bangla* variety had pungent and spicy fragrance, and the *Sanchi* variety had the most intense spicy-pungent odour. Sankar et al. (1996) also reported that fennel (*Foeniculum vulgare* Mill)-like aroma and sweet taste in the *Mitha Calcutta* cultivar was due to the presence of anethole (54.93%), while the characteristic clove-like aroma and pungency of the *Bangla* group of varieties was due to the high concentration of eugenol (45.30%–57.30%) in the oil. Rawat et al. (1989) also reported that the essential oil extracted from different varieties of betel leaf was light to dark yellow in colour with some kind of spicy fragrance. More specifically, the oil extracted from *Mitha* variety was yellowish brown in colour with a fennel-like odour and sweet taste, while the oil of *Kapoori* had yellow colour with a greenish tinge and an aromatic flavour, but the oil of *Bangla* variety had a clove-like spicy odour and a sharp pungent taste. However, colour of the essential oil extracted by CHD from Nepalese variety had a pale yellow colour (Satyal and Setzer 2012), but the oil extracted from *Sirugamani-1* variety had a golden yellow colour with aromatic odour besides pungent taste and refractive index of 1.505 (Periyanayagam et al. 2011).

Dubey and Tripathi (1987) reported some of the important physical properties of the oil extracted from unspecified varieties of betel leaf, such as specific gravity (1.04), refractive index (1.52), acid value (2.50), saponification value (140.25), ester value (137.75), pH (3.35), and solubility (soluble in organic solvents such as acetone, hexane, benzene, butanol, methanol, and solvent ether). In addition, Guha (2003) reported that the freezing point of the oil of *Sada Bangla* variety was very low and ranged from 0 to −5 °C. Gildemeister and Hoffmann (1929) also studied the properties of essential oils extracted from a large number of varieties of betel leaf and found that the specific gravity of the oils varied from 0.958 to 1.057. On the other hand, Arambewela et al. (2005) reported that the specific gravity of five Sri Lankan varieties of betel leaf ranged from 1.03 to 1.05 only, but in a subsequent study, Sugumaran et al. (2011) found that specific gravity of essential oil of *Vellaikodi* variety was 1.0010 only. Caburian and Osi (2010) also studied physical properties of the oil and published the results which are more or less the same as above. In all the cases, the specific gravity of essential oil of betel leaf is mentioned to be equal or nearly equal to that of water. However, current literatures lack information about emulsifying ability of essential oil of betel leaf except a little that was provided by Guha (2010) who stated that this oil formed milky emulsion very rapidly with water. This phenomenon hinders extraction and separation of oil from aqueous medium in hydro-distillation or steam distillation process. However, such striking ability of formation of emulsion may lead to formation of micro- and nanoemulsions, whereby useful physical, chemical, and biological properties of the oil may be augmented by many folds, for example, the antimicrobial efficacy of the oil may be significantly enhanced (Basak and Guha 2017a; Roy and Guha 2018), and the latter authors along with Basak (2017) are also attempting to enhance bioavailability of the active components of the oil by encapsulation, active packaging, fumigation (vapour phase delivery system), etc., and studies in this direction are in progress. This is expected to evolve more appropriate and economically viable utilization strategy in future.

Sharma et al. (1996) concluded that the particular flavours of *Bangla*, *Desawari*, *Kapoori*, *Mitha*, and *Sanchi* cultivars could be attributed, respectively, to the presence of high concentration of phenolics, phenolic ether, and terpenes in combination with isoeugenol, anethole, and phenolic ethers such as safrole.

5.12 Biochemical Composition and other relevant details of Essential Oil of Betel Leaf

It is a widely accepted fact that there are over 100 varieties of betel leaf (Peter 2004; Guha 2006), but according to Ravindran et al. (2002), it accounts to about 150. Among them, some varieties have different names in different geographical locations, for example, *Bangla* variety is variously known as *Godi Bangla*, *Simurali Bangla* (Ramamurthi and Rani 2012), *Calcutta Bangla* (Dhongle and Kogje 2013),

Sada Bangla, *Kali Bangla*, *Ramtek Bangla*, *Desi Bangla*, *Desi Paan*, *Bangla Deshi* (Saxena et al. 2014), and so on in different parts of India, and their exact identity remains mostly doubtful. That apart, plants grown asexually from cuttings (for preserving their genetic makeup) also change their morphological and other characteristics so significantly that they achieve a completely new identity and, hence, known by different names in different geographical locations with different agro-climatic conditions. Such changes in the vine can also occur like any other crops merely due to different management practices, particularly that pertaining to agronomy or horticulture. Therefore, identification of a particular variety becomes a very difficult task. In view of that, several workers have tried to identify a specific variety with the help of biochemical composition of essential oil extracted from the leaves of that particular variety, treating it as a bio-marker. Some scientists have also tried to identify the actual active ingredients, i.e. the biochemical compounds responsible for specific characteristics of any particular variety, such as particular taste or aroma, antimicrobial activity, insecticidal properties, or medicinal effects. Some scientists have also tried to examine the toxic effects of any particular variety by detecting presence of possible harmful biochemical compounds in the plant or in the oil. Further, in search of commercially valuable biochemical compounds, such as eugenol acetate, hydroxychavicol, or chlorogenic acid, some scientists took up studies on biochemical composition of essential oil of betel leaf with the help of advanced technologies such as GC-MS, LC-MS, co-GLC, co-TLC, and NMR technologies, while some others had taken up such studies purely in pursuit of their academic interest.

It is a matter of common understanding that essential oil is not a pure substance in the sense that it is not consisted of just one biochemical compound, rather, it is a mixture of several compounds, which may go up to 300 in betel leaf oil, but it is generally restricted to 20–30 compounds (Sandra and Bicchi 1987), though 40–50 compounds are also not very infrequent (Basak and Guha 2015). However, if merely any one of these compounds is removed partly or totally, then the oil may not retain its original aroma or other characteristics. All these organic compounds can be grouped into about nine classes for the sake of convenience (Table 5.4), such as monoterpenes, sesquiterpenes, alcohols, aldehydes, acids, oxides, phenols, phenolic ether, esters, besides others like ketone as reported by Das et al. (2016a). Examples of some of the biochemical compounds pertaining to essential oil of betel leaf clustered in these groups are also shown in Table 5.4. Additionally, the organoleptic, functional, and biological properties of some of the important compounds are given in Table 5.5, while relevant synonyms are given in Table 5.6, and the molecular structures of some of the prominent compounds with molecular formula and molecular weight are shown in Fig. 5.7.

The earliest report on the examination of essential oil of betel leaf by Guenther (1952) showed that chemical composition of betel oil varied with origin of the leaf and the oils contained up to 55% phenols, mostly chavibetol and sometimes chavicol.

Garg and Jain (1996) identified 21 constituents in the essential oil extracted from *Sagar Bangla* cultivar which was rich in chavicol (48%).

Table 5.4 Classification of biochemical compounds present in the essential oil of betel leaf

S. no.	Monoterpenes	Sesquiterpenes	Alcohols	Aldehydes	Acids	Oxides	Phenols	Phenolic ethers	Esters
1	α-Thujene[b,c]	γ-Cadinene[b,c]	Linalool[b,c]	Decanal (capric aldehyde)[b,c]	Hexadeconoic acid[b,c]	1,8-cineol[b,c]	Eugenol[b,c,e]	Methyl eugenol[b,c,e]	Eugenol acetate[b,c]
2	Camphene[b,c]	Δ-cadinene[b,c,e]	α-Terpineol[b,c]	Decanal (laural aldehyde)[b,c]	Terpinyl acetate[c]	Caryophyllene oxide[b,c]	Iso-eugenol[b,c]	Methyl chavicol[b]	Methyl benzoate[b]
3	Sabinene[b,c]	α-Cadinene[b,c]	Terpinol-1-ol[b,c]	Stearaldehyde[b,c]	Chlorogenic acid[d]		Chavicol[b,c,e]	Anethole[b,c]	Methyl salicylate[e]
4	Trans-sabinene hydrate[e]	β-Salinene[b,c]	α-Costol[b]	n-Decanol[e]			Chavibetol[b,c,e]	Safrole[b,c]	Chavibetol acetate[e]
5	β-Myrcene[b,c]	β-Slemene[b,c,f]	Δ-Cardinol[b,c]				Hydroxy-chavicol[a]		Allylpyrocatecol diacetate[e]
6	Trans-β-ocimene[b,c]	γ-Elemene[b,c]	3,7,11,15-tetra-methyl-2-hexadecane-ol[b]						
7	Bornylene[b,c]	Cis-caryophyllene[b,c,e]	Geraniol[b,c]						
8	β-Pinene[b,c]	Trans-Caryophyllene[b,c,e]	α-Cardinol[e]						
9	Trans-β-ocimene[b,c]	Aromadendrene[b,c]	τ-Muurolol[e]						
10	γ-Terpenine[b,c]	α-Cubebene[b,c]	α-Selinenol[f]						
11	Terpinolene[b,c]	β-Cubebene[b,c]							
12	Allo-ocimene[b,c]	α-Humulene[e]							
13	α-Terpenene[b,c]	γ-Muurolene[e]							
14	β-Phellandrene[b,c]	Germacrene D[f]							
15	Limonene[b,c]	Lepidozene[f]							

16	p-cymene[b,c]								
17	2,6,6-Trimethyl-1-methyl-cyclo-hex-2-ene[b]								
18	α-Pinene[b,c]								

[a]Amonkar et al. (1986)
[b]Rawat et al. (1989)
[c]Sharma et al. (1996)
[d]Guha (2006)
[e]Satyal and Setzer (2012)
[f]Periyanayagam et al. (2011)

Table 5.5 Organoleptic, functional, and biological properties of major components of essential oil of betel leaf

S. no.	Name of the compounds	Organoleptic properties	Functional and biological properties	References
1	Eugenol	Pleasant, spicy, clove-like odour	Anti-inflammatory, antimicrobial, analgesic, antioxidant, antiviral, anti-carcinogenic, antidepressant, antiseptic, anaesthetic in dentistry, anti-mutagenic, insecticidal, fungicides	Rai et al. (2011); Foo et al. (2015); Das et al. (2016b); Kudva et al. (2018); HMDB (2018c)
2	Chavibetol	Spicy odour	Noncentral analgesic, anti-pyretic, anti-inflammatory, antibacterial, antineoplastic, antiperspirants	NCBI (2018); Das et al. (2016b)
3	Hydroxy-Chavicol	Light smell of creosote	Anti-carcinogenic, anti-nitrosation, antimutagenic, anti-inflammatory, antioxidant, antibacterial, antiplatelet, antithrombotic, xanthine oxidase inhibitory, and gastric ulcer-healing activity	Nagabhushan et al. (1989); Sharma et al. (2009); Rathee et al. (2006); Chakraborty et al. (2012); Vikash et al. (2012); Bhalerao et al. (2013); Kumar et al. (2015); Abdullah et al. (2016); Singh et al. (2018)
4	β-Caryophyllene	Spiciness of black pepper, woody-spicy, dry, clove-like aroma	Anti-inflammatory, anti-carcinogenic, pain relief, primary therapeutic against atherosclerosis and osteoporosis, prevents diabetes, endometriosis cerebral ischaemia, anxiety and depression, liver fibrosis, and Alzheimer-like disease	Calleja et al. (2013); Cheng et al. (2014); Mahmoud et al. (2014); Bahi et al. (2014);Chang et al. (2013); Gertsch et al. (2008)
5	Methyl eugenol	Clove-like aroma	Used in aroma therapy and massage oil, anti-inflammatory, cytotoxic against human cell line, insecticidal activity, used as fragrance ingredients in perfumes, toiletries, and detergents	GOC (2010); Joshi (2013); Das et al. (2016b)
6	Cubebene	Citrus type	Anti-inflammatory, antiseptic, antioxidant, immunomodulatory activities, neuroprotection against glutamate-induced oxidative injury, used as surfactant and emulsifier	Lee et al. (2012); HMDB (2018b); Zahin et al. (2018)

(continued)

Table 5.5 (continued)

S. no.	Name of the compounds	Organoleptic properties	Functional and biological properties	References
7	Estragole	Odour reminiscent of anise, sweet taste	Antimutagenic, antifungal against some bacteria, flavouring agent, food additive	EU (2001); Chang et al. (2009); Zielińska and Matkowski (2014); PF (2018)
8	Anethole	Sweet anise-like flavour, 13 times sweeter than sugar	Antimicrobial, antifungal, anthelmintic, insecticidal, anti-inflammatory, antinociceptive, gastroprotective and anti-implantation, secretolytic, expectorant, spasmolytic, estrogenic, sedative, flavouring agent in food industry	Balasubramanyam and Rawat (1990); Marinov and Valcheva-Kuzmanova (2015)
9	Iso-eugenol	Floral odour reminiscent of carnation	Antimicrobial, antioxidant, anti-inflammatory, flavouring agent innonalcoholic drinks, baked foods, and chewing gums	Atsumi et al. (2005); Khan (2014); Hyldgaard et al. (2015)
10	Safrole	Spicy odour	Anti-inflammatory, detoxifying agent, antioxidant, antimicrobial, antimutagenic, immunosuppressive, beverage and candy preparation but carcinogenic to rat (not to humans)	Nagabhushan et al. (1989); Parise-Filho et al. (2011); Da Silveira et al. (2014); Madrid et al. (2014); Das et al. (2016b); Marko (2017)
11	α-Copaene	Woody flavour	Antimicrobial, antiproliferative, antioxidant, antigenotoxic, and anticytotoxic activities	Brito et al. (2005)
12	Chavicol	Phenolic odour	Antimicrobial, antioxidant, antiseptic	Nagori et al. (2011); Murakami et al. (2015); HMDB (2018a)

Thahn et al. (2002) described a new chemotype of betel leaf from Hue area of Vietnam that contained isoeugenol (72%) as the chief component which was followed by isoeugenol acetate (12%). Therefore, the variety may be named isoeugenol chemotype of *Piper betle*.

Sharma et al. (1981) examined essential oil extracted from *Kapoori* variety of betel leaf and found that terpinyl acetate (21.98%) and eugenol (15.83%) were the prominent ingredients.

Sugumaran et al. (2011) examined essential oil obtained from *Vellaikodi* variety of betel leaf which is popular in Tamil Nadu (India) and identified a total of 65 compounds. The major compound was safrole (25.67%) which was followed by eugenol

Table 5.6 Names and synonyms of prominent compounds found in the essential oil of betel leaf

S. no.	Name of the compounds	CAS no.	Synonyms
1	Eugenol	97-53-0	4-Allyl-2-methoxyphenol; p-Allylguaiacol; p-eugenol; eugenic acid
2	Eugenyl acetate	93-28-7	Acetyleugenol; 4-Allyl-2-methoxyphenyl acetate; phenol, 2-methoxy-4-(2-propenyl)-, acetate; Aceto eugenol; 1-Acetoxy-2-methoxy-4-allylbenzene
3	Chavibetol	501-19-9	m-eugenol; 5-Allyl-2-methoxyphenol; 3-Allyl-6-methoxyphenol
4	Hydroxy-chavicol	1126-61-0	4-Allylpyrocatechol, 4-Allylbenzene-1,2-diol; 4-Allylcatechol; Desmethyleugenol
5	β-Caryophyllene	87-44-5	8-Methylene-4,11,11-(trimethyl)bicyclo (7.2.0) undec-4-ene
6	Methyl eugenol	93-15-2	Eugenol methyl ether; 4-Allyl-1,2-dimethoxybenzene; benzene, 1,2-dimethoxy-4-(2-propenyl)-
7	α-Cubebene	1769-14-8	3,7-Dimethyl-4-(propan-2-yl)-3a,3b,4,5,6,7-hexahydro-1 h-cyclopenta[1,3]cyclopropa[1,2]benzene
8	Estragole	140-67-0	4-Allylanisole, methyl chavicol; tarragon; anisole, p-allyl-; Chavicol, O-methyl-; p-Allylanisole; p-Methoxyallylbenzene
9	Anethole	4180-23-8	4-Propenylanisole, Isoestragole, (E)-1-Methoxy-4-(1-propenyl)benzene
10	Iso-eugenol	97-54-1	2-Methoxy-4-propenylphenol, 4-Propenylguaiacol; 2-Methoxy-4-(1-propenyl)phenol
11	Safrole	94-59-7	4 allyl 1,2 methylenedioxybenzene; 1,3-Benzodioxole, 5-(2-propenyl)-; Shikomol; 4-Allylpyrocatechol formaldehyde acetal
12	α-Copaene	3856-25-5	Copaene; Tricyclo[4.4.0.02,7]dec-3-ene,1,3-dimethyl-8-(1-methylethyl)-, stereoisomer
13	Chavicol	501-92-8	4-Allylphenol; p-Hydroxyallylbenzene; 4-(2-propenyl)-phenol
14	Isoxylic acid	610-72-0	Benzoic acid, 2,5-dimethyl-; 2-Carboxy-1,4-dimethylbenzene; 2,5-Dimethylbenzoic acid
15	β-Sitosterol	83-46-5	Cupreol; Quebrachol; α-Dihydrofucosterol; β-Sitosterin; Angelicin; Triastonal; 5-Cholesten-24β-ethyl-3β-ol

(18.27%) and eugenol acetate (8.00%). Another report from Mysore (India) also showed high safrole content (39.74%) in the oil obtained from a local variety (Ramalakshmi et al. 2002).

Basak and Guha (2015) characterized the chemical compounds present in *Tamluk Mitha* variety of East Medinipur district of West Bengal (India) and identified 46 different compounds, among which prominent compounds were chavibetol (22.00%), estragole (15.80%), β-cubebene (13.60%), chavicol (11.80%), and caryophyllene (11.30%). The compositional study showed that the natural sweetening compound anethole was totally absent in this variety. Therefore, it may be possible that estragole contributed (Table 5.5) the peculiar sweet aroma and taste to this vari-

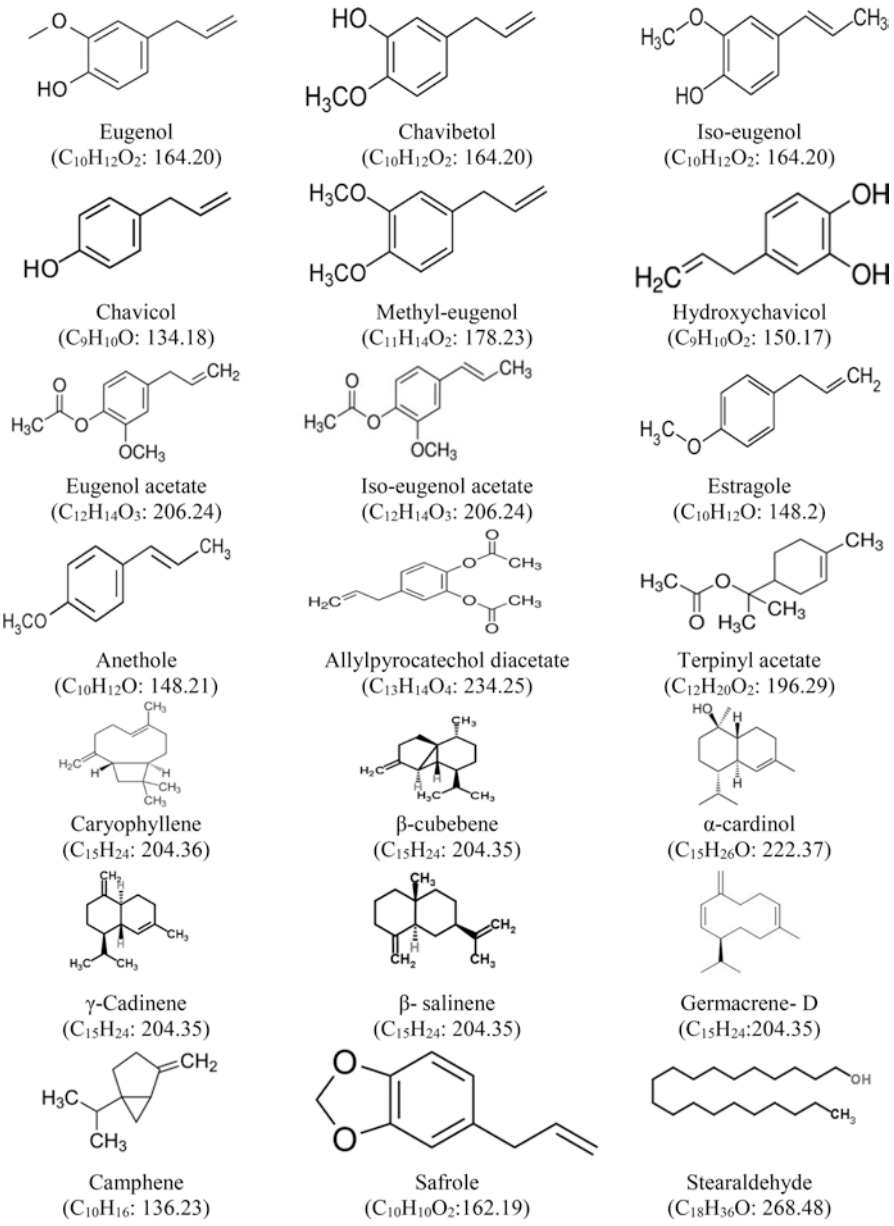

Fig. 5.7 Molecular structure, formula and weight of major compounds of essential oil of different varieties of betel leaf

ety. Rawat et al. (1989) found that essential oil of *Mitha* variety contained anethole (19.31%) and cis-caryophyllene (10.64%), whereas the oil of *Sanchi* variety contained stearaldehyde (2.69%) which was not present in any other variety examined together, namely, *Bangla*, *Mitha*, *Desawari*, and *Kapoori*. Therefore, presence of stearaldehyde may be used as a biomarker for identification of *Sanchi* cultivar.

Mohottalage et al. (2007) reported that Sri Lankan betel leaf oil contained safrole (52.70%) as the major constituent which was followed by allylpyrocatechol diacetate (15.40%), eugenol (6.40%) and eugenol acetate (5.85%).

Prakash et al. (2010) identified 32 compounds from essential oil of *Maghi* variety of betel leaf from Varanasi (India). The major constituent was eugenol (63.39%) which was followed by acetyl eugenol (14.05%), while the other components cumulatively contributed less than three percent.

Karak et al. (2018) analysed essential oils from seven varieties of betel leaf from West Bengal, India. They identified 45 constituents in total, which included 14 monoterpenes, 23 sesquiterpenes, and 8 phenyl propanes. In *Bangla*, *Bagerhati*, *Manikdanga*, and *Ghanagate* varieties, the prominent components were eugenol acetate (31.46–43.97%) and eugenol (13.13–33.06%), whereas in *Mitha* variety, it was chavicol (23.85%), while in *Chhaanchi* variety, safrole (42.77%) was the chief ingredient. In absence of a large proportion of anethole in *Mitha* variety, the sweet fragrance was probably contributed by the presence of estragole (Basak and Guha 2015) since it is also reported to possess similar organoleptic characteristics (Table 5.5).

Sugumaran et al. (2011) extracted essential oil from *Sirugamani* variety of betel leaf and found 67 compounds, among which the major compound was safrole (32.79%) followed by eugenol (16.17%), eugenol acetate (8.01%), τ-gurjunene (4.14%), and sabinene (3.43%). On the other hand, Periyanayagam et al. (2011) extracted essential oil from a similarly named betel leaf variety of Tamil Nadu (India), i.e. *Sirugamani-1*, and found that the oil was constituted of 59 biochemical compounds. The major compound was germacrene-D (16.07%) followed by lepidozene (14.99%), β-caryophyllene (9.86%), 1,3,4-eugenol (7.17%), β-elemene (5.75%), γ-murrolene (3.18%), α-seleninol (3.07%), β-cadinene (2.82%), and cineol (2.80%). By comparing the composition of the above two varieties, it may be concluded that similarity in the varietal names does not ensure similarity in composition of essential oils of the varieties. This also indicates that the nomenclature of the betel leaf varieties has not been scientifically accomplished. Therefore, research work in this area becomes essential for proper identification of the varieties of betel leaf.

Saxena et al. (2014) identified 25 and 35 components in the essential oils of *Bangladeshi* and *Desawari* varieties of betel leaf representing only 85.40% and 86.11% of the oil, respectively. The prominent components identified were eugenol, α-celinene, α- farnesene, p-celinene, methyl eugenol, and germacrene-D in both the varieties, but safrole and isosafrole were present only in *Desawari* variety. Safrole is commonly thought to be a potential carcinogenic agent, but that is a misconception. This is because safrole is quickly metabolized in human body into di-hydroxychavicol and eugenol, which are excreted along with urine (Chang et al. 2002).

Preethy et al. (2017) extracted essential oils from five varieties of betel leaves available in Malappuram district of Kerala (India). They identified a maximum of 56 compounds in total. In all the varieties, hydroxychavicol (39.50–45.50%) was the principal compound followed by eugenol (11.00–20.80%) though other compounds like methyl isoeugenol (0.10%–1.50%), methyl eugenol (0–0.80%), and isoeugenol (0.80–1.00%) were also present. The names of the varieties, percentage of hydroxychavicol, and number of compounds therein were as follows: *Karinadan* (45.50%, 39), *Pathukodi* (45.30%, 55), *Nadan* (44.60%, 56), *Muvattupuzha local* (41.10%, 55), and *Chelan* (39.50%, 56).

Das et al. (2016a) studied essential oils of eight landraces of betel leaf collected from coastal areas of Odisha (India) and identified 50 compounds in total, among which eugenol was the chief ingredient in all the landraces; however, its percentage varied, such as in *Chandrakala* (34.61%), *Karpada local* (39.84%), *Godibangla* (44.04%), *Nahua* (44.96%), *Balia* (45.85%), *Desibangla* (46.47%), *Dandabalunga* (55.49%), and *Maghai* (71.87%). From this work of Das et al. (op. cit.), it may be concluded that all these varieties possess clove-like pungency which increased with increasing proportion of eugenol in these varieties. Therefore, these results may serve as an excellent example of increasing order of organoleptic properties, i.e. clove-like pleasant pungency, which may be introduced in different products requiring appropriate amount of pungency. For example, a new product, a lozenge, for soothing throat problems (Vijay 2015), for infants should have minimum pungency, but for the elderly, the maximum amount of pungency may be introduced. This is because senses in the infants are very acute, whereas it becomes gradually blunt with age in the elderly.

Sankar et al. (1996) examined essential oil from 13 cultivars of betel vines of Andhra Pradesh (India) and found that in most of the varieties, eugenol (45.33–57.39%) was the chief ingredient present in different proportions as shown in parentheses of the foregoing lines. The varieties included *Karapaku* (57.39%), *Pachaikodi* (56.09%), *Bangla* (54.66%), *Kariele* (50.89%), *Maghai* (50.77%), *Kakair* (47.20%), *Gaachi Paan* (47.13%), *Mitha* cum *Bangla* (45.33%), and *Godi Bangla* (45.10%). However, in some varieties, anethole (54.93%) was the chief ingredient as in *Mitha Calcutta* variety, wherein proportion of eugenol (20.27%) was the second most abundant component next to anethole. On the other hand, terpinyl acetate, in different proportions, as shown in the parentheses, was the chief ingredient in some other varieties, such as *Tellaku of Pannur* (48.76%), *Tellaku of Utukur* (37.43%), and *Tellaku of Chennur* (34.21%). However, these three varieties also contained eugenol to the extent of 17.00%, 23.16%, and 18.42%, respectively. In all these 13 varieties, methyl eugenol and α-terpineol were also present in the proportions ranging from 5.11% to 0.07%. Based on the chemical constituents of betel leaf oils, all these 13 cultivars can be classified into four groups, namely, *Bangla*, *Mitha*, *Sanchi*, and *Kapoori*. However, this grouping is not complete, since there are some overlapping.

Muhammed (2007) extracted essential oil from three varieties of betel leaf of Keralian (India) origin, namely, *Nadan*, *Kuzhikkodi*, and *Selan*, and identified 40, 43, and 38 compounds, respectively. The major compound in *Nadan* variety was

safrole (38.10%) which was followed by eugenol (20.60%) and 4-allyl-1,2-diacetoxybenzene (9.70%). Similarly, the major component of *Kuzhikkodi* variety was safrole (35.60%) which was followed by eugenol (16.20%). On the other hand, the major constituent of *Selan* variety was eugenol (58.00%) which was followed by eugenol acetate (4.80%) and 4-allyl-1,2-diacetoxybenzene (3.80%).

Sharma et al. (1996) carried out chemical analysis of essential oils of 84 types of betel leaf. They identified 45 compounds including 15 monoterpenes, 10 sesquiterpenes, and 20 oxygenated derivatives including alcohols, aldehydes, acids, phenols, and phenolic derivatives. These 84 cultivars were categorized into five major flavour groups, namely, *Bangla*, *Desawari*, *Kapoori*, *Mitha*, and *Sanchi*. Among these, the oil of the *Bangla* group was constituted of five components, namely, eugenol (63.56%), eugenol acetate (18.68%), methyl eugenol (6.90%), isoeugenol (5.20%), and chavicol (1.07%), whereas essential oil of *Desawari* was constituted of 24 compounds, among which safrole (45.30%) was the major compound followed by eugenol (20.47%). However, essential oil of *Kapoori* variety was constituted of 26 compounds, among which eugenol (33.22%) was the major compound followed by terpenyl acetate (11.00%), isoeugenol (10.59%), laural aldehyde (7.10%), and eugenol acetate (6.45%). On the other hand, essential oil of *Sanchi* variety was constituted of 23 compounds, among which eugenol (25.90%) was the chief ingredient followed by safrole (22.75%), terpenyl acetate (8.70%), caryophyllene (7.78%), and β-salinene (6.36%). Lastly, essential oil of *Mitha* variety was constituted of 17 compounds, of which anethole (32.20%) was the chief ingredient followed by eugenol (18.92%), caryophyllene (10.64%), and γ-cadinene (9.44%).

Satyal and Setzer (2012) reported that the major component of Nepalese betel leaf was chavibetol (80.50%) which was followed by chavibetol acetate (11.70%) and allyl-pyrocatechol diacetate (6.20%), whereas four more compounds, namely, chavicol, eugenol, methyl eugenol, and (E)-caryophyllene, were also present in a very low concentration (0.40% each), while another seven compounds were present only as traces, namely, trans-sabinene hydrate, Δ-cadinene, α-humulene, γ-muurolene, α-cardinol, τ-muurolol, and methyl salicylate. Similar report available from Malaysia also reveals that essential oil of *Piper betle* contained 15 compounds, among which chavibetol (69.00%) was the chief ingredient followed by eugenol acetate (8.3%), chavicol (6.0%), β-carryophyllene (2.4%), and γ-cadinene (1.6%) (Jantan et al. 1994).

In view of the current review of literature, particularly Satyal and Setzer (2012) and Dwivedi and Tripathi (2014) related to chemotypic classification of betel leaf varieties based on composition of essential oils collected from various parts of the world and connected dominant characteristics, they can be categorized into eight different chemotypes as shown in Table 5.7. However, this categorization is not complete because there is some overlapping which needs to be studied well in future for more accurate categorization. The categorization should be based on the rare biochemical compound or by the highest concentration of specific biochemical compound produced by a particular variety which is not comparable to any other variety and, hence, specific to only one variety. Here, the trace compounds may also play a significant role.

Table 5.7 Classification of different varieties of betel leaf into the major chemotypic groups

S. no.	Chemotypic group	Name of variety and percentage of chief components	References
1	Chavicol	Sagar Bangla (48%)	Garg and Jain (1996)
		Mitha (23.85%)	Karak et al. (2018)
2	Germacrene-D	Sirugamani-1 (16.07%)	Periyanayagam et al. (2011)
3	Isoeugenol	Vietnamese variety (72%)	Thahn et al. (2002)
4	Chavibetol	Betel leaf from the Philippines (53.10%)	Rimando et al. (1986)
		Malaysian variety (69.0%)	Jantan et al. (1994)
		Nepal variety (80.50%)	Satyal and Setzer (2012)
5	Eugenol	Bangla (63.56%)	Rawat et al. (1989)
		Kapoori (33.22%)	
6	Anethole	Mitha (19.30%)	Rawat et al. (1989)
7	Safrole	Sri Lankan variety (52.70%)	Mohottalage et al. (2007)
		Taiwanese (inflorescence) varieties (28%)	Dwivedi and Tripathi (2014)
		Desawari (45.34%)	Rawat et al. (1989)
		Sanchi (22.75%)	
		Chhaanchi or Sanchi (42.77%)	Karak et al. (2018)
8	Eugenol acetate	Kali Bangla (22.16%)	Karak et al. (2018)
		Manikdanga (44.03%)	
		Bangla (35.77%)	
		Ghanagete (43.97%)	
		Bagerhati (31.46%)	

5.13 Uses of Betel Leaf and its Essential Oil

This edible leaf has achieved an esteemed position in human society right from the dawn of civilization, particularly in most of the Asian countries (Khoshoo 1981; Samanta 1994; Jana 1996; Sharma et al. 1996; Guha 2006), and also in different other countries of the world among the Asian immigrants. The leaves are traditionally used for chewing in their natural raw condition along with many taste-enhancing ingredients like sliced areca nuts, *Kattha* (thick paste of wood extract of *Acacia catechu* L.), slaked lime, etc., for obtaining mainly refreshing, stimulating, mood-elevating, digestive, and aphrodisiac effects (CSIR 1969; Garg and Jain 1996; Guha 1997, 2006, Chu 2001). These beneficial effects may be attributed mainly to the essential oil present in the leaves (Guha 1997; Khanra 1997) which is constituted of a large number of biochemical compounds with distinct bioactivity (Pradhan et al. 2013; Basak and Guha 2015; Roy and Guha 2018). Such useful properties of the oil indicate a promising industrial future for manufacturing of a large number of cosmetics, medicines, pharmaceuticals (Guha 1997, 2000, 2002), insecticides (Tabacchi

and Guerin 2007; Vasantha-srinivasan et al. 2017), fungicides (Ansari et al. 2017), food preservatives (Basak 2018a, b, c; Basak and Guha 2017a), food flavouring agents (Roy and Guha 2015), and food products, such as *paan masala* (spiced and processed betel leaf), cold drinks, *gutkha* (non-tobacco-based chewable mouth freshener), chocolates (Guha 1997, 2000), *suji halwa* (Bhagath and Guha 2014), chili bo (Wendy et al. 2014), cupcake (Roy and Guha 2015), and many other novel products.

The distinct bioactivities of essential oil of betel leaf which are most relevant to the food sector are antioxidant and antimicrobial activities which are discussed in the foregoing paragraphs.

5.14 Antioxidant Activity of Essential Oil of Betel Leaf

The antioxidants in relation to food are the substances which minimize, delay, or prevent oxidation of the target molecules (Halliwell and Gutteridge 1995; Halliwell 2007) like lipids, proteins, nucleic acids, and polysaccharides (Duan and Kasper 2011). The concentration of the antioxidants is normally very low compared to that of the target molecules. The examples of common (nonenzymatic) antioxidants are quercetin, vitamin E (α-tocopherol), vitamin C, β-carotene, lycopene, lutein, selenium, polyphenols, carotenoids, etc.

Oxidation of components of food changes the physical, biochemical, nutritional, and organoleptic properties of the food articles and make it unacceptable and, hence, renders it unfit for human consumption. Oxidation is caused by the free radicals, and it is one of the most prominent ways of spoilage of food that reduces the shelf life of the food articles. The free radicals are ions, atoms, or molecules with unpaired electrons which make them highly reactive and thereby unstable. These radicals may originate from elements like oxygen, nitrogen, and sulphur, which produce reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive sulphur species (RSS), respectively. Free radicals have a variety of chemical mechanisms such as electron donation, reduction of radicals, electron acceptance, oxidation of radicals, hydrogen abstraction, addition reaction, self-annihilation, and so on (Slater 1984). These mechanisms are disrupted by nonenzymatic antioxidants, while the antioxidant enzymes (e.g. catalase) destroy the free radicals by various other ways like metabolization, neutralization, catalytic break down, etc. in presence of some co-factors like copper, zinc, manganese, etc. When these mechanisms are disrupted or the free radicals are destroyed, shelf life of the food articles is enhanced. For these purposes, synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG), and tert-butylhydroxyquinone (TBHQ) are used in a large scale in the commercial food products. Unfortunately, all these synthetic antioxidants have one or more adverse effects (Kahl and Kappus 1993; Lorenzo et al. 2018), many of which have not been explored or disclosed in order to protect commercial interest. Therefore, some other potential natural antioxidants like essential oils are being explored as an alternative

to the synthetic chemicals. Such antioxidant properties are also reported with respect to essential oil of betel leaf (Arambewela et al. 2006; Suppakul et al. 2006; Prakash et al. 2010; Das et al. 2016a). Several components of the leaf and its essential oil, such as vitamin C, vitamin A, vitamin E, terpenes and its derivatives, phenols and its derivatives, etc., are reported to be the active ingredients contributing to such antioxidant property (Guha 2006; Rathee et al. 2006; Swapna et al. 2012; Nagababu et al. 2014; Bhargava et al. 2015; Chauhan et al. 2016; Chitnis 2017; Sarma et al. 2018). Therefore, it may be concluded that there is a general agreement among the scientists that betel leaf and its oil possess strong antioxidant activity. Consequently, several workers have attempted to explore antioxidant capability of the essential oil of betel leaf for extending shelf life of several food products like apple juice (Basak 2018a), tomato paste (Basak 2018b), ghee (Gupta and Guha 2018), and several other products. That apart, manufacturing of some novel products can be taken up like food supplements and pharmaceuticals for treatment of cancer (Shukla et al. 2018; Kudva et al. 2018).

Another distinct bioactivity of essential oil of betel leaf, which is also very much relevant to the food sector, is the antimicrobial activity which is discussed in the foregoing paragraph.

5.15 Antimicrobial Activity of Essential Oil of Betel Leaf

We need food for mitigating hunger and for obtaining nutrition, and also for various other purposes like treatment of illness, convalescence, fun, pass time, social entertainment of guest, etc. Therefore, continuous supply of food is needed, for which it has to be stored for a long time that requires enhancement of shelf life. However, the microbes pose a serious threat to this. They not only spoil the food items but also release microbial toxins into the food which cause several types of diseases and even death. Therefore, attempts have been made to stop or minimize the harmful microbial growth to an acceptable limit in food by various means including incorporation of antimicrobial agents in the food or in the packaging materials. Some of the most common synthetic antimicrobial agents and their permissible limits are as follows: benomyl (<100 μg/kg/day body weight, EHC 1993; Malhotra et al. 2015), benzoic acid (<1000 ppm, FSSAI 2011), chlorotetracycline (<5 ppm, Sivasankar 2003), imazalil (<61 μg/kg/day, EPA 2005; Malhotra et al. 2015), natamycin (<500 ppm, HMDB 2011), nisin (<12.5 ppm, FSSAI 2011), oxytetracycline (<5 ppm, Sivasankar 2003), paraben (<10 ppm of body weight per day, EFSA 2004; Anand and Sati 2013), etc. These and many more antimicrobial agents are used during production of primary food or its processing, packaging, and storage to make the food articles safe for human consumption. These compounds though effective may pose several side effects ranging from simple ailments to incurable chronic diseases like cancer (EPA 2005; HMDB 2011; Anand and Sati 2013) which ultimately leads to painful unnatural death. That apart, many of the synthetic preservatives have pollutive effect and undesirably long persistence conducive to development

of resistant strains requiring progessively higher doses for controlling the microbes with lapse of time. Therefore, several hundreds of synthetic compounds are discarded every year (Dubey and Tripathi 1987). Moreover, continuous increase in public demand for minimally processed food with extended shelf life (Suppakul et al. 2006) and green consumerism are compelling the manufacturers to find out safe alternatives, such as nontoxic natural essential oils derived from the plants which are generally recognized as safe (Burt 2004; Burdock and Carabin 2004; Gutierrez et al. 2009).

There is a general agreement among the research workers that betel leaf possesses significant antimicrobial activity against a large number of microorganisms including both fungus and bacteria; the gram-positive bacteria being more susceptible than the gram-negative bacteria (Sugumaran et al. 2011; Agarwal et al. 2012; Pradhan et al. 2013; Bhargava et al. 2015; Chauhan et al. 2016). Prakash et al. (2010) reported that essential oil of betel leaf has a special merit in terms of antifungal and aflatoxins suppressive characteristics, which are the most desirable characteristics of an ideal food preservative for extending shelf life of edible commodities during storage and processing.

Mazumder et al. (2016) reviewed effect of different solvent extracts of betel leaf and found that it was effective against a wide range of microorganisms. However, there have been more works for exploring the antimicrobial activity of solvent extracts of betel leaf than its oil. The bioactive compounds responsible for such antimicrobial activity in the extracts are actually derived from the components of essential oil present in the leaves. Therefore, when oil is extracted from these leaves, the same is reasonably expected to be more powerful in its antimicrobial functions than the extracts at relatively lower doses since the concentration of the active ingredients is more in the oil compared to the leaf extracts. This is more so when the oil is reported to be thermo- and baro-stable, capable of withstanding high temperature (100 °C for 1 h) and subsequent autoclaving (121 °C at 15 psi for 20 minutes) treatments (Rath and Mohapatra 2015). These findings are corroborative to the conclusions drawn by Dubey and Tripathi (1987) who reported earlier that the antimicrobial properties of the oil were not affected by autoclaving temperature, duration of storage, and even inoculum density.

5.16 Affected Microorganisms and Corresponding Doses

Prakash et al. (2010) examined efficacy of essential oil of betel leaf against 1651 fungal isolates belonging to 14 species including six species of *Aspergillus* and found that the minimum inhibitory concentration (MIC) was as low as 0.70 µl/ml and aflatoxin production was completely inhibited at a concentration of 0.60 µl/ml.

Dubey and Tripathi (1987) found that the oil was toxic at a very low concentration of 0.20–0.50 μl/ml to 13 fungi including *Alternaria*, *Aspergillus*, *Botryodiplodia* (syn. *Lasiodiplodia*), *Penicillium*, and *Rhizopus* species. In a similar study, Garg and Jain (1992) reported that the essential oil of betel leaf (var. *Sagar Bangla*) was effective against nine strains of fungi including five strains of *Aspergillus* along with *A. niger* at a very low concentration (5 μl/ml). Apart from being effective against fungi, the oil was also found by them to be effective against three strains of gram-positive bacteria (*Bacillus subtilis*, *B. pumilus*, and *Staphylococcus aureus*) and two strains of gram-negative bacteria (*Salmonella typhi* and *Vibrio cholerae*). In further studies, the oil was also found to be toxic to *Penicillium expansum* in the experiments conducted by Basak and Guha (2015) who estimated the E_{max} (minimum concentration of oil at which mold growth was inhibited) and MIC values to be in the order of 0.56 and 0.74 μl/ml in both the potato dextrose agar medium (PDA) and apple juice agar medium. In continuation of this study, Basak (2018c) also found that essential oil of betel leaf (var. *Tamluk Mitha*) inhibited germination of both *A. flavus* and *P. expansum* at very low concentrations of 0.65 and 0.54 μl/ml, respectively, in PDA.

Apart from the Indian reports as above, similar reports are also available from foreign countries. Suppakul et al. (2006) found that essential oil of betel leaf from Thailand was highly effective against 13 strains of microorganisms including 5 strains of gram-positive bacteria, 5 strains of gram-negative bacteria, and 3 strains of yeast at a very low concentration (12.50–100 μl/ml) except *Pseudomonas aeruginosa* which was not sensitive to this oil even at the highest concentration (200 μl/ml). The susceptible microorganisms included *Bacillus cereus*, *Escherichia coli*, *Salmonella enteritidis*, and *Staphylococcus aureus* besides *Candida albicans* and *Saccharomyces cerevisiae.* It is, however, suggested that anti-quorum sensing properties of the active ingredients of essential oil may be explored for reducing the virulency of *P. aeruginosa* (Umar et al. 2018) which is reported to be resistant to this oil (Suppakul et al. 2006).

Caburian and Osi (2010) also explored the essential oil of Filipino betel leaf and concluded that the oil was effective against four harmful microorganisms. The minimum inhibitory concentrations were 250.00, 125.00, 15.60, and 1.95 μg/ml for *Candida albicans*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Trichophyton mentagrophytes*, respectively.

Satyal and Setzer (2012) experimented with chavicol-rich Nepalese betel oil and found that several microorganisms were susceptible to the oil at a very low concentration (MIC values shown in parentheses as μg/ml) such as *Candida albicans* (1250), *Escherichia coli* (625), *Pseudomonas aeruginosa* (625), *Staphylococcus aureus* (625), and *Aspergillus niger* (313). In another study from India on yeast, Rath and Mohapatra (2015) also found that essential oil of betel leaf was highly effective against four strains of *Candida* species at a very low concentration and the MIC ranged from 31.25 to 125.00 μl/ml.

Roy and Guha (2018) reported further that the antimicrobial efficacy of the oil (var. *Tamluk Mitha*) was enhanced by formulating a nano-emulsion with Tween 20 dispersed in water (3% oil, 3–6% Tween 20, and rest water) compared to macro-emulsion against three strains of gram-negative bacteria, namely, *Escherichia coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*. For nano-emulsion, the MIC ranged from 0.50 to 1.00 μl/ml, whereas for macro-emulsion, it was 0.75–1.25 μl/ml. Similarly, for nano-emulsion, the minimum bactericidal concentration ranged from 1 to 2 μl/ml, whereas for macro-emulsion, it was 1.50–2.50 μl/ml. In another study on two strains of fungi (*Aspergillus flavus* and *Penicillium expansum)*, Basak and Guha (2017b) concluded that micro-emulsion of essential oil of betel leaf (var. *Tamluk Mitha*) was highly toxic at a lower concentration (0.40 μl/ml), whereas sporicidal activity was found at a relatively higher concentration (15 μl/ml).

5.17 Mechanisms of Action and Active Ingredients

Basak and Guha (2017b) studied the ultramicroscopic structures of the treated spores and found that the oil affected the membrane integrity of the spores causing release of the internal cellular materials. Vintilla (2018) also reported modification of permeability or integrity of the cytoplasm or cell structures. That apart, there were cytoplasmic coagulation, shrinkage, granulation, and serious morphological damages. Several other authors also observed the destruction of cytoplasmic membrane leading to coagulation of cellular materials and other serious damages (Pauli 2001; Suppakul et al. 2006; Rath and Mohapatra 2015; Roy and Guha 2018). It is clear from these studies that the mode of antimicrobial action is similar in all the cases irrespective of type of cells and microorganisms, such as fungal hyphae, spores, both gram-positive and gram-negative bacterial cells, and even the budded cells of the yeasts. In all the cases, detrimental effects, deformation, and death are caused which is reported to take just a minute in case of *Candida* (Rath and Mohapatra 2015), but the time required for other species needs to be investigated and quantified. The active ingredients responsible for such detrimental effects are reported to be the derivatives of terpenes and phenols such as carvacrol, caryophyllene, chavibetol, eugenol, limonene, methyl eugenol, pinene, safrole, and some sterols (α-cardinol, linalool, β-sitosterol, etc.), which are present in the essential oil of betel leaf in different proportions, such as chavicol (0.40–48.00%), eugenol (6.40–63.56%), safrole (22.75–52.70%), etc. depending upon the varieties and environmental, edaphic, and managerial factors.

There are only a few reports available in the current literature for the confirmation of efficacy and mode of action of the supposed active ingredients discussed above. Therefore, the active ingredients in pure form and its systematic dilutions should be experimented with different strains of microorganisms separately. That apart, studies on the combination of active ingredients should also be taken up for exploring their synergistic, additive, or antagonistic effects. Such experiments will generate relevant information conducive for commercial exploitation of antimicrobial formulations from natural sources like betel leaf in future.

5.18 Food Product Development

Betel leaf is intended for human consumption due to its nutritional, organoleptic, and several other useful qualities. Therefore, it comes within the domain of food. The essential oil contained in the leaves has multiple unique natural characteristics relevant to the food industries, such as unique aroma and taste besides antimicrobial, antioxidant, and many other characteristics of high therapeutic significance. These characteristics provide a challenging opportunity to the food scientists and technologists to develop novel food products with enhanced food safety, extended shelf life, attractive sensory, therapeutic, prophylactic, functional, and other desirable qualities. However, research work in this domain is very limited. Therefore, research work was taken up at IIT, Kharagpur, and a few novel products have been developed with incorporation of essential oil of betel leaf ranging from 0.005% to 0.50% of the main ingredients. These developed products include ice cream (Jain 2012), chocolate (Godbole 2013), *suji halwa* (Bhagath and Guha 2014), cupcake (Roy and Guha 2015), lozenge (Vijay 2015), *rosogolla*, i.e. sweet balls of milk solids (Rajput 2017), etc. These products are not only novel but also expected to include most of the beneficial effects of the oil including the digestive one contributing to better health benefits to the consumers. That apart, the textural and organoleptic properties of all these developed products were also found to be better than or comparable to the bestselling commercial products available in the market. Such product development is envisaged to be economic, self-sustaining, and profitable to the entrepreneurs. This is also envisaged to minimize the wastage of surplus leaves which may range from 10% to 70% of the total annual production (Rao and Narasimham 1977; Guha and Jain 1997; Guha 2006) mainly during the glut season and thereby increase profitability of the betel vine farmers and the traders as well (Guha 2014).

Roy and Guha (2015) manufactured a novel cupcake with common ingredients using essential oil of betel leaf as a novel food additive (0.005% v/w). The textural, sensory, and economic data indicated that the betel-flavoured cupcake was comparable to the bestselling commercial cupcakes available in the market. Similar is the case with biscuit (Maurya 2014). This is the first time that two bakery products have been developed with essential oil of betel leaf and that such entrepreneurship could be profitable and self-sustaining.

Basak (2018b) reported that essential oil of betel leaf (var. *Tamluk Mitha*) has an enormous potential as a natural preservative in the food sector due to its safety and antimicrobial effectivity at a very low concentration (0.25 mg/g in micro-emulsion form) without adversely affecting sensory qualities of the food products. At this concentration, shelf life of the product (tomato paste) could be extended by 14 more days compared to control in an accelerated storage study (temperature 39 °C ± 1 °C and relative humidity 89% ± 1.2%). Therefore, it is envisaged that this oil has a tremendous future potential in the food processing industries dealing with tomato and its preparations. Similarly, this oil has also a tremendous future potential in another food processing industry dealing with apple and its products, particularly the apple juice which is a highly demanded nutritional drink all over the world. This industry is under a great threat of a bio-toxin called Patulin produced by the

toxicogenic fungus *Penicillium expansum* (Liewen and Bullerman 1992; Welke et al. 2011). This harmful microorganism is found to be highly susceptible to essential oil of betel leaf even at a very low concentration (0.56–0.74 μl/ml) which does not adversely affect the sensory qualities of the product (Basak 2018b). Thus, from these studies, it can be concluded that the essential oil of betel leaf would completely revolutionize the apple juice industry all over the world. Yet another food processing industry where the essential oil of betel leaf may play a significant role is the *Hokkein* noodle industry. The product, yellow alkaline noodles, when added with 15% betel leaf extract had shown the best sensory acceptability among the consumers (Nouri et al. 2015). This high dose (15%) may be reduced substantially if essential oil of betel leaf is used instead of the leaf extract which may also otherwise enhance shelf life and improve the quality of the food products. However, this needs a planned and detailed scientific study. Similar is the case with three products formulated from the spent or dried betel leaves such as biscuit (Laji 2015), tea (Nalkar 2015), and roti (Sriharsha 2017) besides one more product formulated from discarded petioles, namely, *Kathi Goja*, meaning sweet finger-shaped confectionary (Janaiah 2017).

In another study, Nouri and Nafchi (2014) formulated a sago starch-based edible film incorporating betel leaf extract and found that addition of 20% extract had significantly positive and acceptable impact on different mechanical and barrier properties of the film. Due to incorporation of betel leaf components, the film attained antimicrobial properties against both gram-positive and gram-negative bacteria except *Pseudomonas aeruginosa*. Here again, this high dose (20%) may be reduced substantially if essential oil of betel leaf is used instead of the leaf extract which may also otherwise improve the antimicrobial properties of the edible film. However, this needs further investigation for confirmation of the conception. Similarly, two products developed from *aloe vera* gel and potato chips incorporating betel leaf extracts (Arambewela et al. 2006) could also be improved by substituting the extracts with essential oil of betel leaf. This proposition of substitution of leaf extracts with essential oil can be supported with the fact that increase in sensory quality has been found to be directly proportional to the percentage of essential oil present in the leaves which can be found in the experiments conducted by Dastane et al. (1958). In their studies, they found that curing of betel leaf increased essential oil content from 1.23% to 4.20%. Curing is one of the procedure traditionally followed in India for enhancing shelf life and sensory qualities of the leaves to a great extent (Guha and Jain 1997; Sadhukhan and Guha 2011). Obviously, the cured leaves had much higher scores of sensory qualities mainly due to enhanced proportion of the oil in it which has already been discussed in the previous paragraphs.

Apart from the food products discussed above, some nonfood products have also been formulated by incorporating essential oil of betel leaf, such as toothpaste (Ekka 2015), soap (Lohra 2015), incense cone (Shah 2015), shampoo (Godbole 2012), etc.

In view of the entire discussions, it may be concluded that the essential oil of betel leaf has a potential future in the world food sector.

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References

Abdullah NF, Mohamad Hussain R, Amom Z (2016) Effect of APC on killing of *Staphylococcus aureus* by oxidative stress agents. J Teknol 78(5-5):39–44

Agarwal T, Singh R, Shukla AD et al (2012) Comparative analysis of antibacterial activity of four *Piper betle* varieties. Adv Appl Sci Res 3(2):698–705

Amaresh A, Guha P, Khan S et al (2017) Comparative study of microwave assisted hydro-distillation with conventional hydro-distillation for extraction of essential oil from *Piper betle* L. Biosci Biotechnol Res Asia 14(1):401–407

Amonkar AJ, Nagabhushan M, D'Souza AV et al (1986) Hydroxychavicol: a new phenolic antimutagen from betel leaf. Food Chem Toxicol 24(12):1321–1324

Anand SP, Sati N (2013) Artificial preservatives and their harmful effects: looking towards nature for safer alternatives. Int J Pharmaceu Sci Res 4(7):2496–2501

Ansari MJ, Al-ghamdi A, Usmani S et al (2017) *In vitro* evaluation of the effects of some plant essential oils on *Ascosphaera apis*, the causative agent of Chalkbrood disease. Saudi J Biol Sci 24:1001–1006

Arambewela L, Kumaratunga KGA, Dias K (2005) Studies on *Piper betle* of Sri Lanka. J Natl Sci Found Sri Lanka 33(2):133–139

Arambewela L, Arawwawala M, Rajapaksa D (2006) *Piper betle*: a potential natural antioxidant. Int J Food Sci Technol 41(1):10–14

Atsumi T, Fujisawa S, Tonosaki K (2005) A comparative study of the antioxidant/pro-oxidant activities of eugenol and isoeugenol with various concentrations and oxidation conditions. Toxicol In Vitr 19(8):1025–1033

Bahi A, Mansouri SA, Al Memari E et al (2014) β-caryophyllene, a CB_2 receptor agonist produces multiple behavioral changes relevant to anxiety and depression in mice. Physiol Behav 135:119–124

Balasubramanyam VR, Rawat AKS (1990) Studies on morphology and chemistry of *Piper betle* L. J Plantation Crops 18:78–87

Barnett C (2015) Making perfume from the rain. Available at: https://www.theatlantic.com/international/archive/2015/04/making-perfume-from-the-rain/391011/. Retrieved on 01/07/2018

Basak S (2017) Antifungal efficacy of essential oil of betel leaf (*Piper betle* L.) on *Aspergillus flavus* and *Penicillium expansum* and its potential as food preservative. PhD thesis (Under the guidance of Prof. P. Guha), Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur (W. B.), India

Basak S (2018a) The use of fuzzy logic to determine the concentration of betel leaf essential oil and its potency as a juice preservative. Food Chem 240:1113–1120

Basak S (2018b) Shelf life extension of tomato paste through organoleptically acceptable concentration of betel leaf essential oil under accelerated storage environment. J Food Sci 83(5):1396–1403

Basak S (2018c) Modelling the effect of betel leaf essential oil on germination time of *Aspergillus flavus* and *Penicillium expansum* spore population. LWT-Food Sci Technol 95:361–366

Basak S, Guha P (2015) Modelling the effect of essential oil of betel leaf (*Piper betle* L.) on germination, growth, and apparent lag time of *Penicillium expansum* on semi-synthetic media. Int J Food Microbiol 215:171–178

Basak S, Guha P (2017a) Betel leaf (*Piper betle* L.) essential oil microemulsion: characterization and antifungal activity on growth, and apparent lag time of *Aspergillus flavus* in tomato paste. LWT-Food Sci Technol 75:616–623

Basak S, Guha P (2017b) Use of predictive model to describe sporicidal and cell viability efficacy of betel leaf (*Piper betle* L.) essential oil on *Aspergillus flavus* and *Penicillium expansum* and its antifungal activity in raw apple juice. LWT-Food Sci Technol 80:510–516

Bevilacqua A, Speranza B, Perricone M et al (2018) Bioactivity of essential oils towards fungi and bacteria: mode of action and mathematical tools. In: Hashemi SMB, Khaneghah AM, de Sant'Ana AS (eds) Essential oils in food processing: chemistry, safety and application. Wiley, New York

Bhagath BVVB, Guha P (2014) Development of novel Sooji Halwa with unique properties of essential oil of betel leaf. Int J Agric Food Sci Technol 5:87–93

Bhalerao SA, Verma DR, Gavankar RV et al (2013) Phytochemistry, pharmacological profile and therapeutic uses of *Piper betle* Linn. – an overview. J Pharmacogn Phytochem 1(2):10–19

Bhargava P, Uppoor AS, Pralhad S et al (2015) An *in-vitro* study of determination of anti-bacterial, antioxidant, anti-inflammatory potential of *Piper betle* essential oil. Natl J Integr Res Med 6(2):37–44

Böhme K, Barros-Velázquez J, Calo-Mata P et al (2014) Antibacterial, antiviral and antifungal activity of essential oils: mechanisms and applications. In: Villa TG, Veiga-Crespo P (eds) Antimicrobial compounds. Springer, Berlin, pp 51–81

Brito MVH, de Moreira RJ, Tavares MLC et al (2005) Copaiba oil effect on urea and creatinine serum levels in rats submitted to kidney ischemia and reperfusion syndrome. Acta Cir Bras 20(3):243–246

Burdock GA, Carabin IG (2004) Generally recognized as safe (GRAS): history and description. Toxicol Lett 150(1):3–18

Burkill IH (1966) A dictionary of economic products of the Malaysian peninsula, Vol. II. Ministry of Agriculture, Malaysia, pp 1767–1772 (Reprint)

Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods – a review. Int J Food Microbiol 94:223–253

Caburian AB, Osi MO (2010) Characterization and evaluation of antimicrobial activity of the essential oil from the leaves of *Piper betle* L. E-Intl Sci Res J 2(1):2–13

Calleja MA, Vieites JM, Montero-Meterdez T et al (2013) The antioxidant effect of β-caryophyllene protects rat liver from carbon tetrachloride-induced fibrosis by inhibiting hepatic stellate cell activation. Brit J Nutr 109(3):394–401

de Candolle A (1884) Origin of cultivated plants. Kegan P, London (Quoted from Handbook of Herbs and Spices Vol. 2 edited by Peter KV, CRC Press)

Chakraborty D, Shah B (2011) Antimicrobial, anti-oxidative and anti-hemolytic activity of *Piper betle* leaf extracts. International J Pharm Pharmaceu Sci 3:192–198

Chakraborty JB, Mahato SK, Joshi K et al (2012) Hydroxychavicol, a *Piper betle* leaf component, induces apoptosis of CML cells through mitochondrial reactive oxygen species-dependent

JNK and endothelial nitric oxide synthase activation and overrides imatinib resistance. Cancer Sci 103(1):88–99
Chang MC, Uang BJ, Wu HL et al (2002) Inducing the cell cycle arrest and apoptosis of oral KB carcinoma cells by hydroxychavicol: roles of glutathione and reactive oxygen species. Br J Pharmacol 135:619–630
Chang CL, Cho IK, Li QX (2009) Insecticidal activity of basil oil, trans-anethole, estragole, and linalool to adult fruit flies of *Ceratitis capitata*, *Bactrocera dorsalis*, and *Bactrocera cucurbitae*. J Econ Entomol 102:203–209
Chang HJ, Kim JM, Lee JC et al (2013) Protective effect of β-caryophyllene, a natural bicyclic sesquiterpenes, against cerebral ischemic injury. J Med Food 16(6):471–480
Chattopadhyay SB, Maity S (1967) Diseases of betel vine and spices. ICAR, New Delhi
Chauhan ES, Aishwarya J, Singh A et al (2016) A review: nutraceuticals properties of *Piper betle* (Paan). Am J Phytomedicine Clin Ther 4(2):28–41
Cheng Y, Dong Z, Liu S (2014) β-Caryophyllene ameliorates the Alzheimer-like phenotype in APP/PS1 mice through CB_2 receptor activation and the PPARγ pathway. Pharmacology 94(1–2):1–12
Chitnis KS (2017) Quantitation of eugenol in betel leaf varieties by HPTLC. Int J Pharm Sci Res 8(11):4858–4862
Chu NS (2001) Effects of betel chewing on the central and autonomic nervous systems. J Biomed Sci 8(3):229–236
CSIR (Council of Scientific and Industrial Research, New Delhi) (1969) The Wealth of India. CSIR, New Delhi, pp 84–94
Da Silveira ESRDC, Andrade LN, De Oliveira RDRB et al (2014) A review on anti-inflammatory activity of phenylpropanoids found in essential oils. Molecules 19(2):1459–1480
Das S, Parida R, Sandeep IS et al (2016a) Chemical composition and antioxidant activity of some important betel vine landraces. Biologia 71(2):128–132
Das S, Parida R, Sriram IS et al (2016b) Biotechnological intervention in betel vine (*Piper betle* L.): a review on recent advances and future prospects. Asian Pac J Trop Med 9(10):938–946
Dastane NG, Patil RG, Chaugule BA (1958) This business of bleaching betel leaves. Indian Farming 7(10):10–12
Devi MP, Chakrabarty S, Ghosh SK et al (2015) Essntial oil: its economic aspect, extraction, importance, uses, hazards and quality. In: Sharangi AB, Datta S (eds) Value addition of horticultural crops: recent trends and future directions. Springer, India, pp 269–273
Dhongle SS, Kogje KK (2013) Comparative pharmacognostic study of leaves of two varieties of *Piper betle* L. namely Calcutta Bangla and Assam Kapoori. Int J Herb Med 1(3):115–119
Duan J, Kasper DL (2011) Oxidative depolymerization of polysaccharides by reactive oxygen/nitrogen species. Glycobiology 21(4):401–409
Dubey P, Tripathi SC (1987) Studies on antifungal, physico-chemical and phytotoxic properties of the essential oil of *Piper betle*. Dis Prot 94(3):235–241
Dwivedi V, Tripathi S (2014) Review study on potential activity of *Piper betle* L. J Pharmacogn Phytochem 93(34):93–98
EFSA (2004) EFSA advises on the safety of paraben usage in food. Available at: https://www.efsa.europa.eu/en/press/news/040929. Retrieved on 27/08/2018
EHC (1993) Environmental Health Criteria 148, Benomyl. Available at: http://www.inchem.org/documents/ehc/ehc/ehc148.htm. Retrieved on 27/08/2018
Ekka A (2015) Development of novel toothpaste with essential oil of betel leaf. B. Tech thesis (under the supervision of Prof. P. Guha), Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur (W. B.), India
EPA (US Environmental Protection Agency) (2005) US EPA archive document, R.E.D Facts. Imazalil, pp 1–8
EU (European Commission) (2001) Opinion of the scientific committee on food on estragole (1-allyl-4-methoxybenzene). SCF/CS/FLAV/FLAVOUR/6 ADD2 FINAL. Available at: https://ec.europa.eu/food/sites/food/files/safety/docs/fs_food-improvement-agents_flavourings-out104.pdf. Retrieved on 01/08/2018

Ferreres F, Oliveira AP, Gil-Izquierdo A et al (2014) *Piper betle* leaves: profiling phenolic compounds by HPLC/DAD-ESI/MS and anti-cholinesterase activity. Phytochem Anal 25(5):453–460

Foo LW, Salleh E, Nur S et al (2015) Extraction and qualitative analysis of *Piper betle* leaves for antimicrobial activities. Int J Eng Technol Sci Res 2:1–8

FSSAI (2011) Food Safety and Standards (Food Product Standards and Food Additives) Regulation, 2011. Available at: http://fsdaup.gov.in/writereaddata/images/pdf/act-and-rules/fss-regulation/Food-safety-and-standards-Food-product-standards-and-Food-Additives-regulation-2011.pdf. Retrieved on 27/08/2018

Garg SC, Jain R (1992) Biological activity of the essential oil of *Piper betle* L. J Essent Oil Res 4(6):601–606

Garg SC, Jain R (1996) Chavicol rich essential oil of *Piper betle* L., cultivar Sagar Bangla. Euro Cosmet 5:27–28

Gertsch J, Leonti M, Raduner S et al (2008) β-caryophyllene is a dietary cannabinoid. Proc Natl Acad Sci 105(26):9099–9104

Gildemeister E, Hoffmann F (1929) Die Atherischen Ole, vol Vol. II, 3rd edn. Wiley, New York, p 474

GOC (Government of Canada) (2010) Risk management scope for Benzene, 1,2-dimethoxy-4-(2-propenyl)-methyl eugenol. Chemical Abstract Service Registry Number (CAS RN): 93-15-2. Environment Canada Health. Available at: http://www.ec.gc.ca/substances/ese/eng/challenge/batch9/batch9_93-15-2_rm_en.pdf. Retrieved on 19/07/2018

Godbole AH (2012) Development of novel shampoo with essential oil of betel leaf. B. Tech thesis (under the supervision of Prof. P. Guha), Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur (W. B.), India

Godbole AH (2013) Development of novel chocolate with unique properties of essential oil of betel leaf (*Piper betle* L.). M. Tech thesis (under the supervision of Prof. P. Guha), Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur (W. B.), India

Gopalan C, Rama SBV, Balasubramanian SC (1984) Nutritive value of Indian food. National Institute of Nutrition, ICMR, Bangalore, India, p 108

Guenther E (1948) The essential oils, vol I. D. Van Nostrand & Co., Inc., New York

Guenther E (1952) The essential oils, 5th edn. D. Van Nostrand & Co, Inc., New York, pp 160–161

Guha P (1997) "*Paan Theke Kutir Silpa Sambhabana*" (In Bengali). "Exploring Betel Leaves for cottage industry", pp. 15–19, In: *Krishi, Khadya-O- Gramin Bikash Mela* – a booklet published by the Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur, India

Guha P (1998) Extraction of essential oil from betel leaves cultivated in and around Medinipur district. In: Annual report of All India Coordinated Research Project on Postharvest Technology (ICAR). Indian Institute of Technology, Kharagpur, pp 53–58

Guha P (1999) Extraction of essential oil from betel leaves. In: Annual report of All India Coordinated Research Project on Postharvest Technology (ICAR). Indian Institute of Technology, Kharagpur, pp 41–57

Guha P (2000) Commercial exploitation of oil from betel leaves. In Proc. 6th Regional workshop on oil seeds and oils, held at IIT, Kharagpur, West Bengal, India on 26th February, 2000, pp 56-57

Guha P (2002) Essential oil extraction from betel leaves: A promising options for rural employment. In: National Symposium on Information Technology for Sustainable Agriculture held during Jan 28–30, 2002 at Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur, pp 174–175

Guha P (2003) Extraction of essential oil from betel leaves grown in and around Medinipur district. In: Annual report of All India Coordinated Research Project on Postharvest Technology (ICAR). Indian Institute of Technology, Kharagpur, pp 15–23

Guha P (2006) Betel leaf: the neglected green gold of India. J Hum Ecol 19(2):87–93

Guha P (2007a) Extraction of essential oil: an appropriate rural technology for minimizing wastage of surplus betel leaves. Agric Mech Asia, Africa Lat Am 38(4):47–50

Guha P (2007b) Extraction of essential oil from betel leaf for manufacturing of food, medicinal, and cosmetics products. In: Proceedings of Indian Convention of Food Scientist and Technologists held during 31/12/2007 to 02/01/2008 at Indian Institute of Technology, Kharagpur, p 44

Guha P (2008) Processing, packaging and marketing of betel leaf (*Piper betle* L.). In national seminar on Piperaceae-harnessing agro-technologies for accelerated production of economically important *Piper* species, IISR, Calicut, Kerala, India, pp 258–269

Guha P (2009) Postharvest physiology of cured betel leaf. In: National Conference on Frontiers in Plant Physiology. Jorhat, Assam, India, p 25

Guha P (2010) Design, development, testing and comparative evaluation of the betel leaf oil extractor. Agric Mech Asia, Africa Lat Am 41(4):28–35

Guha P (2012) Employment generation through cultivation and processing of betel leaves in Chhattisgarh. In: National Seminar on role of Science and Technology for sustainable development of Chhattisgarh, Bhilai, India, p 01

Guha P (2014) Postharvest management of betel leaf (*Piper betle* L.): a review. In: Compendium of national meet on betel vine held at IIHR, Bangalore, India, pp 72–75

Guha P, Jain RK (1997) Status report on production, processing and marketing of betel leaf (*Piper betle* L.). Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur, India

Gupta N, Guha P (2018) Efficacy of essential oil of betel leaf (*Piper betle* L.) essential oil as a natural antioxidant for ghee preservation. International conference on Recent Advances in Food Processing Technology held at Indian Institute of Food Processing Technology, Thanjavur, Tamil Nadu, India, during 17–19th August, 2018. Poster Presentation. Poster code- C163

Gupta D, Singh A (2016) *Piper betle* and some Indian plant for antidepressant activity: a review. Res J Pharm Biol Chem Sci 7(2):1670–1678

Gutierrez J, Bourke P, Lonchamp J et al (2009) Impact of plant essential oils on microbiological, organoleptic and quality markers of minimally processed vegetables. Innov Food Sci Emerg Technol 10(2):195–202

Halliwell B (2007) Biochemistry of oxidative stress. Biochem Soc Trans 35:1147–1150

Halliwell B, Gutteridge JM (1995) The definition and measurement of antioxidants in biological systems. Free Radic Biol Med 18:125–126

Hans N (2017) Comparison of ultrasound assisted and conventional methods of extraction of essential oil from betel leaf (*Piper betle* L.). M. Tech thesis (under the supervision of Prof. P. Guha), Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur (W.B.), India

Hemalatha DH (2017) Microwave assisted extraction of essential oil from dried betel leaves (*Piper betle* L.). B. Tech thesis (under the supervision of Prof. P. Guha), Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur (W.B.), India

HMDB (The Human Metabolome Database) (2011) Natamycin, Material Safety Data Sheet Version 2.0 (Revised on 28/09/2011). Available at: http://www.hmdb.ca/system/metabolites/msds/000/013/627/original/Natamycin_MSDS.pdf?1368650610. Retrieved on 27/08/2018

HMDB (The Human Metabolome Database) (2018a). Chavicol. Available at: http://www.hmdb.ca/metabolites/HMDB0034107. Retrieved on 28/07/2018

HMDB (The Human Metabolome Database) (2018b). Cubebene. Available at: http://www.hmdb.ca/metabolites/HMDB0036413. Retrieved on 28/07/2018

HMDB (The Human Metabolome Database) (2018c). Eugenol. Available at: http://www.hmdb.ca/metabolites/HMDB0005809. Retrieved on 28/07/2018

Hyldgaard M, Mygind T, Piotrowska R et al (2015) Isoeugenol has a non-disruptive detergent-like mechanism of action. Front Microbiol 6:1–14

Islam QMS, Martin MA, Rashid MA et al (2015) Profitability of betel leaf (*Piper betle* L.) cultivation in some selected sites of Bangladesh. Bangladesh J Agric Res 40:409–420

Jain G (2012) Development of Novel Ice-cream with unique properties of essential oil of betel leaf (*Piper betle* L.). M. Tech thesis (under the supervision of Prof. P. Guha), Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur (W.B.), India

Jana BL (1995) *Gram Banglar Arthakari Phasal-Paan* (In Bengali). Betel leaf: a cash crop of villages of Bengal. Asaboni, Flat 203, 184, B B Chatterji Road, Calcutta, West Bengal, India.

Jana BL (1996) Improved technology for betel leaf cultivation. A paper presented in the Seminar-cum-Workshop on betel leaf marketing held at state cashew nut farm, Directorate of Agricultural Marketing, Digha, Midnapur (W.B.), India, June 5–6, 1996

Janaiah K (2017) Development of a novel *Kathi Goja* fortified with discarded petioles of betel leaf industry. M. Tech thesis (under the supervision of Prof. P. Guha), Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur (W.B.), India

Jantan I, Ahmad AR, Ahamd AS et al (1994) Comparative study of the essential oils of five *Piper* species from Peninsular Malaysia. Flavour Fragr J 9:339–342

Jeng JH, Chen SY, Liao CH et al (2002) Modulation of platelet aggregation by areca nut and betel leaf ingredients: roles of reactive oxygen species and cyclooxygenase. Free Radic Biol Med 32(9):860–871

Joshi RK (2013) Chemical composition, *in-vitro* antimicrobial and antioxidant activities of the essential oils of *Ocimum gratissimum*, *O. sanctum* and their major constituents. Indian J Pharm Sci 75(4):457–462

Kahl R, Kappus H (1993) Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E. Z Lebensm Unters Forsch 196:329–338

Karak S, Acharya J, Begum S et al (2018) Essential oil of *Piper betle* L. leaves: chemical composition, anti-acetylcholinesterase, anti-β-glucuronidase and cytotoxic properties. J Appl Res Med Aromat Plants. Available at: https://doi.org/10.1016/j.jarmap.2018.06.006. Retrieved on 08/09/2018

Khan TH (2014) Isoeugenol accelerates wound healing by attenuating pro-inflammatory markers and chemokine expression in diabetic mice. In: 5th World Congress on Bioavailability and Bioequivalence held at DoubleTree by Hilton Baltimore-BWI Airport, USA from September 29 to October 01, 2014, p 4172.

Khanra S (1997) *Paan Vittik Silpakendra* (In Bengali). "Betel leaf based industry". Nabanna Bharati 30(2):169

Khoshoo TN (1981) Welcome address, pp. xvii–xx. In: Proc of group discussion on Improvement of Betel Vine Cultivation. Khanduja SD, Balasubrahmanyam VR (eds.). National Botanical Research Institute, Lucknow, India

Kraft P (2004) Aroma Chemicals (4th ed): Musks. In: Rowe DJ (ed) Chemistry and technology of flavours and fragrances. Blackwell, UK, pp 143–145

Kudva AK, Rao S, Rao P et al (2018) *Piper betle* Linn. in cancer: past, present and future. In: Akhtar MS, Swamy MK (eds) Anticancer plants: properties and application, vol 1. Springer Singapore Pte Ltd., pp 327–348

Kumar S, Pathania AS, Satti NK et al (2015) Synthetic modification of hydroxychavicol by Mannich reaction and alkyne-azide cycloaddition derivatives depicting cytotoxic potential. Eur J Med Chem 92:236–245

Laji S (2015) Development of novel medicinal biscuit incorporated with betel leaf powder (*Piper betle* L.). M. Tech thesis (under the supervision of Prof. P. Guha), Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur (W.B.), India

Lee SK, Kim SD, Lee HY et al (2012) Iso-cubebene, a natural compound isolated from *Schisandra chinensis* fruit, has therapeutic benefit against polymicrobial sepsis. Biochem Biophys Res Commun 426(2):226–231

Liewen MB, Bullerman LB (1992) Toxigenic fungi and fungal toxins. In: Vanderzant C, Splittstoesser DF (eds) Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, DC, pp 811–819

Lohra S (2015) Development of novel soap with essential oil of betel leaf. B. Tech thesis (under the supervision of Prof. P. Guha), Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur (W.B.), India

Lorenzo JM, Pateiro M, Domínguez R et al (2018) Berries extracts as natural antioxidants in meat products: a review. Food Res Int 106:1095–1104

Madrid A, Espinoza L, Pavéz C et al (2014) Antioxidant and toxicity activity *in vitro* of twelve safrole derivatives. J Chil Chem Soc 59(3):2598–2601

Mahmoud MF, S-El S, Hasan RA et al (2014) Role of cannabinoid receptors in hepatic fibrosis and apoptosis associated with bile duct ligation in rats. Eur J Pharmacol 742:118–124
Maity S (1989) Extension bulletin: The Betel Vine. All India Coordinated Research Project on betel vine. Indian Institute of Horticultural Research, Hessarghatta, Bangalore, India
Malhotra B, Keshwani A, Kharkwal H (2015) Antimicrobial food packaging: potential and pitfalls. Front Microbiol 6:1–9
Marinov V, Valcheva-Kuzmanova S (2015) Review on the pharmacological activities of anethole. Scr Sci Pharm 2(2):14–19
Marko K (2017) Sassafras reduces inflammation, pain, toxins and more. Available at: https://www.thealternativedaily.com/sassafras-reduces-inflammation-and-pain/. Retrieved on 21/07/2018
Maurya S (2014) Development of novel biscuit with unique properties of essential oil of betel leaf (*Piper betle* L.). M. Tech thesis (under the supervision of Prof. P. Guha), Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur (W.B.), India
Mazumder S, Roychowdhury A, Banerjee S (2016) An overview of betel leaf (*Piper betle* L.): A review. Ann Food Sci Technol 17(2):367–376
MHMC (Malaysian Herbal Monograph Committee) (2015) Malaysian Herbal Monograph, Sireh Leaves. Institute of Medical Research, Ministry of Health, Malaysia, pp 416–427
Mohanto S, Datta S, Mandal S (2017) *Piper betle* Linn: a brief study. Int J Curr Med Pharm Res 3(2):1290–1296
Mohottalage S, Tabacchi R, Guerin PM (2007) Components from Sri Lanka *Piper betle* L. leaf oil and their analogues showing toxicity against the housefly *Musca domestica*. Flavour Fragr J 22:130–138
Muhammed AM (2007) Isolation, structure elucidation and properties of secondary metabolites in plants. PhD thesis (under the guidance of Prof. P. Mohamed Shafi), Department of Chemistry, University of Calicut, Kerala, India
Murakami Y, Kawata A, Ito S et al (2015) The radical scavenging activity and cytotoxicity of resveratrol, orcinol and 4-allylphenol and their inhibitory effects on *Cox-2* Gene expression and Nf-κb activation in RAW264. 7 cells stimulated with *Porphyromonas gingivalis*-fimbriae. In Vivo 29:341–350
Nagababu E, Rifikind JM, Boindala S et al (2014) Assessment of antioxidant activity of eugenol *in vivo* and *in vitro*. In: Rao MU, Subramanyam NM, William AP, Narasimham LP (eds) Free radicals and antioxidant protocol. Humana Press, New York, pp 1–5
Nagabhushan M, Amonkar AJ, Nair UJ, D'Souza AV, Bhide SV (1989) Hydroxychavicol: a new anti-nitrosating phenolic compound from betel vine leaf. Mutagenesis 4:200–204
Nagori K, Singh MK, Alexander A et al (2011) *Piper betle* L.: a review on its ethnobotany, phytochemistry, pharmacological profile and profiling by new hyphenated technique DART-MS (Direct Analysis in Real Time Mass Spectrometry). J Pharm Res 4(9):2991–2997
Nalkar P (2015) Development of novel herbal Tea from processed betel leaf. M. Tech thesis (under the supervision of Prof. P. Guha), Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur (W.B.), India
NCBI (National Center for Biotechnology Information) (2018). Chavibetol. Available at: https://alsaha.ru/C10H12O2.pdf. Retrieved on 24/07/2018
Nouri L, Nafchi AM (2014) Antibacterial, mechanical, and barrier properties of sago starch film incorporated with betel leaves extract. Int J Biol Macromol 66:254–259
Nouri L, Nafchi AM, Karim AA (2015) Mechanical and sensory evaluation of noodles incorporated with betel leaf extract. Int J Food Eng 11(2):221–227
Parise-Filho R, Pastrello M, Pereira CCE et al (2011) The anti-inflammatory activity of dillapiole and some semisynthetic analogues. Pharm Biol 49(11):1173–1179
Pauli A (2001) Antimicrobial properties of essential oil constituents. Int J Aromather 11(3):126–131
Periyanayagam K, Dhanalakshmi S, Karthikeyan V et al (2011) Phytochemical studies and GC/MS analysis on the isolated essential oil from the leaves of *Piper betle* L. var. Sirugamani1 (SGM1). J Nat Prod Plant Resour 4(7):2411–2413

Periyanayagam K, Jagadeesan M, Kavimani S et al (2012) Pharmacognostical and Phytophysicochemical profile of the leaves of *Piper betle* L. var. Pachaikodi (Piperaceae) – valuable assessment of its quality. Asian Pac J Trop Biomed 2:S506–S510

Peter KV (2004) Handbook of Herbs and Spices, 2nd edn. Woodhead Publishing Limited, CRC Press, Washington, DC, pp 85–89

PF (PerfumesWorld) (2018) Estragole. Available at: https://www.perfumersworld.com/product/estragole-4PS00169. Retrieved on 03/08/2018

Pradhan D, Suri KA, Pradhan DK et al (2013) Golden heart of the nature: *Piper betle* L. J Pharmacogn Phytochem 1(6):147–167

Prakash B, Shukla R, Singh P et al (2010) Efficacy of chemically characterized *Piper betle* L. essential oil against fungal and aflatoxin contamination of some edible commodities and its antioxidant activity. Int J Food Microbiol 142(1-2):114–119

Preethy TT, Elsy CR, Beena C (2017) Profiling of essential oil in Tirur vettilai (*Piper betle* L.), a group of unique betel vine land races from Malappuram district, Kerala. J Pharmacogn Phytochem 6(3):774–778

Rafferty JP (2012) Civet. Encyclopedia Britannica. Available at: https://www.britannica.com/animal/civet-mammal-Viverridae-family. Retrieved on 27/08/2018

Rai MP, Thilakchand KR, Palatty PL et al (2011) *Piper betle* Linn (betel vine), the maligned Southeast Asian medicinal plant possesses cancer preventive effects: time to reconsider the wronged opinion. Asian Pac J Cancer Prev 12:2149-2156.

Rajput SS (2017) Development of novel Rosogolla fortified with essential oil of betel leaf (*Piper betle* L.). M. Tech thesis (under the supervision of Prof. P. Guha), Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur (W. B.), India

Ramalakshmi K, Sulochanamma G, Rao JM et al (2002) Impact of drying on quality of betel leaf (*Piper betle* L.). J Food Sci Technol 39(6):619–622

Ramamurthi K, Rani OU (2012) Betel leaf: nature's green medicine. Facts You:8–10

Rana VS (2015) Production of fragrant raw materials. In FFDC (ed) A report on training cum workshop on essential oil, perfumery & aromatherapy held at New Delhi on March 14–16, 2015. http://www.ffdcindia.org/pdf/production_fragrant_19032015.pdf. Retrieved on 04/07/2018

Rao VM, Narasimham B (1977) Prolonging storage life of betel leaves. Indian J Hortic 34(2):175–182

Rath CC, Mohapatra S (2015) Susceptibility characterization of *Candida* spp. to four essential oils. Indian J Med Microbiol 33:93–97

Rathee JS, Patro BS, Mula S et al (2006) Antioxidant activity of *Piper betle* leaf extract and its constituents. J Agric Food Chem 54(24):9046–9054

Raut JS, Karuppayil SM (2014) A status review on the medicinal properties of essential oils. Ind Crop Prod 62:250–264

Ravindran PN, Johny AK, Nirmal BK (2002), Spices in our daily life. Satabdi Smaranika Vol. 2. Arya Vaidya Sala, Kottakka. (Quoted from Handbook of Herbs and Spices Vol. 2 Edited by Peter KV, CRC Press)

Rawat AKS, Tripathi RD, Khan AJ et al (1989) Essential oil components as markers for identification of *Piper betle* L. cultivars. Biochem Syst Ecol 17(1):35–38

Rayaguru K, Khan MK, Mohanty SN et al (2007) Effect of drying and storage on quality of betel leaf. J Agric Eng 44(1):64–68

Regnault-Roger C, Vincent C, Arnason JT (2012) Essential oils in insect control: low-risk products in a high-stakes world. Annu Rev Entomol 57(1):405–424

Rimando AM, Han BH, Park JH et al (1986) Studies on the constituents of Philippine *Piper betle* leaves. Arch Pharm Res 9(2):93–97

Roy A, Guha P (2015) Development of a novel cup cake with unique properties of essential oil of betel leaf (*Piper betle* L.) for sustainable entrepreneurship. J Food Sci Technol 52(8):4885–4894

Roy A, Guha P (2018) Formulation and characterization of betel leaf (*Piper betle* L.) essential oil based nanoemulsion and its *in vitro* antibacterial efficacy against selected food pathogens. J Food Proces Preserv, e13617. https://doi.org/10.1111/jfpp.13617.

Sadgrove N, Jones G (2015) A contemporary introduction to essential oils: chemistry, bioactivity and prospects for Australian agriculture. Agriculture 5:48–102

Sadhukhan S, Guha P (2011) Post-harvest technology of betel leaf (*Piper betle* L.): effect of curing on quality and physiological parameters. Agric Mech Asia Africa Lat Am 42(3):47–51

Samanta C (1994) *Paan chaser samasyabali-o-samadhan*: *Ekti samikkha* (In Bengali): A report on the problems and solutions of betel vine cultivation. A booklet published by Adhikari HR, C-2/16, Karunamoyee, Salt Lake City, Kolkata-64 (W.B.), India

Sandra P, Bicchi C (1987) Capillary gas chromatography in essential oil analysis. Alfred Huethig Verlag, Heidelberg/Basel/New York

Sankar CR, Sridevi D, Babu MK (1996) Studies on essential oil and oil constituents of betel vine cultivars. Andhra Agric J 43(1):24–26

Sarma C, Kumari S, Rasane P et al (2018) Exploring nutraceutical potential of selected varieties of *Piper betle* L. Carpathian J Food Sci Technol 10(1):64–73

Satyal P, Setzer WN (2012) Chemical composition and biological activities of Nepalese *Piper betle* L. Int J Prof Holist Aromather 1(2):23–26

Saxena M, Khare NK, Saxena P et al (2014) Antimicrobial activity and chemical composition of leaf oil in two varieties of *Piper betle* from northern plains of India. J Sci Ind Res 73(2):95–99

SDAMM (Superintendent, Directorate of Agricultural Marketing, Medinipure) (1996) *Paaner Kotipoy Tathya* (In Bengali). Some aspects of betel leaf. A paper presented in the Seminar-cum-Workshop on betel leaf marketing held at the state cashew nut farm, Directorate of Agricultural Marketing, Digha, Medinipure (W.B.), India

Sengupta K (1996) *Paan Patar Sangraksan* (in Bengali). Nabana Bharati 28(12):580–582

Sengupta R, Banik JK (2013) A review on betel leaf (Pan). Int J Pharm Sci Res 4(12):4519–4524

Shah B (2015) Development of a novel incense cone using unique properties of essential oil extracted from surplus betel leaves (*Piper betle* L.). M. Tech thesis (under the supervision of Prof. P. Guha), Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur (W.B.), India

Sharma ML, Rawat AKS, Balasubraminan VR et al (1981) Essential oil of betel leaf (*Piper betle* L.) cv. Kapoori. In: Khanduja SD, Balasubrahmanyam VR (eds) Improvement in betel vine cultivation. Proc of group discussion on Improvement of Betel Vine Cultivation. National Botanical Research Institute, Lucknow, pp 161–164

Sharma ML, Rawat AKS, Khanna RK et al (1996) Flavour characteristics of betel leaves. Euro Cosmet 5:22–24

Sharma S, Khan IA, Ali I et al (2009) Evaluation of the antimicrobial, antioxidant, and anti-inflammatory activities of hydroxychavicol for its potential use as an oral care agent. Antimicrob Agents Chemother 53(1):216–222

Shukla SV (2015). Global Scenario, market potential & business opportunities in essential oil, fragrance & flavour. In: Flavour and Fragrance Development Center, Kannuaj, UP, India, (ed) A report on training cum workshop on essential oil, perfumery and aromatherapy held at New Delhi 14–16, March 2015. Available at: http://www.ffdcindia.org/pdf/global_scenario_19032015.pdf. Retrieved on 04/07/2018

Shukla D, Johri J, Srivastava S et al (2018) Assessment of anticancer properties of betel vine. In: Akhtar MS, Swamy MK (eds) Anticancer plants: properties and application, vol 1. Springer Singapore Pte Ltd., pp 195–206

Singh KK, Balasubrahmanyam VR, Kochhar VK (1990) Effect of different packing methods, temperature conditions, treatment with chemicals on the senescence and storage behaviour of betel (*Piper betle* L.) leaves. J Plant Crop 18(1):23–28

Singh D, Narayanamoorthy S, Gamre S et al (2018) Hydroxychavicol, a key ingredient of *Piper betle* induces bacterial cell death by DNA damage and inhibition of cell division. Free Radic Biol Med 120:62–71

Sivasankar B (2003) Food processing and preservation. Prentice Hall of India Private Limited, New Delhi, p 118

Slater TF (1984) Free-radical mechanisms in tissue injury. Biochem J 222:1–15

Speranza B, Corbo MR (2010) Essential oils for preserving perishable foods: possibilities and limitations. In: Bevilacqua A, Corbo MR, Sinigaglia M (eds) Application of alternative food preservation technologies to enhance food safety & stability. Bentham Publisher, Sharjah (UAE), pp 35–57

Sriharsha Y (2017) Utilization of spent betel leaves from essential oil industry. M. Tech thesis (under the supervision of Prof. P. Guha), Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur (W.B.), India

Sugumaran M, Gandhi S, Sankarnarayanan M et al (2011) Chemical composition and antimicrobial activity of Vellaikodi variety of *Piper betle* Linn leaf oil against dental pathogens. Int J Pharm Tech Res 3:2135–2139

Suppakul P, Sanla-ead N, Phoopuritham P (2006) Antimicrobial and antioxidant activities of betel oil. Kasetsart J Natural Sci 40:91–100

Swapna NL, Ammani K, Prasad SHKR (2012) Antioxidant activity of Mokkathotapapada leaves of *Piper betle* L. cv. Kapoori. Free Rad Antiox 2(4):68–72

Tabacchi SMR, Guerin PM (2007) Components from Sri Lankan *Piper betle* L. leaf oil and their analogues showing toxicity against the housefly, *Musca domestica*. Flavour Fragrance 22:130–138

Thahn L, Dung NX, Luu HV et al (2002) Chemical composition of the leaf oil from *Piper betle* L. cultivated in Vietnam. J Essent oil Bear plants 5(1):38–42

TNEB (The New Encyclopedia Britannica) (1974) Musk Deer. p 448

TOI (Times of India) (2014) Sterilization could make betel leaves bacteria-free: experts. Available at: https://timesofindia.indiatimes.com/india/Sterilization-could-make-betel-leaves-bacteria-free-Experts/articleshow/37189046.cms. Retrieved on 08/07/2018

Umar RA, Sanusi NA, Zahary MN et al (2018) Chemical composition and the potential biological activities of *Piper betle* – a review. Malaysian J Appl Sci 3(1):1–8

Van de Braak SAAJ, Leijten GCJJ (1999) Essential oils and oleoresins: a survey in the Netherlands and other major markets in the European Union. Centre for the Promotion of Imports from Developing Countries, Rotterdam, p 116

Vasantha-srinivasan P, Senthil-nathan S, Selin-rani S et al (2016) Developmental response of *Spodoptera litura* Fab. to treatments of crude volatile oil from *Piper betle* L. and evaluation of toxicity to earthworm, *Eudrilus eugeniae* Kinb. Chemosphere 155:336–347

Vasantha-srinivasan P, Senthil-nathan S, Ponsankar A et al (2017) Comparative analysis of mosquito (Diptera: Culicidae: *Aedes aegypti* Liston) responses to the insecticide Temephos and plant derived essential oil derived from *Piper betle* L. Ecotoxicol Environ Saf 139:439–446

Vijay N (2015) Development of herbal novel Lozenge with flavour of essential oil of betel leaf. B. Tech thesis (under the supervision of Prof. P. Guha), Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur (W.B.), India

Vikash C, Tripati S, Verma NK et al (2012) PIPER BETEL: Phytochemistry, traditional use & pharmacological activity – a review. Int J Pharm Res Dev 4(4):216–223

Vintilla L (2018) Basic structure, nomenclature, classification and properties of organic compound of essential oil. In: Hashemi SMB, Khaneghah AM, de Sant'Ana AS (eds) Essential oils in food processing: chemistry, safety and application. Wiley, New York, pp 173–177

Welke JE, Hoeltz M, Dottori HA et al (2011) Patulin accumulation in apples during storage by *Penicillium expansum* and *Penicillium griseofulvum* strains. Braz J Microbiol 42:172–180

Wendy VWY, Ghali NA, Rukayadi Y et al (2014) Application of betel leaves (*Piper betle* L.) extract for preservation of homemade chili bo. Int Food Res J 21(6):2399–2403

Wikipedia (2018a) Deer Musk. Available at: https://en.wikipedia.org/wiki/Deer_musk. Retrieved on 01/07/2018

Wikipedia (2018b) Kannuaj perfume. Available at: https://en.wikipedia.org/wiki/Kannauj_Perfume. Retrieved on 26/08/2018

Wikipedia (2018c) Geosmin. Available at: https://en.wikipedia.org/wiki/Geosmin. Retrieved on 26/08/2018

Zahin M, Khan MS, Qais FA et al (2018) Antioxidant properties and anti-mutagenic potential of *Piper cubeba* fruit extract and molecular docking of certain bioactive compounds. Drug Chem Toxicol 41(3):358–367

Zielińska S, Matkowski A (2014) Phytochemistry and bioactivity of aromatic and medicinal plants from the genus *Agastache* (Lamiaceae). Phytochem Reviews 13(2):391–416

Chapter 6
Artemisia annua and *Artemisia afra* Essential Oils and Their Therapeutic Potential

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Abbreviations

ACT Artemisinin combination therapy
EO Essential oil

6.1 Introduction

For millennia, *Artemisia annua* L. was used by the Chinese to treat fever, which was often thought to be malaria (Hsu 2006; Tu 2011). The sesquiterpene lactone, artemisinin, is considered the main antimalarial phytochemical in *A. annua* (Fig. 6.1). However, many constituents of the plant's essential oils (EOs) including 1,8-cineole (eucalyptol), limonene, myrcene, α- and β-pinenes, and nerolidol also are known to be antimalarial as isolated chemicals, albeit with much less effective inhibitory concentrations (IC_{50} values) than artemisinin (see reviews by Weathers et al. 2014, 2017). There is evidence, however, suggesting that at least in some cases, the EO fraction per se is more potent than its individual constituents (Radulović et al. 2013). *A. afra* has also been used by native Africans to treat malaria (Liu et al. 2009; Watt and Breyer-Brandwijk 1962). While considerable information is known about the breadth of the medicinal properties of *A. annua* to treat not only malaria as well as other diseases, *A. afra* has only recently attracted more attention for its healing properties (Patil et al. 2011). Although their composition is somewhat different, constituents of the EOs of each species seem to play a role in the therapeutic efficacy of both plant species. Here, we summarize what is currently known about the EO fraction of these two important medicinal plant species and how the phytochemicals therein may affect therapeutic outcomes.

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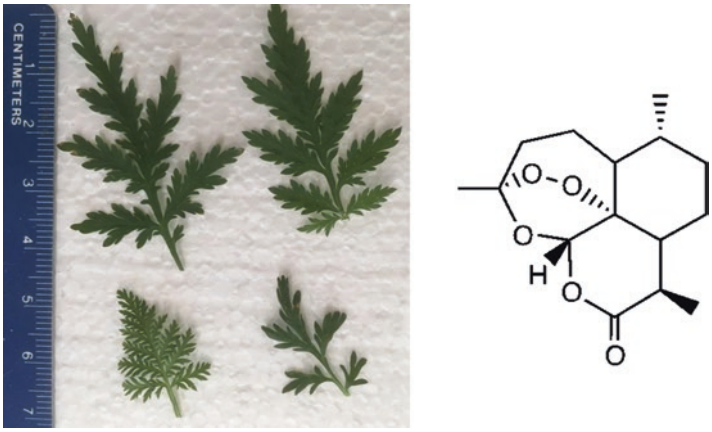

Fig. 6.1 Representative leaves of the same developmental age from three cultivars of *Artemisia annua* and one *A. afra* along with structure of artemisinin. Clockwise from top left: *A. annua* SAM, #15, GLS, *A. afra*

6.2 Essential Oil Content of *A. annua*

6.2.1 Cultivar Content Differences

One of the major difficulties in studying EOs from *A. annua* is the inconsistency of the oil contents. From cultivar to cultivar, the major constituents of the oil can change dramatically. Even within cultivars, factors including geographic location, growth conditions, method of propagation, and stage of development at harvest can change the contents of the oil. For example, our lab performed a phytochemical analysis of *A. annua* essential oil sourced from the United States or China. The resulting peak areas from the GC-MS analysis can be seen in Table 6.1 (Desrosiers and Weathers 2017). As expected, both oils contained mostly monoterpenes; however, the oil sourced in the United States contained almost 31% thujone, a compound not detectable in the Chinese-sourced oil. Although thujone is characteristically found in *A. afra* and *A. absinthium*, *A. annua* is consistently reported to be thujone-free, which aids in its validation as a generally recognized as safe (GRAS) plant (Duke 2001). The presence of thujone, therefore, suggests the US-sourced EO was adulterated with oil from other *Artemisia* species. The US-sourced oil contained about 30% camphor, while the Chinese oil only contained about 14% of the same. Such dramatic differences in EO composition even within the same plant species (see below) make studying the oil and establishing reliable scientific conclusions difficult. Comparisons between labs are nearly impossible, and this raises questions about therapeutic uses of the oil if a consistent product cannot be produced on a large scale. This also highlights the dangers of using unregulated EO products to treat medical conditions. An EO of undefined chemical makeup cannot be trusted to consistently treat any medical condition and may even have undesirable health effects.

Table 6.1 Relative abundance of some phytochemicals identified by GC-MS in *A. annua* essential oil from US and Chinese sources (Desrosiers and Weathers 2017)

Phytochemical	US EO source % of total peak area	Chinese EO source % of total peak area
1,8-Cineole (eucalyptol)	16.5	27.4
α-Pinene	0.3	17.5
β-Pinene	2.5	4.7
Borneol	3.7	1.8
Camphene	13.2	5.5
Camphor	30.3	14.1
Carene	Nd	2.2
Caryophyllene	1.0	6.2
Caryophyllene oxide	0.9	Nd
Copaene	Nd	1.1
Humulene	Nd	5.5
Limonene	Nd	5.2
Myrcene	0.1	Nd
Phellandrene	Nd	8.5
Santolina triene	0.1	0.2
Stigmasterol	0.1	Nd
Terpineol	0.2	Nd
Thujone	30.9	Nd

EO essential oil, *Nd* not detected

6.2.2 *Changes Throughout Plant Development*

As *A. annua* growth shifts from the vegetative to reproductive stage, EO components change, and maturity of the leaf is also a factor (Bagchi et al. 2003; Rana et al. 2013; Towler and Weathers 2015; Yang et al. 2012). For example, 1,8-cineole content decreases for the *A. annua* SAM clonal line in vegetative leaves versus leaves from plants in the budding (reproductive) stage. Vegetative leaves also have no detectable α-pinene, but it is present in floral budding plants, and camphor is nearly threefold higher in young leaves of the shoot apical meristem region versus mature leaves (Towler and Weathers 2015). However, as previously noted, different cultivars have varying EO profiles, along with unpredictable responses to the morphological changes associated with plant maturation. Each cultivar would need to be studied in a particular environment and growth stage in order to obtain a reasonable description of its "typical" EO content. Such developmental variations in EOs would also be expected for *A. afra*.

6.2.3 Artemisia afra

Why *A. afra*? This species is native to southern Africa and has been used as a tea infusion by indigenous peoples to treat fevers, especially associated with malaria (Watt and Breyer-Brandwijk 1962). Many other medicinal properties are ascribed to the plant; these are well summarized in two relatively recent reviews (Liu et al. 2009; Patil et al. 2011). Recently, tea infusions of *A. afra* and *A. annua* performed faster than praziquantel in treating schistosomiasis in a human clinical trial (Munyangi et al. 2018). The oil also shows antimicrobial activity against some bacteria and yeast species. Unfortunately, most of the studies express antibiotic activity through a series of oil dilutions or by zones of inhibition; only a few studies provide IC_{50} values, statistical analysis of minimum inhibitory concentration (MIC), or minimum inhibitory percentage (MIP). In a recent malaria clinical trial, a tea infusion of *A. afra* performed similarly to *A. annua,* and both were better than artemisinin combination therapy (ACT) in their therapeutic efficacy and reduction of gametocyte carriage (Munyangi et al. 2019). Interestingly, *A. afra* used in that study only had trace amounts of artemisinin.

Over 130 volatile chemicals constituting *A. afra* EO were identified and documented in a review by Liu et al. (Liu et al. 2009). The most common volatiles in *A. afra* include artemisyl acetate, 1,8-cineole, α- and β-thujone, artemisia ketone, α-copaene, camphor, santolina alcohol, borneol, and camphene (Liu et al. 2009). As observed for *A. annua*, these EO phytochemicals change in quantity and quality with plant part, among cultivars, throughout development, with cultivation, and with processing method. For example, surveying some of the volatile constituents of dried leaf samples of three cultivars analyzed in our lab, camphor can vary by threefold and thujone can be present or absent (Table 6.2). Drying methods also substantially alter volatile components from 0.18% to 1.88% from fresh to dried material (Asekun et al. 2007). Considering that fresh material has about ten times the water of dried material, these percent values are not particularly different. However, drying did change the relative composition of individual phytochemicals within the oil. As an example, artemisia ketone was present in fresh material but absent in air- and sun-dried material (Asekun et al. 2007). When comparing the effects of microwave-drying against air-, sun-, and oven-dried material, the monoterpenes, 1,8-cineole and β-thujone (but not α-thujone) decreased, while several other compounds increased, particularly *trans*-caryophyllene (Ashafa and Pitso 2014).

6.2.4 Caveats About Extraction and Analysis

Methods of producing EOs include steam distillation, solvent extraction, CO_2 extraction, maceration, enfleurage, cold-press extraction, and water distillation. Factors such as temperature, pressure, and processing time all affect the quality and

Table 6.2 Example of essential oil variation in *A. afra* cultivars

	Phytochemical (mg g DW^{-1})		
Cultivar	PAR	SEN	WPI
1,8-Cineole (eucalyptol)	0.47	0.27	2.68
a-Pinene	Nd	Nd	0.02
β-Neoclovene #	0.51	0.13	4.32
β-Pinene	Nd	Nd	0.02
Borneol #	0.67	0.07	0.55
Camphor	3.26	0.72	2.90
Caryophyllene	Nd	Nd	Nd
Caryophyllene oxide	Nd	Nd	Nd
Spathulenol #	0.12	Nd	0.28
Thujone	0.86	Nd	Nd
Other important phytochemicals			
Artemisinin	Nd	0.05	Nd
Total flavonoids +	3.74	3.03	7.95

Methylene chloride extract assayed directly by GC-MS. Each of the three cultivars were dried leaves from plants grown in Senegal (SEN), Paris (PAR), or at Worcester Polytechnic Institute (WPI), with the latter sourced from Companion Plants, Athens, OH, USA. Each plant cultivar had an $n = 6$; Nd = not detected; all identified using NIST library; #, expressed as camphor equivalents; +, expressed as quercetin equivalents; all others quantified with authentic standards

composition of the resulting EO. In terms of identification and quantification of the phytochemicals in a given EO product, it can be very difficult to make comparisons among content claims. Most notably, it is important to be aware that, particularly for GC-MS analysis, the ion current generated by a compound depends on its characteristics and is not a true measure of quantification (Tzenkova et al. 2010). EOs are often described by listing identifiable components and their respective peak areas, however, this is an estimation. In addition, compound identification is not infallible. Reference standards are needed for accurate quantification, and it is nearly impossible or prohibitively expensive to procure them for every compound present within an EO mixture. Of note, the source of the *A. annua* EO from China shown in Table 6.1 claimed that it had a high content of artemisia ketone; however, it was undetectable.

6.3 Therapeutic Efficacy of *A. annua* Essential Oils

6.3.1 Diversity of Therapeutic Efficacy

Artemisinin from *A. annua* has been widely studied for its antimalarial activity, and several derivatives have been developed as ACTs and are in use to combat malaria worldwide. However, the EO produced by *Artemisia* species also has wide-ranging antimicrobial properties. Many have pondered the evolutionary benefits of producing

EO, as well as artemisinin, to the *A. annua* plant. Some have speculated these compounds acted as antimicrobials and insecticides to deter herbivorous insects and pathogenic microbes. There is indeed evidence for these hypotheses, as several studies have shown *A. annua* EO to have activity against common bacterial and fungal strains, with examples shown in Table 6.3.

While the EO of *A. annua* has shown some promise in vitro, there are few studies in vivo and there are still several questions surrounding the use of EOs as a therapeutic. For example, the proper way to deliver EOs is not clear. For certain external infections, topical application may suffice, but in vivo studies and clinical trials would have to be performed to establish efficacy, dosage, and safety. Furthermore, it is unclear whether or not it is economically feasible to produce EOs on a large enough scale to be used as a therapeutic. Large-scale production of EOs requires steam distillation of large amounts of plant material to produce a small amount of oil, and for this reason, it may simply be too expensive to rely on EOs as antimicrobials.

Table 6.3 Antimicrobial activity of *A. annua* essential oil

Microbial species	Activity	Reference
Candida albicans *Saccharomyces cerevisiae* *Enterococcus hirae*	Growth inhibition	Juteau et al. (2002)
Listeria innocua	No activity	Viuda-Martos et al. (2010)
Staphylococcus aureus *Bacillus cereus* *Enterococcus faecalis* *Pseudomonas aeruginosa* *Escherichia coli*	Growth inhibition and bactericidal	Massiha et al. (2013)
Staphylococcus aureus *Escherichia coli* *Bacillus subtilis* *Bacillus thuringiensis*	Growth inhibition	Li et al. (2011)
Staphylococcus aureus *Escherichia coli* *Saccharomyces cerevisiae* *Candida albicans*	Growth inhibition	Verdian-Rizi et al. (2008)
Pseudomonas aeruginosa	No activity	
Bacillus cereus *Staphylococcus aureus* *Sarcina lutea* *Escherichia coli* *Klebsiella pneumoniae* *Salmonella enteritidis* *Shigella* sp. *Candida albicans* *Aspergillus fumigatus*	Growth inhibition	Radulović et al. (2013)

6.3.2 Toxicity: Humans

Although any pure EO can be toxic, consumption of *A. annua* EOs as part of dried leaf material or a tea infusion is nontoxic; the plant is GRAS, and tea infusions have been consumed for millennia. *A. afra* may contain thujone in its EO, which is considered toxic and, therefore, regulated. Thujones act on the central nervous system as antagonists of γ-aminobutyric acid-A ($GABA_A$) (Höld et al. 2000) and 5-hydroxytryptamine (5-HT_3, or serotonin) (Deiml et al. 2004), but the toxicity of *A. afra* is debatable. Interestingly, the EU restricts thujone to 0.5 mg kg^{-1} in food prepared with *Artemisia* species, but in those made with sage, which also contains the monoterpene, the limit is 25 mg kg^{-1}. There are also similar types of restrictions for foods and beverages in the USA and Canada, again with exceptions for use of the herb, sage. In rats, the per os LD50 is 500 mg kg^{-1}, and thus, naturally occurring levels of thujone in *A. afra* are too low from orally consumed plant material either as powdered dried leaves or as tea infusions to be considered toxic (Table 6.2). Additionally, only a small fraction of the thujone present in plant material is extracted into water compared to an ethanolic formulation (Tegtmeier and Harnischfeger 1994).

6.3.3 Process Caveats: Losses with Drying, Storage, Powdering, Tableting

Fresh *A. annua* plant material has a different EO profile versus dried, processed leaves, mainly due to the volatile nature of the monoterpene components. After fresh leaves were dried, sieved, and powdered, camphor and 1,8-cineole content decreased. Only camphor remained detectable after powdered leaves were processed into tablets via mechanical compression (Table 6.4; adapted from Weathers and Towler 2014). Powdering dried leaf material with a blade mill generates only slight heat when performed in short pulses. However, operation of the machinery required to form the tablets can cause an increase in processing temperature, which can

Table 6.4 Selected compounds in *A. annua* and effect of drying, granulation, and tablet formation

Compound	Fresh leaves[a] mg g^{-1} DW^{-1}	Dried and sieved mg g^{-1} DW^{-1}	Powdered mg g^{-1} DW^{-1}	Tablets mg g^{-1} DW^{-1}
1,8-cineole	0.30	0.03	0.03	Nd
Camphor	3.57	2.10	1.67	0.19
Artemisinin	11.38	15.90	17.31	17.18
Total flavonoids #	1.55	2.78	5.05	10.97

[a]DW calculated using DW/FW ratio of 0.25. Methylene chloride extract assayed by GC-MS, except total flavonoids assayed by colorimetric aluminum chloride assay. Each condition had $n \geq 6$; nd = not detected; #, expressed as quercetin equivalents; all others quantified with authentic standards and identified using NIST library

account for the loss of monoterpenes. We simulated different processing methods of *A. annua* leaves on a small scale by comparing a commercial coffee mill that has a flat blade (Kitchen Aide) to a mortar and pestle, representing cutting and crushing, respectively. Compared to powdering via cutting, analysis of five components of the *A. annua* EO powdered using mortar and pestle showed a percent loss of 40, 100, 100, 15, and 0 for camphor, α-pinene, eucalyptol, caryophyllene, and phytol, respectively. Artemisinin and total flavonoid content responded differently; drying and processing had a neutral or positive effect on the measured concentrations (Table 6.4). Processing variations also affected *A. afra* EOs (Ashafa and Pitso 2014).

We also monitored the stability of the dried leaf material in storage by tracking the total flavonoid content and several EO monoterpenes in dried *A. annua* leaves kept in a sealed plastic bag at room temperature in dim light. While the amount of artemisinin and total flavonoids remained relatively stable for over a year, the monoterpene fraction declined over time. After 2 months, we observed that camphor and 1,8-cineole content dropped by 25%; after 2 years, camphor content decreased by over 60% and 1,8-cineole by nearly 90%.

This information collectively emphasizes the importance of selecting the appropriate processing equipment and performing assays on the final consumer-ready product and not just the starting material. The EO profile is particularly susceptible to processing changes.

6.4 Bioavailability

Beyond its direct bioactivity against microbes, the EO of *A. annua* has effects on the bioavailability of the main drug of interest in *A. annua*, artemisinin, at least indirectly increasing the therapeutic efficacy of dried leaf treatment. The oral bioavailability of any drug is influenced by several factors as illustrated in Fig. 6.2. An orally delivered drug is first subject to low pH and digestive enzymes in the stomach. It is then subject to more enzymatic activity, bile, and more neutral pH in the small intestine. Most absorption occurs in the intestine where solubility of the drug has significant effects on how well the drug is absorbed. The drug also is subjected to limited first-pass metabolism as it is transported across the intestine and then travels

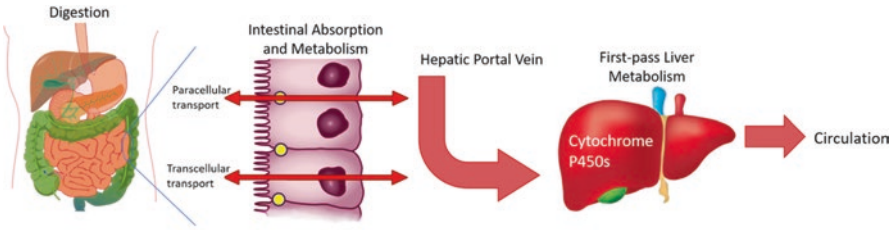

Fig. 6.2 Schematic of the steps orally delivered drugs take to reach systemic circulation

through the hepatic portal vein to the liver where it is then subjected to significant metabolism before reaching systemic circulation. *A. annua* EO may have effects at each stage in this process, and those effects are discussed here. For a more comprehensive review of oral drug bioavailability, please see Song et al. (2004).

6.4.1 Solubility Effects

A. annua EOs are largely found in the glandular trichomes where they co-localize with artemisinin, allowing it to stay in solution. Traditionally, artemisinin is known to have low bioavailability due largely in part to its low aqueous solubility. In its pure form, the drug does not readily solubilize in aqueous environments like the stomach or intestine, leading to very low absorption into the blood. As a result, pure artemisinin treatment is only marginally effective against malaria because most of the drug does not reach systemic circulation where it is required to act on the *Plasmodium* parasites. Consequently, the derivatives of artemisinin currently used in ACTs were developed to have increased aqueous solubility. Interestingly, when artemisinin is delivered as plant material, artemisinin solubility is about fourfold higher in simulated intestinal fluid (Desrosiers and Weathers 2016). EO from the plant is largely responsible for this effect. When *A. annua* EO was combined with pure artemisinin and subjected to simulated digestion experiments, the solubility in simulated intestinal fluid increased 2.5-fold (Desrosiers and Weathers 2016). This effect on solubility partially explains why artemisinin delivered as dried *A. annua* leaves was >40× more bioavailable than pure drug in mice (Weathers et al. 2011). Other antimalarial phytochemicals in *A. annua* also have low aqueous solubility; for example, the flavonoids, which are reported to synergistically enhance antimalarial efficacy of artemisinin (Liu et al. 1992; Suberu et al. 2013). Bioavailability of these phytochemicals may also be increased. Thus, by increasing solubility of artemisinin and other phytochemicals, the EO in the plant also increases the bioavailability of these compounds, further enhancing the antiplasmodial efficacy of artemisinin delivered as powdered dried plant leaves.

6.4.2 Intestinal Transport

Besides altering artemisinin solubility, the EO of *A. annua* altered the intestinal permeability of artemisinin. Artemisinin is transported across the intestine through simple diffusion (Augustijns et al. 1996), and our group has shown its intestinal permeability is increased by 37% when delivered as digested *A. annua* leaves (Desrosiers and Weathers 2017). In studies using the Caco-2 cell model of the intestinal epithelium, *A. annua* EO that had been subjected to simulated digestion decreased the intestinal permeability of artemisinin. However, when EO from *A. annua* and dried leaves of an *A. annua* mutant lacking glandular trichomes and

artemisinin were digested together with pure artemisinin using the simulated system, the permeability of artemisinin was unchanged compared to pure artemisinin controls (Desrosiers and Weathers 2017). These data suggested that *A. annua* EO decreased permeability on its own and that this decrease was nullified by the bulk of the plant matrix.

6.4.3 Potential Effects on the Liver

The full story of how *A. annua* EOs affect artemisinin bioavailability is not complete without understanding how EOs affect metabolism in the liver, where artemisinin is known to undergo significant first-pass metabolism. In this scenario, artemisinin is metabolized into four metabolites: deoxyartemisinin, deoxydihydroartemisinin, 9,10-dihydrodeoxyartemisinin, and crystal 7 (Lee and Hufford 1990). None has any antimalarial activity mainly due to the loss of the endoperoxide bridge responsible for the drug's potent activity against *Plasmodium*. In addition to solubility concerns, the high first-pass metabolism of artemisinin is another reason for the development of the semi-synthetic artemisinin derivatives currently used in ACTs. These semi-synthetic derivatives are metabolized in the liver into dihydroartemisinin, which retains the endoperoxide bridge and potent antiplasmodial activity (Lee and Hufford 1990; Navaratnam et al. 2000).

Several compounds found in the EO of *A. annua* may, however, modulate first-pass liver metabolism of artemisinin in a way that allows more of the drug to reach systemic circulation. For example, camphor, one of the components found in both *A. annua* and *A. afra* EOs, inhibits CYP2B6, the main enzyme responsible for artemisinin metabolism (Seo et al. 2008; Svensson and Ashton 1999). By inhibiting CYP2B6, camphor present in the oil would allow more artemisinin to bypass metabolism in the liver and reach systemic circulation. Borneol, limonene, and cineol also inhibited CYP2B6. More studies are needed to determine if other components in *A. annua* EO inhibit CYP2B6 as well as CYP3A4, which is also partially involved in artemisinin metabolism.

6.5 Repellency and Insecticidal Activity

Besides having a myriad of antimicrobial activities, *A. annua* EOs have been reported to have various insecticidal and repellant qualities, including activities against stored product beetles, codling moths (*Cydia pomonella*), blowflies (*Calliphora vomitoria*), *E. paenulata,* and *S. eridania* (Table 6.5).

As with their use as antimicrobials, questions remain about the economic feasibility of these compounds as insecticides. Nevertheless, EOs may offer a more environmentally friendly alternative to some common synthetic insecticides. Many have also postulated that *A. annua* EO may function as an effective mosquito repellant;

Table 6.5 Repellant and insecticidal activity of *A. annua* essential oil and essential oil components on various insect species

A. annua component	Species	Activity	Source
A. annua alcoholic extract	*Cydia pomonella*	Repellant	Durden et al. (2011)
Artemisinin			
1,8-Cineole			
A. annua EO	*Calliphora vomitoria*	Insecticidal	Bedini et al. (2017)
A. annua EO	*Tribolium castaneum*	Repellant and insecticidal	Tripathi et al. (2000)
	Callosobruchus maculatus	Insecticidal	

however, to our knowledge, no reliable studies have been conducted to date to validate this claim. Many of the EOs found in *A. annua* are also in *A. afra* and would be expected to provide similar responses.

6.6 Conclusions

While EOs per se are not recommended as direct therapeutic agents, their inclusion in an herbal or other medicinal preparation may have profound effects on therapeutic outcomes. Evidence shows that EOs from *A. annua* and *A. afra* may not only have direct therapeutic effects against various ailments including infectious diseases but also enhance the bioavailability of more potent phytochemical drugs, e.g., artemisinin. The mechanism of action of EO effects include improved solubility of otherwise poorly soluble compounds and possible inhibition of liver metabolism by cytochrome P450s. Overall, this information enhances our understanding of the role of EOs in therapeutic medicinal applications.

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References

Asekun OT, Grierson DS, Afolayan AJ (2007) Variations in the quality and yield of the essential oil from *Artemisia afra* using different drying methods. J Essent Oil Bear Pl 10(1):5–9

Ashafa AOT, Pitso TR (2014) Effects of microwave drying on the yield and chemical composition of essential oils of *Artemisia afra* Jacq. Ex willd from the Eastern Free State South Africa. J Essent Oil Bear Pl 17(6):1087–1093

Augustijns P, D'Hulst A, Van Daele J, Kinget R (1996) Transport of artemisinin and sodium artesunate in Caco-2 intestinal epithelial cells. J Pharm Sci 85(6):577–579

Bagchi GD, Haider F, Dwivedi PD, Singh A, Naqvi AA (2003) Essential oil constituents of *Artemisia annua* during different growth periods at monsoon conditions of subtropical north Indian plains. J Essent Oil Res 15(4):248–250

Bedini S, Flamini G, Cosci F, Ascrizzi R, Echeverria MC, Guidi L, Landi M, Lucchi A, Conti B (2017) Artemisia spp. essential oils against the disease-carrying blowfly Calliphora vomitoria. Parasit Vectors 10(1):80

Deiml T, Haseneder R, Zieglgänsberger W, Rammes G, Eisensamer B, Rupprecht R, Hapfelmeier G (2004) α-Thujone reduces 5-HT 3 receptor activity by an effect on the agonist-induced desensitization. Neuropharmacology 46(2):192–201

Desrosiers MR, Weathers PJ (2016) Effect of leaf digestion and artemisinin solubility for use in oral consumption of dried *Artemisia annua* leaves to treat malaria. J Ethnopharmacol 190:313–318

Desrosiers MR, Weathers PJ (2017) Artemisinin permeability via Caco-2 cells increases after simulated digestion of *Artemisia annua* leaves. J Ethnopharmacol

Duke JA (2001) Handbook of phytochemical constituents of GRAS herbs and other economic plants. CRC Press LLC, Boca Raton, FL

Durden K, Sellars S, Cowell B, Brown JJ, Pszczolkowski MA (2011) *Artemisia annua* extracts, artemisinin and 1, 8-cineole, prevent fruit infestation by a major, cosmopolitan pest of apples. Pharm Biol 49(6):563–568

Hsu E (2006) The history of qing hao in the Chinese materia medica. Trans R Soc Trop Med Hyg 100(6):505–508

Höld KM, Sirisoma NS, Ikeda T, Narahashi T, Casida JE (2000) α-Thujone (the active component of absinthe): γ-aminobutyric acid type A receptor modulation and metabolic detoxification. Proc Natl Acad Sci 97(8):3826–3831

Juteau F, Masotti V, Bessière JM, Dherbomez M, Viano J (2002) Antibacterial and antioxidant activities of *Artemisia annua* essential oil. Fitoterapia 73(6):532–535

Lee I-S, Hufford CD (1990) Metabolism of antimalarial sesquiterpene lactones. Pharmacol Ther 48(3):345–355

Li Y, Hu H-b, Zheng X-d, Zhu J-h, Liu L-p (2011) Composition and antimicrobial activity of essential oil from the aerial part of *Artemisia annua*. J Med Plant Res 5(16):3629–3633

Liu KC-SC, Yang S-L, Roberts MF, Elford BC, Phillipson JD (1992) Antimalarial activity of *Artemisia annua* flavonoids from whole plants and cell cultures. Plant Cell Rep 11(12):637–640

Liu NQ, Van der Kooy F, Verpoorte R (2009) *Artemisia afra*: a potential flagship for African medicinal plants? S Afr J Bot 75(2):185–195

Massiha A, Majid Khoshkholgh-Pahlavian M, Issazadeh K, Bidarigh S, Zarrabi S (2013) Antibacterial activity of essential oils and plant extracts of Artemisia (*Artemisia annua* L.) in vitro. Zahedan J Res Med Sci 15(6):14–18

Munyangi J, Cornet-Vernet L, Idumbo M, Lu C, Lutgen P, Perronne C, Ngombe N, Bianga J, Mupenda B, Lalukala P, Mergeai G, Mumba D, Towler M, Weathers P (2018) Effect of Artemisia annua and Artemisia afra tea infusions on schistosomiasis in a large clinical trial. Phytomedicine 51:233–240

Munyangi J, Cornet-Vernet L, Idumbo M, Lu C, Lutgen P, Perronne C, Ngombe N, Bianga J, Mupenda B, Lalukala P, Mergeai G, Mumba D, Towler M, Weathers P (2019) Artemisia annua and Artemisia afra tea infusions vs. artesunate-amodiaquine (ASAQ) in treating Plasmodium falciparum malaria in a large scale, double blind, randomized clinical trial. Phytomedicine 57:49–56

Navaratnam V, Mansor SM, Sit N-W, Grace J, Li Q, Olliaro P (2000) Pharmacokinetics of artemisinin-type compounds. Clin Pharmacokinet 39(4):255–270

Patil GV, Dass SK, Chandra R (2011) *Artemisia afra* and modern diseases. J Pharmacogenomics Pharmacoproteomics 2(3)

Radulović NS, Randjelović PJ, Stojanović NM, Blagojević PD, Stojanović-Radić ZZ, Ilić IR, Djordjević VB (2013) Toxic essential oils. Part II: chemical, toxicological, pharmacological and microbiological profiles of *Artemisia annua* L. volatiles. Food Chem Toxicol 58:37–49

Rana VS, Abirami K, Blázquez MA, Maiti S (2013) Essential oil composition of *Artemisia annua* L. at different growth stages. J Spices Aromat Crops 22(2):181–187

Seo K-A, Kim H, Ku H-Y, Ahn H-J, Park S-J, Bae SK, Shin J-G, Liu K-H (2008) The monoterpenoids citral and geraniol are moderate inhibitors of CYP2B6 hydroxylase activity. Chem Biol Interact 174(3):141–146

Song N-N, Zhang S-Y, Liu C-X (2004) Overview of factors affecting oral drug absorption. Asian J Drug Metab Pharmacokinet 4(3):167–176

Suberu JO, Gorka AP, Jacobs L, Roepe PD, Sullivan N, Barker GC, Lapkin AA (2013) Antiplasmodial polyvalent interactions in *Artemisia annua* L. aqueous extract – possible synergistic and resistance mechanisms. PLoS One 8(11):e80790

Svensson USH, Ashton M (1999) Identification of the human cytochrome P450 enzymes involved in the in vitro metabolism of artemisinin. Br J Clin Pharmacol 48(4):528–535

Tegtmeier M, Harnischfeger G (1994) Methods for the reduction of thujone content in pharmaceutical preparations of Artemisia, Salvia and Thuja. Eur J Pharm Biopharm 40(5):337–340

Towler MJ, Weathers PJ (2015) Variations in key artemisinic and other metabolites throughout plant development in *Artemisia annua* L. for potential therapeutic use. Ind Crop Prod 67:185–191

Tripathi AK, Prajapati V, Aggarwal KK, Khanuja SPS, Kumar S (2000) Repellency and toxicity of oil from *Artemisia annua* to certain stored-product beetles. J Econ Entomol 93(1):43–47

Tu Y (2011) The discovery of artemisinin (qinghaosu) and gifts from Chinese medicine. Nat Med 17(10):1217–1220

Tzenkova R, Kamenarska Z, Draganov A, Atanassov A (2010) Composition of *Artemisia annua* essential oil obtained from species growing wild in Bulgaria. Biotechnol Biotechnol Equip 24(2):1833–1835

Verdian-Rizi MR, Sadat-Ebrahimi E, Hadjiakhoondi A, Fazeli MR, Pirali Hamedani M (2008) Chemical composition and antimicrobial activity of *Artemisia annua* L. essential oil from Iran. J Med Plant 1(25):58–62

Viuda-Martos M, El Gendy AE-NGS, Sendra E, Fernandez-Lopez J, Abd El Razik KA, Omer EA, Pérez-Alvarez JA (2010) Chemical composition and antioxidant and anti-Listeria activities of essential oils obtained from some Egyptian plants. J Agric Food Chem 58(16):9063–9070

Watt JM, Breyer-Brandwijk MG (1962) The medicinal and poisonous plants of Southern Africa and Eastern Africa: being an account of their medicinal and other uses, chemical composition, pharmacological effects and toxicology in man and animal. E.S. Livingstone, Edinburgh

Weathers PJ, Arsenault PR, Covello PS, McMickle A, Teoh KH, Reed DW (2011) Artemisinin production in *Artemisia annua*: studies in planta and results of a novel delivery method for treating malaria and other neglected diseases. Phytochem Rev 10(2):173–183

Weathers PJ, Cambra HM, Desrosiers MR, Rassias D, Towler MJ (2017) Artemisinin the nobel molecule: from plant to patient. In: Studies in natural products chemistry. Elsevier, Amsterdam, pp 193–229

Weathers PJ, Towler M, Hassanali A, Lutgen P, Engeu PO (2014) Dried-leaf *Artemisia annua*: a practical malaria therapeutic for developing countries? World Journal of Pharmacology 3(4):39–55

Weathers PJ, Towler MJ (2014) Changes in key constituents of clonally propagated *Artemisia annua* L. during preparation of compressed leaf tablets for possible therapeutic use. Ind Crop Prod 62:173–178

Yang Z-n, Zhu S-q, Yu Z-w (2012) Comparison of terpene components from flowers of *Artemisia annua*. Bangladesh J Pharmacol 7(2):114–119

Chapter 7
Outstanding Efficacy of Essential Oils Against Oral Pathogens

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7.1 Introduction

The oral cavity is a favorable environment for various microorganisms where they exist in multispecies communities (Borges et al. 2015). Bacterial community, which is attached to organic or inorganic surfaces, is called biofilm. Bacteria are embedded in an extracellular polymeric matrix which they produce themselves (Mashima and Nakazawa 2014).

An exquisite example of naturally formed biofilm is a dental plaque (Mancl et al. 2013). It is considered the main cause of tooth decay (caries) that can lead to root canal infections; and also biofilm can affect the tooth supporting tissues and cause

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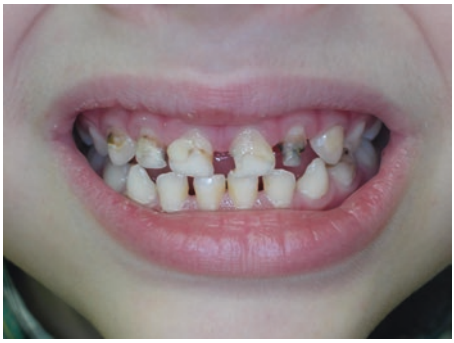

Fig. 7.1 *S. sanguinis* and *S. salivarius* interactions with other bacteria in biofilm community involved in caries development process: an example of early childhood caries with a number of carious lesions

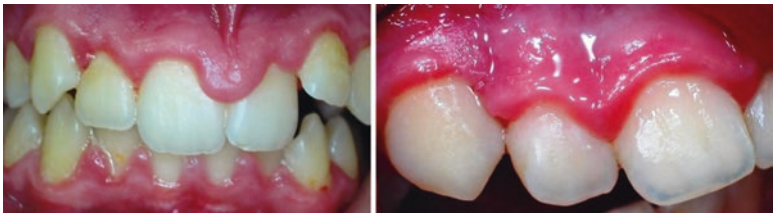

Fig. 7.2 *S. sanguinis* and *S. salivarius*: members of biofilm community. Both *species* have an important role in first-stage development of disease of tooth-supporting tissues. An example of gingivitis and gingival hypertrophy

periodontitis (Figs. 7.1 and 7.2). *Streptococcus sanguinis* and *Streptococcus salivarius* are known for their contribution to dental plaque (Seow et al. 2009). Their specific behavior and interactions with other bacteria in such a complex community make them the pathogens of specific importance.

In the incoming era of well-documented antimicrobial and antibiotic resistance, modern science is opening to investigate natural products for a possible solution. With regard to this, essential oils (EOs) particularly seem promising due to a range of well-documented biological activities (Marković 2011). Their efficacy against *S. sanguinis* and *S. salivarius* is already proven in many recent studies (Bersan et al. 2014; Crevelin et al. 2015; Mahboubi and Kazempour 2011; Nikolic et al. 2014, 2016; Nikolic 2015; Zomorodian et al. 2015), implying that the EOs with appropriate chemical composition may serve as antimicrobial alternatives to standard endodontic therapy agents that are already documented as agents lacking in efficiency toward these two pathogens (Fig. 7.3) (Lew et al. 2015; Sakamoto et al. 2007).

The aim of this chapter is to present EOs with the most significant in vitro activity against two oral pathogens, *S. sanguinis* and *S. salivarius*, associated with the obstacles of standard endodontic procedure, and to discuss their chemical composition and constituents with regard to their responsibility in the achieved outstanding efficacy.

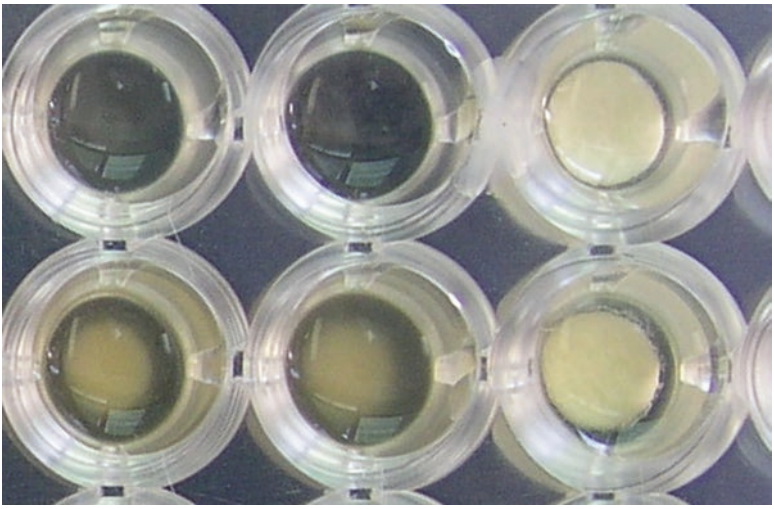

Fig. 7.3 *Streptococcus salivarius* (first row) and *Streptococcus sanguinis* (second row)

7.2 Multiple Roles of *Streptococcus sanguinis* and *Streptococcus salivarius*

As specific behavior of these oral pathogens and their interactions with other bacteria make them very important in dental biofilm formation, their multiple roles deserve to be described in more detail.

7.2.1 Anti-biofilm Role of Streptococcus sanguinis *(Anti-*Streptococcus mutans *and Anti-periodontopathogenic)*

Development of a dental biofilm which further leads to caries is initiated by the adherence of "early colonizers" to the salivary proteins and glycoproteins on the outer tooth layer, the tooth enamel (Mashima and Nakazawa 2014). Since the early colonizers provide substrate for the attachment of the other ones, the process of adherence seems to be very important for biofilm formation as it substantially influences its succeeding developmental stages (Li et al. 2004).

S. sanguinis has been recognized as an early colonizer due to its unique cell surface polymeric structure named pili, which enables its adhesion (Okahashi et al. 2010; Rosan and Lamont 2000). Its initial colonization usually takes place during a discrete "window of infectivity," at the age of 9 months, which correlates to the primary teeth emergence (Caufield et al. 2000). Although some reports implicate *Streptococcus mutans* as a principal etiological agent in caries (Thenisch et al. 2006), the role of *S. sanguinis* in development of caries, achieved through its relative incidence and interaction with *S. mutans*, must not be neglected (Ge et al. 2008).

According to Caufield et al. (2000) and Kreth et al. (2005), there is antagonism between *S. sanguinis* and *S. mutans*. Bearing in mind that different *Streptococcus* species compete for similar surviving and growing conditions (Zhu and Kreth 2010), the role of *S. sanguinis* in caries development might be defined as antagonistic toward *S. mutans*; its activity is mainly based on inhibition of *S. mutans* which is achieved by the release of hydrogen peroxide it produces (Carlsson et al. 1983; Kreth et al. 2005), in addition to the fact that it also secrets antimicrobial agent named bacteriocin, sanguicin (Fujimura and Nakamura 1979). Similarly, *S. mutans* developed anti-*S. sanguinis* strategy which includes production of antimicrobial peptides mutacin I and IV, both capable of inhibiting *S. sanguinis* (Kreth et al. 2005). An obvious antagonism between these two pathogens, particularly pronounced under the aerobic conditions, is controlled by the fact which one will be first inoculated, and it is crucial for the entire caries development procedure (Kreth et al. 2008). The synthesis of hydrogen peroxide by *S. sanguinis* lies in its capability to metabolize carbohydrates and produce lactate and pyruvate via glycolytic pathways. The enzyme pyruvate oxidase catalyzes conversion of pyruvate, inorganic phosphate, and oxygen to hydrogen peroxide, carbon dioxide, and acetyl phosphate; this provides *S. sanguinis* an effective H_2O_2 mechanism which enables it to resist other bacteria, at the same time being resistant to itself (Carlsson and Edlund 1987; Zheng et al. 2011; Zhu and Kreth 2012).

Antagonism of *S. sanguinis* is not specific only against cariogenic bacteria *S. mutans* but is also observed toward the periodontopathogens which cause the most common oral disease, periodontitis. This might be illustrated by the prevalence of *S. sanguinis* in healthy subjects in comparison with patients with periodontitis (Stingu et al. 2008). Periodontopatoghens, such as *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Aggregatibacter actinomycetemcomitans*, showed to be sensitive to the hydrogen peroxide produced by *S. sanguinis*; the achieved inhibition seems to be time dependent and was only observed when *S. sanguinis* were spotted 24 h before spotting the other pathogens. Also, the inhibition was favored under the aerobic then anaerobic condition (Herrero et al. 2016).

7.2.2 *Anti-biofilm Role of* Streptococcus salivarius *(Anti-*Streptococcus mutans *and Anti-periodontopathogenic)*

Another early colonizer in biofilm development is *S. salivarius*; it becomes established in oral cavity within the first 48 h, where it remains as a predominant commensal inhabitant (McCarthy et al. 1965; Kaci et al. 2014).

Next to *S. sanguinis*, whose antagonism is already discussed, *S. salivarius* is also frequently occurring human oral cavity inhabitant (Barretto et al. 2012). Its capability to produce bacteriocins makes it a good probiotic candidate—live microorganism which when administered in adequate amounts confers a health benefit on the host (Araya et al. 2002). Bacteriocins, as antimicrobials with relatively specific killing activity, are capable of suppressing growth of bacteria which are phyloge-

netically closely related to bacteriocin-producing strain; such a specific killing ability differ them from classic antibiotics which extend to microbial species phylogenetically distant from its producing strain (Gregori et al. 2016). Probiotic bacteria can achieve its full impact by competing for adhesion, nutrients, and growth factors, by production of inhibiting molecules or enzymes which are lethal to pathogenic ones or by supporting the host immune system (Wescombe et al. 2012).

Different generations of *S. salivarius* as a probiotic were examined. The first type was obtained with *S. salivarius* strain K12, which was isolated from saliva of a healthy child (Barretto et al. 2012); it successfully reduced occurrence of a recurrent pharyngitis and halitosis (Daniels 2015; Di Pierro et al. 2016; Masdea et al. 2012) due to its potent activity toward *S. pyogenes* (Hyink et al. 2007) and *S. pneumoniae* (Power et al. 2008) and through secretion of salivaricins A2 and B (Hyink et al. 2007; Tagg 2008; Tagg and Dierksen 2003). However, this strain had relatively weak in vitro inhibitory activity against *S. mutans* (Walker et al. 2016). This imposed necessity to develop strain M18, which was originally isolated from a healthy adult female (Chen et al. 1996; Di Pierro et al. 2016; Heng et al. 2011; Ohnishi et al. 1995); the improvement of this strain was achieved with a fact that, apart from producing several bacteriocins, including salivaricins A2, M, MPS, and 9, it also produces dextranase, the enzymes which antagonize biofilm formation, as well as urease, which rise the local pH value (Barbour et al. 2013; Burton et al. 2013; Wescombe et al. 2012). Mechanism of salivaricin 9 is quite known; it penetrates the cytoplasmic membrane of the targeted cells and induces pore formation (Barbour et al. 2013). Although *S. salivarius* M18 proved to be efficient toward *S. mutans*, its efficacy toward periodontopathogenic bacteria still remains unsolved. In addition, *P. gingivalis* and *P. intermedia* already proved not to be inhibited by this strain (Burton et al. 2013). Novel candidate for oral probiotic *S. salivarius* strain JH was found to be more potent anti-*S. mutans*, as it produces salivaricin E (SalE), in addition to the fact that it produces more dextranase than any other strain of *S. salivarius* (Walker et al. 2016). However, its effectiveness toward periodontopathogenic bacteria is not estimated yet.

7.2.3 *Roles of* Streptococcus sanguinis/Streptococcus salivarius *in Endocarditis*

Although previous lines were dedicated to *S. sanguinis* and *S. salivarius* found in the oral cavity, in a manner of semi-beneficial bacteria in a complex microbial community competing for space and nutrients, their pathogenicity gets its full impact in case of their incidence in naturally sterile environment, such as a bloodstream. Now when they are not in position to compete with other pathogenic bacteria, they may present their full pathogenic impact and be the main cause of systemic diseases, such as bacterial endocarditis, mycotic aneurysm infection (destruction of blood vessel walls) (Kadowaki et al. 2013), systemic vasculitis (Behçet's disease) (Kaneko et al. 2011), and hepatic encephalopathy (Bajaj 2016). Infective endocarditis is a

serious and potentially fatal infection obtained through interaction of the two oral pathogens, *S. sanguinis* and *S. salivarius*, with matrix molecules and platelets at sites of endocardial cell damage (Di Filippo et al. 2006; Kao et al. 2013; Wilson et al. 2008).

The oral cavity is the entrance gate for these two bacteria. Bacteremia can be provoked by some every day routine activities, such as tooth brushing, flossing, usage of wooden toothpicks and chewing a food (Wilson et al. 2008), or it can appear following a common dental treatment such as a tooth cleaning (Di Filippo et al. 2006; Laura et al. 2014) or tooth extraction (Wilson et al. 2008). Also, important source and provoking cause of bacteremia may be a dental infection, originating weather from the tooth or its supporting tissues, and it is considered as a dental focus (Di Filippo et al. 2006; Wilson et al. 2008; Wisniewska-Spychala et al. 2012). *S. sanguinis* and *S. salivarius* related to subacute endocarditis were isolated from the following dental foci: teeth with necrotic pulp or with its necrotic decay, resected teeth with poorly filled root canals due to anatomical or other difficulties, and teeth without evident periapical lesions and with seemingly well-filled root canal, such as endo-perio syndrome (Wisniewska-Spychala et al. 2012). All these lead to the fact that endocarditis is certainly related to untreated or badly treated *S. sanguinis*- and *S. salivarius*-rich infected root canals.

If we consider that only sterile root canal is the healthy one, then none of the beneficial bacteria nor their interaction with more or less pathogenic bacteria may find its place or role in the root canal system. With regard to this, the dental root canal resembles the blood stream; only the sterile ones may guarantee survival.

7.2.4 The Roles of Streptococcus salivarius/Streptococcus sanguinis *in Infected Dental Root Canal (Oral Focus)*

Streptococcus spp., including *S. sanguinis* and *S. salivarius*, are well known by its presence in infected root canal (Fouad et al. 2003). They use to be found in primary infected dental root canals where infection is caused by microorganisms that initially invade and colonize the necrotic pulp tissue (Gomes et al. 2004; Lew et al. 2015; Siqueira and Rôças 2009) or they use to be isolated from the root canals of already treated teeth (Gomes et al. 2004; Łysakowska et al. 2016, Rocas and Siqueira 2012). Further, they can be part of a canal flora in cases of canal infection complicated with symptomatic or asymptomatic, acute or chronic periapical periodontitis (Jacinito et al. 2003; Provenzano et al. 2015; Rolph et al. 2001; Rocas and Siqueira 2012; Siqueira et al. 2007; Tatikonda et al. 2017).

S. sanguinis proved to be capable of infecting the root canal system within a week time period (Shovelton 1959). The SEM microscopy analysis confirmed that, while in combination with *Fusobacterium nucleatum* or with *Actinomyces oris*, it penetrates into the dental tubules (Stauffacher et al. 2017), which usually occurs between the 20th and 28th day. Its penetration length ranges from 150 to 792 μm

(Berkiten et al. 2000; Perez et al. 1993). Bearing in mind that bacteria localized deep inside the dentin tubules could not be removed by the endodontic procedure, as they are not easily accessed by the instruments or irrigation solutions (Matsuo et al. 2003), it's easy to understand why the members of *Streptococcus* spp. represent crucial portion of dental flora associated to root infections (Gajan et al. 2009; Gomes et al. 2004; Lysakowska et al. 2016; Siqueira and Rocas 2008).

Resistance of *S. sanguinis* and *S. salivarius* to endodontic treatment is related to their capabilities to survive chemo-mechanical root canal preparation (Lew et al. 2015; Sakamoto et al. 2007), while the intra-canal medication with $Ca(OH)_2$ paste following the chemo-mechanical procedure represents the last stage of defense against these two bacteria prior to dental canal filling (Siqueira and Rocas 2008). Apart from already known resistance of *Enterococcus faecalis* (Abbaszadegan et al. 2016), *S. sanguinis* and *S. salivarius* also proved to be alkaline-tolerant and capable to survive $Ca(OH)_2$ dressing (Lew et al. 2015; Sakamoto et al. 2007), which leads to a conclusion that standard endodontic procedure lacks in efficiency with regard to the mentioned canal bacteria. Although not every patient with infected root canal necessarily suffer bacteremia, severity of described systemic diseases makes bacterial eradication very important.

Bearing in mind emerging antimicrobial resistance to antimicrobial agents, accompanied with a fact that EOs are proved to be good alternative toward standard antimicrobial therapy with no resistance issues, we are searching for EO which will possess high potential toward both *S. sanguinis* and *S. salivarius* and be capable of disabling their ability of forming biofilm and consequently avoid most common oral diseases.

7.3 Procedure for Screening the Literature on High-Efficacy Essential Oils Against Two Oral Pathogens

Original papers have been selected from the available index bases, the Web of Science, Scopus, and Medline, in order to select results from available scientific literature and study them together with results from our previous investigation (Nikolić 2015; Nikolić et al. 2014, 2016). The keywords used in the searching procedure were essential oil and *Streptococcus sanguinis/Streptococcus sanguis*; essential oil and *S. sanguinis/sanguis*; essent* oil* and *S. sanguinis/sanguis*; essential oil and *Streptococcus salivarius*; essential oil and *S. salivarius*; and essent* oil* and *S. salivarius.* There was no time span limitation. All manuscripts presenting antimicrobial effect of EOs on *S. sanguinis* and/or *S. salivarius*, fulfilling the following pre-set criteria were included:

- With chemical composition of tested EOs
- With clinical isolates of *S. sanguinis* and/or its ATCC 10556 referent strain
- With clinical isolates of *S. salivarius* subsp. *salivarius* and/or its ATCC 9222 referent strain

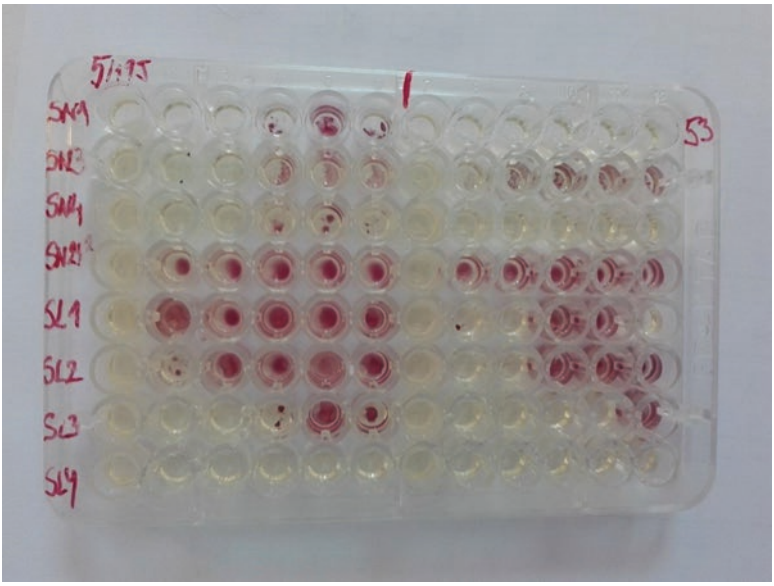

Fig. 7.4 Microdilution method for MIC values determination

- With the CLSI recommendation standard for *Streptococcus* spp. *Viridans* group (CLSI 2013) (which includes ATCC referent strains of *S. sanguinis* and *S. salivarius*)
- With *Streptococcus sanguinis* ATCC® 10556™ manufacturer strain product sheet recommendation
- With *S. salivarius* subsp. *salivarius* ATCC® 9222™ manufacturer strain product sheet recommendation
- With the use of microdilution method (Fig. 7.4) for MIC values determination, in which the MIC values were ≤250 μL of EO mL^{-1} of growing medium

According to the achieved MIC values, selected EOs were divided into two groups: the EOs with *strong* antimicrobial activity (MIC ranged from 0.0001 to 10 μL mL^{-1}) and the EOs with *good* antimicrobial activity (MIC values ranged from 10 to 250 μL mL^{-1}).

7.4 Antimicrobial Activity of Essential Oils and Their Constituents Toward *Streptococcus salivarius* and *Streptococcus sanguinis*, Clinical and ATCC Strains

Following the thorough screening of the available peer-review literature, 30 EOs tested on *S. sanguinis* and 21 EOs on *S. salivarius* were selected, all of them expressing satisfactory antimicrobial activity on clinical isolates or corresponding referent strains of these two crucial dental root canal pathogens in humans.

7.4.1 *General Variation in Response of* Streptococcus sanguinis *and* Streptococcus salivarius *to Selected Essential Oils*

Out of 21 selected EOs (Table 7.1), 12 EOs showed identical MIC values against both pathogens: ten EOs (*Thymus serpyllum*, *T. algeriensis*, *Leptospermum petersonii*, *Eucalyptus citriodora*, *Melaleuca quinquenervia*, *Rosa centifolia*, *Syzygium aromaticum*, *Hyssopus officinalis*, *Rosmarinus officinalis*, and *Pelargonium graveolens*) against the clinical isolates and the remaining two EOs (*Satureja khuzestanica* and *Carum copticum*) against the referent ATCC strains.

Table 7.1 Comparative presentation of the MIC values of 21 EOs with satisfactory efficacy (MIC£250 μg mL[1]) on two oral *Streptococcus* species (clinical isolates and corresponding ATCC strains)

	Essential oils	MIC values		Reference
	(EOs)	*S. salivarius*	*S. sanguinis*	
Clinical isolates	*Thymus serpyllum*	3 μg mL^{-1}	3 μg mL^{-1}	Nikolic et al. (2014)
	Thymus algeriensis	40 μg mL^{-1}	40 μg mL^{-1}	Nikolic et al. (2014)
	Satureja montana	60 μg mL^{-1}	30 μg mL^{-1}	Nikolic (2015)
	Leptospermum petersonii	60 μg mL^{-1}	60 μg mL^{-1}	Nikolic (2015)
	Thymus vulgare	80 μg mL^{-1}	160 μg mL^{-1}	Nikolic et al. (2014)
	Eucalyptus citriodora	80 μg mL^{-1}	80 μg mL^{-1}	Nikolic (2015)
	Melaleuca quinquernervia	130 μg mL^{-1}	130 μg mL^{-1}	Nikolic (2015)
	Rosa centifolia	130 μg mL^{-1}	130 μg mL^{-1}	Nikolic (2015)
	Syzygium aromaticum	130 μg mL^{-1}	130 μg mL^{-1}	Nikolic (2015)
	Hyssopus officinalis	160 μg mL^{-1}	160 μg mL^{-1}	Nikolic et al. (2016)
	Rosmarinus officinalis	160 μg mL^{-1}	160 μg mL^{-1}	Nikolic et al. (2016)
	Pelargonium graveolens	250 μg mL^{-1}	250 μg mL^{-1}	Nikolic (2015)
ATCC strains	*Trachyspermum copticum*	0.06 μL mL^{-1}	1 μL mL^{-1}	Mahboubi and Kazempour (2011)
	Satureja hortensis	0.125 μL mL^{-1}	2 μL mL^{-1}	Mahboubi and Kazempour (2011)
	Satureja khuzestanica	0.125 μL mL^{-1}	0.125 μL mL^{-1}	Zomorodian et al. (2015)
	Zataria multiflora	0.125 μL mL^{-1}	0.25 μL mL^{-1}	Zomorodian et al. (2015)
	Carum copticum	0.25 μL mL^{-1}	0.25 μL mL^{-1}	Zomorodian et al. (2015)
	Satureja bachtiarica	0.25 μL mL^{-1}	0.5 μL mL^{-1}	Zomorodian et al. (2015)
	Salvia mirzayanii	0.5 μL mL^{-1}	0.125 μL mL^{-1}	Zomorodian et al. (2015)
	Ocimum sanctum	0.5 μL mL^{-1}	0.25 μL mL^{-1}	Zomorodian et al. (2015)
	Artemisia sieberi	4 μL mL^{-1}	1 μL mL^{-1}	Zomorodian et al. (2015)

Analysis of the MIC values revealed the following major observations with regard to differences in response of tested pathogens to selected EOs:

1. *S. sanguinis* was more sensitive than *S. salivarius* on EOs of *S. montana*, *S. mirzayanii*, *O. sanctum,* and *A. sieberi*; their major constituent was *1,8*-cineole whose content ranged from 20.8% to 49.3%.
2. *S. salivarius* was more sensitive than *S. sanguinis* on EOs of *T. vulgare*, *T. copticum*, *S. hortensis*, *Z. multiflora,* and *S. bachtiarica* EOs; their major constituent was thymol whose content ranged from 28.0% to 49.1%.
3. Interestingly, none of the above mentioned four*1,8*-cineole-rich EOs also contained thymol nor the other five thymol-rich EOs also contained *1,8*-cineole.

Comparing to *S. sanguinis*, the two cineole-rich oils, *S. Montana* (49.3%) and *O. sanctum* (20.8%), showed about 2 times higher MIC values against *S. salivarius*, while those from *S. mirzayanii* and *A. sieberi* with similar cineole content (41.2% and 21.1%, respectfully) showed about 4 times higher MIC values. These observations suggest that the difference in susceptibility between the two *Streptococcus* species is not only due to their cineole content; it would be wiser to assume that the *cineole-rich oils lacking thymol are more efficient against S. sanguinis than S. salivarius*. The exact sensitivity ratio to the oils between the two pathogens has to be precisely determined as it could be useful not only for their possible practical implementation but also for avoiding undesired defects (Nikolic 2015; Zomorodian et al. 2015).

Similar observation was noticed in the group of even more efficient five EOs; all of them were thymol-rich but had no *1,8*-cineole content. Regardless of their variability in thymol content (*T. vulgare* 49.1%, *Z. multiflora* 37.8%, and *S. bachtiarica* 28.0%), their MIC values revealed that *S. sanguinis* was always about twice more sensitive than *S. salivarius*. In case of other two thymol-rich oils (*T. copticum* 45.9% and *S. hortensis* 28.2%), the observed sensitivity pattern between the two *Streptococci* was even more pronounced (*S. sanguinis* was about 17 and 16 times more sensitive to these two oils, respectively). Comparative analysis confirmed that the thymol-rich oils are highly efficient against the two pathogenic oral *Streptococcus* species, so determination of their exact sensitivity ratio to the oils would be crucial due to many practical reasons (Nikolic et al. 2014; Mahboubi and Kazempour 2011; Zomorodian et al. 2015).

7.4.2 *Differences Between Clinical and Referent ATCC Strains of* Streptococcus sanguinis *and* Streptococcus salivarius *in Their Sensitivity to Essential Oils*

The best efficacy toward *S. sanguinis* (ATCC 10556) exhibited EOs from *Salvia mirzayanii* and *Satureja khuzestanica,* both with MIC = 0.125 μL mL^{-1} (Zomorodian et al. 2015), while the most efficient against corresponding clinical isolates was the EO of *Thymus serpyllum* with MIC = 3 μg mL^{-1} (Nikolic et al. 2014); comparison

of efficacy of these two EOs revealed that clinical isolates required 24 times higher MIC then their corresponding referent ATCC strain (Table 7.1).

Similar pattern in sensitivity between the clinical isolates and corresponding referent strain was observed for *S. salivarius*; the most efficient oil against the referent strain was *Trachyspermum copticum* with MIC = 0.06 μL mL^{-1} (Zomorodian et al. 2015), which proved to be about 50 times lower than the most efficient against corresponding clinical isolates, the oil of *Thymus serpyllum* with MIC = 3 μg mL^{-1} (Nikolic et al. 2014) (Table 7.1).

The least efficient EOs included in this study, with MIC = 250 μg mL^{-1}, were *Pelargonium graveolens* against the clinical isolates of both pathogens, as well as *Cymbopogon martinii* against the *S. sanguinis* isolate (Nikolic 2015) and *Cyperus articulatus* EO against *S. sanguinis* ATCC 10556 (Bersan et al. 2014), while the least efficient on *S. salivarius* ATCC 9222 was *Artemisia sieberi* EO, with a quite lower MIC = 4 μL mL^{-1} (Zomorodian et al. 2015).

Comparison of selected results confirmed that antimicrobial activity of selected EOs is statistically different between oils tested on clinical and ATCC strain types, implicating that the clinical isolates of both *Streptococcus* species generally show lower sensitivity to EOs than their corresponding referent strains (Table 7.2).

From the total EOs tested, 39% achieved strong antimicrobial activity toward ATCC 10556 and ATCC 9222 strains, while in case of clinical isolates, that percentage was only 9.3%.

Although Becerril et al. (2012) reported no significant differences in sensitivity, between the clinical isolates and referent strains of some gram-negative bacteria (*E. coli*, *S. marcescens*, *M. morganii*, *P. mirabilis*, and *P. aeruginosa*) to the activity of *Origanum vulgaris* and *Cinnamomum zeylanicum* EOs, data summarized in our study show the opposite trend, regardless the strength of the achieved antimicrobial activity (Table 7.2). The most efficient EOs toward the both referent strains (*S. sanguinis* and *S. salivarius*) had MIC values several times lower (Zomorodian et al. 2015; Mahboubi and Kazempour 2011) than those of their corresponding clinical isolates (Nikolić et al. 2014), which is also confirmed in our other study on *E. faecalis* (Bogojevic et al. 2016).

As it is quite known that the clinical isolates between themselves may vary in their sensibility toward any tested agent, and that testing on the referent strains revealed that they are quite different in sensitivity than the clinical ones, any conclusion based on results on either of them separately may bring us to a dead end.

Table 7.2 Response of two *Streptococcus* spp. strain types to selected antimicrobial essential oils (EOs)

Parameters		Antimicrobial activity of EOs (X ± SD) (Med; min-max)/*n* (%)		Statistical significance
		Strong	Good	
Strain type	ATCC strain	32 (39.0%)	50 (61.0%)	$p = 0.000^{a,b}$
	Clinical isolates	16 (9.3%)	156 (90.7%)	

[a]Chi square test
[b]Statistically significant

On the other hand, results that came out from simultaneous testing on both, the clinical isolates and corresponding referent strains, and application of the same agent (i.e., essential oil of a known chemical composition) would be more confident and useful for further studies. Since, to the best of our knowledge, there were no reports on simultaneous testing of facultative anaerobes such as *Streptococcus* spp., with EOs of presented chemical composition, once again we propose simultaneous testing procedure with regard to their antimicrobial activity, expressing results on the ATCC strains just as a referent point in comparison.

7.4.3 *Some Other Parameters Influencing Essential Oils Efficacy Toward Two* Streptococcus *spp.*

Further statistical analysis explains weather the content of EO constituents and the chemical class to which they belong to (the terpene class) influence antimicrobial activity of EOs (Tables 7.3 and 7.4).

Content of EO constituents is statistically different between strong and good EOs tested on both ATCC strains and clinical isolates. Strong EOs in general had higher constituent amount compared with good EOs (Table 7.3).

Apart from the previously described influence of the strain types (clinical isolate/referent strain) and the amount of the oil component (Fig. 7.5), the chemical class (terpenes) to which components chemically corresponds was also analyzed as possible influencing parameter (Table 7.4).

Chemical class, to which EOs constituents chemically belong to, statistically showed no difference between the groups of EOs with strong and with good antimicrobial efficacy tested on both ATCC strains and clinical isolates (Table 7.4).

Logistic regression analysis was used for identification of parameters that may predict differences between EOs with strong and good antimicrobial activity.

Logistic regression analysis showed influence of EOs, content of single oil constituents, strain type, and chemical class to which oil constituents belong to, on antimicrobial activity of selected EOs tested toward two *Streptococcus* spp. When univariate predictors (Table 7.5) were introduced in a multivariate model, the

Table 7.3 Influence of the amount of EO constituent (%) on antimicrobial activity of tested EOs towards two *Streptococcus* spp.

	EO antimicrobial efficacy (MIC values) (X ± SD) (Med; min-max)		
Parameters	Strong	Good	Statistical significance
Content of EO constituent (%)	15.30 ± 16.69 (10.86; 1.00–87.70)	8.74 ± 14.22 (3.25; 1.00–83.10)	$p = 0.003$[a,b]

[a]Mann–Whitney test
[b]Statistically significant

Table 7.4 Influence of the chemical class to which components belong to (terpene class) on antimicrobial activity of selected EOs tested towards two *Streptococcus* spp.

Chemical class (terpenes) (%)	Antimicrobial activity of EOs		Statistical significance[a]
	Strong	Good	
Monoterpene hydrocarbons	19 (26.8%)	52 (73.2%)	0.075
Oxygenated monoterpenes	23 (19.3%)	96 (80.7%)	
Sesquiterpene hydrocarbons	5 (10.9%)	41 (89.1%)	
Oxygenated sesquiterpenes	1 (5.6%)	17 (94.4%)	

[a]Chi square test

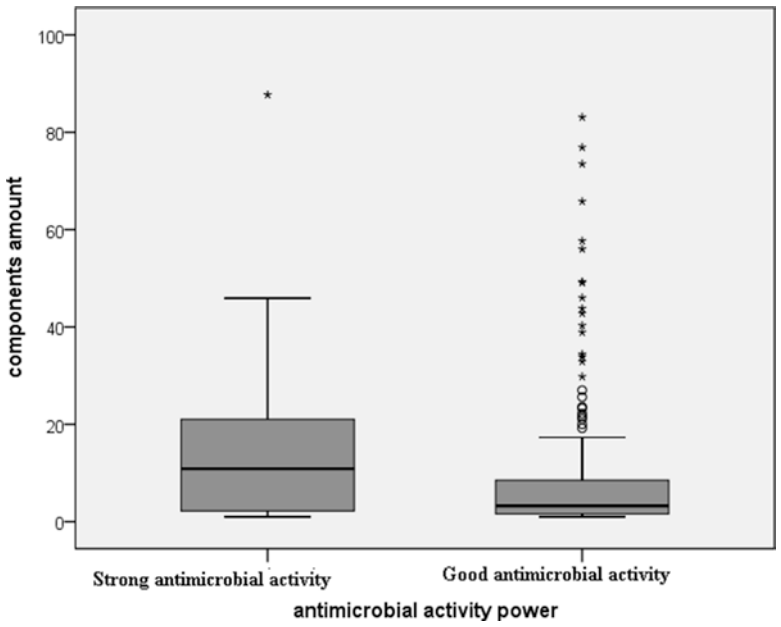

Fig. 7.5 Constituent amount impact on antimicrobial activity of tested EOs toward two *Streptococcus* spp

amount of the oil constituents, together with the strain types and classes to which components chemically belong, proved to be predictor of difference. Results implicate that higher amounts of constituents obtain better antimicrobial efficiency. The strain type as a predictor of difference may be explained with a fact that antimicrobial efficacy of oils tested on ATCC strains was seven times stronger than the efficiency on clinical isolates (Table 7.6). Also, the EOs rich in monoterpene hydrocarbons were the most efficient ones, while the less efficient were EOs with predominating content of oxygenated sesquiterpenes (Table 7.6).

Table 7.5 Logistic regression analysis of the impact of observed factors on antimicrobial activity of selected EOs tested towards two *Streptococcus* spp.

	Logistic regression analysis			
	Uni-		Multi-	
Parameters	expB (95% C.I.)	*p*	expB (95% C.I.)	*p*
EO	0.983 (0.951–1.017)	0.327	/	/
Components amount	0.976 (0.959–0.994)	0.009[a]	0.979 (0.959–0.999)	0.040[a]
Strain type	6.240 (3.163–12.310)	0.000[a]	6.801 (3.341–13.845)	0.000[a]
Class of terpenes	1.728 (1.140–2.620)	0.010[a]	1.984 (1.259–3.126)	0.003[a]

[a]Statistically significant

Table 7.6 Influence of the chemical classes of EOs on the type of *Streptococcus* spp. strains (clinical isolate/referent strain)

	Antimicrobial power of EO			
	Referent ATCC strain		Clinical isolate	
Chemical class/terpene class (%)	Strong	Good	Strong	Good
Monoterpene hydrocarbons	12 (50%)	12 (50%)	7 (14.9%)	40 (85.1%)
Oxygenated monoterpenes	16 (50%)	16 (50%)	7 (8.0%)	80 (92.0%)
Sesquiterpene hydrocarbons	3 (16.7%)	15 (83.3%)	2 (7.1%)	26 (92.9%)
Oxygenated sesquiterpenes	1 (12.5%)	7 (87.5%)	0 (0.0%)	10 (100.0%)
Statistical significance	$p = 0.030$[a]		$p = 0.379$	

[a]Statistically significant

Analysis of chemical composition of the efficient EOs toward clinical isolates showed that classes to which the EOs constituents chemically correspond did not show to statistically influence the antimicrobial activity against *Streptococcus* spp.

On the other hand, analysis of chemical composition of the efficient EOs toward the both referent strains showed that the class to which the oil components chemically correspond statistically differs between the EOs with strong and good antimicrobial activities (Table 7.7); stronger efficiency against both referent *Streptococcus* species showed monoterpenoids (i.e., monoterpene hydrocarbons and oxygenated monoterpenes). In addition, the EOs rich indifferent terpenes but classified as monoterpenoids were evenly distributed between the strong and good antimicrobial activity EOs, while the terpenes classified as sesquiterpenoids (i.e., sesquiterpene hydrocarbons and oxygenated sesquiterpenes) predominated in the EOs with good antimicrobial activity (Table 7.7).

More detailed analysis of chemical class distribution confirmed following: in 9 strong antimicrobial EOs on both oral pathogens similar pattern is observed; 7 EOs are composed mainly of oxygenated monoterpenes which ranged from 36.5 to 87.7% (Tables 7.7 and 7.8). In the remaining EOs which belong to MIC-good EOs just for *S. sanguinis* (Table 7.7), in 3 out of 5 EOs prevailed content of sesquiterpene hydrocarbons, ranging from 17.6 to 59.7%, while in the remaining 2 EOs, again prevailed oxygenated monoterpenes, in a quite high percentage.

Table 7.7 Influence of chemical class of EOs on *S. sanguinis* ATCC 10566

Chemical class to which EO components belong to (Terpene-class)	MIC-strong (μL mL^{-1})									MIC-good (μL mL^{-1})				
	0.13	0.13	0.25	0.25	0.25	0.50	1.00	1.00	2.00	62.0	62.5	125	250	250
	Sm	*Sk*	*Os*	*Zm*	*Cc*	*Sb*	*As*	*Tc*	*Sh*	*Mg*	*Pn*	*Ls*	*Cs*	*Ca*
Monoterpene hydrocarbons	0.0	0.0	0.0	0.0	57.6	0.0	13.1	41.5	46.8	2.1	32.6	17.3	2.7	10.4
Oxygenated monoterpenes	51.9	87.7	36.5	65.0	36.7	41.2	37.7	45.9	40.8	0.0	1.2	72.3	86.2	14.7
Sesquiterpene hydrocarbons	0.0	0.0	21.0	0.0	0.0	0.0	0.0	1.1	1.5	59.7	44.5	10.5	0.0	17.6
Oxygenated sesquiterpenes	0.0	0.0	0.0	0.0	0.0	17.0	0.0	0.0	0.0	11.4	15.6	0.0	0.0	3.4
Sum	*51.9*	*87.7*	*57.5*	*65.0*	*94.3*	*58.2*	*50.8*	*88.5*	*89.1*	*73.2*	*93.9*	*100.0*	*88.9*	*46.1*

Sm S. mirzayanii, *Sk S. khuzestanica*, *Os O. sanctum*, *Zm Z. multiflora*, *Cc C. copticum*, *Sb S. bachtiarica*, *As A. sieberi*, *Tc T. copticum*, *Sh S. hortensis*, *Mg M. glomerata*, *Pn P. neochilus*, *Ls L. sidoides*, *Cs C. sativum*, *Ca C. articulates*

Table 7.8 Influence of chemical class of EOs on *S. salivarius* ATCC 9222

Chemical class to which EO components belong to (Terpene-class)	MIC-strong (μL mL^{-1})								
	0.06	0.125	0.125	0.125	0.25	0.25	0.50	0.50	4.00
	Tc	*Sh*	*Sk*	*Zm*	*Cc*	*Sb*	*Sm*	*Os*	*As*
Monoterpene hydrocarbons	41.5	46.8	0.0	0.0	57.6	0.0	0.0	0.0	13.1
Oxygenated monoterpenes	45.9	40.8	87.7	65.0	36.7	41.2	51.9	36.5	37.6
Sesquiterpene hydrocarbons	1.1	1.5	0.0	0.0	0.0	0.0	0.0	21.0	0.0
Oxygenated sesquiterpenes	0.0	0.0	0.0	0.0	0.0	17.0	0.0	0.0	0.0
Sum	*88.5*	*89.1*	*87.7*	*65.0*	*94.3*	*58.2*	*51.9*	*57.5*	*50.7*

Tc Trachyspermum copticum, *Sh Satureja hortensis*, *Sk Satureja khuzestanica*, *Zm Zataria multiflora*, *Cc Carum copticum*, *Sb Satureja bachtiarica*, *Sm Salvia mirzayanii*, *Os Ocimum sanctum*, *As Artemisia sieberi*

Although the oxygenated monoterpenes prevail in more than 75% examined EOs, clear distinction of this chemical class, as solely responsible for antimicrobial efficacy on the two tested *Streptococcus* species, is not possible, as the constituents from other classes seem to be also involved. Our findings are in agreement with those of Yang et al. (2013) who investigated antimicrobial activity of *Litsea cubeba* EO components on *S. sanguinis* and revealed that class of oxygenated monoterpenes did express the highest antimicrobial impact against this pathogen compared to other chemical classes present in this oil.

7.4.4 *Single Essential Oil Constituents Influencing Efficacy Toward Two* Streptococcus *spp.*

If we want to get a general clue what would be the most favorable composition of EO of the outstanding efficacy on both *Streptococcus* spp., we might decide to study them just on the referent strains, as in case of clinical isolates there is only one MIC-strong EO (*Thymus serpyllum*) tested against both clinical isolates of targeted oral pathogens that fulfilled pre-set criteria (Table 7.1). However, it is interesting to underline that thymol was common constituent in both, the MIC-strong and the MIC-good oils, and always with high contribution (28–65%), implicating it does play certain role, possibly in controlled interaction with other components.

Monoterpenoides (monoterpene hydrocarbons and oxygenated monoterpenes), present in all EOs effective against referent strains of both oral pathogens, are presented in Table 7.9.

Analysis of data for MIC-strong group of EOs revealed that, within the class of monoterpene hydrocarbons, we should look for the presence of myrcene, α-thujone, α-phellandrene and *o*-cymene, while within the oxygenated monoterpenes, that would be camphor, *1,8*-cineole, carvacrol, eugenol and linalyl acetate. γ-terpinene, *p*-cymene and thymol are common in all EOs, regardless they belongs to MIC-strong or MIC-good group of EOs; their interaction with the above mentioned 9 constituents should be a subject of a further investigation.

Table 7.9 Common monoterpene hydrocarbons and oxygenated monoterpenes, tested on ATCC strains of *Streptococcus* spp.

	ATCC 10556 and ATCC 9222	
EO constituents	MIC-strong (%)	MIC-good (%)
Monoterpene hydrocarbons		
Myrcene	**100**	0.0
α-thujone	**100**	0.0
α-phellandrene	**100**	0.0
o-cymene	**100**	0.0
γ-terpinene	75.0	25.0
p-cymene	66.7	33.3
β-pinene	50.0	50.0
Sabinene	50.0	50.0
Oxygenated monoterpenes		
Camphor	**100**	0.0
1,8-cineole	**100**	0.0
Carvacrol	**100**	0.0
Eugenol	**100**	0.0
Linalyl acetate	**100**	0.0
Thymol	83.3	16.7
Terpinen-4-ol	50.0	50.0

Thymol, carvacrol, and *p*-cymene. They are already well-known antimicrobials (Dorman and Deans 2000). Findings of the Lambert et al. (2001) that thymol and carvacrol in the mixture have additive antimicrobial effect was also confirmed in our investigation; following MIC-strong EOs against both oral pathogens (clinical and referent strains), contained them both: *Satureja hortensis*, *Zataria multiflora*, *S. bachtiarica*, and particularly *T. serpyllum*. The third constituent, monoterpene hydrocarbon *p*-cymene facilitate efficacy of thymol and carvacrol, thus adding to the overall antimicrobial achievement; Delgado et al. (2004), Ultee et al. (1998, 2002) proved that *p*-cymene increases activity of thymol by its hydrophobic nature, as is able to incorporate in the lipid bilayer of bacterial cell, dissolve in cytoplasmic membrane between the lipid acyl chains, and facilitate transport of thymol across the membrane (Juven et al.1994; Juliano et al. 2000; Cosentino 1999). This supports our findings with regard to the importance of these three constituents in MIC-strong EOs against targeted oral pathogens.

1.8-**cineole**. It showed to be a constituent common in MIC-strong EOs, at the same time serving as a marker for sensitivity difference between *S. sanguinis* and *S. salivarius*. When tested alone, if isolated from the oil of *R. officinalis*, it showed to be a moderate antimicrobial with MIC = 400 μg mL^{-1} toward both *S. salivarius* ATCC 25975 and *S. sanguinis* ATCC 10556 (Bernardes et al. 2010), while when isolated from the oil of *Artemisia iwayomogi* in tests against *S. sanguinis* ATCC 10556, it

showed quite lower activity with MIC = 12.8 mg mL^{-1} (Cha 2007). As we study EOs with strong activity against *S. sanguinis* ATCC, we must point out that there must be some hidden synergistic interactions of cineole with other oil constituents that has to be further investigated in order to understand the true nature of the entire antimicrobial mechanism and to be precise about what enables cineole to be a major component of MIC-strong oils and makes it a weak antimicrobial while isolated.

Myrcene. It is another monoterpene hydrocarbon which showed to be a common constituent of the MIC-strong EOs against clinical isolates of *S. sanguinis* and *S. salivarius*. Again, we point out on hidden synergistic interactions of this constituent with some other ones. While tested as an isolated constituent toward the referent strains, it expressed moderate antimicrobial activity against both *S. salivarius* ATCC25975 (MIC = 400 μg mL^{-1}) and *S. sanguinis* ATCC 10556 (MIC = 1500 μg mL^{-1}) (Bernardes et al. 2010).

Camphor. This constituent, while isolated from EO *Artemisia feddei*, alone exhibited low antimicrobial activity (12.8 mg mL^{-1}) against *S. sanguinis* ATCC 10556 (Cha et al. 2007), while when isolated from the oil of *R. officinalis*, it showed to be quite stronger (400 μg mL^{-1}) against both referent strains, *S. salivarius* ATCC 25975 and *S. sanguinis* ATCC 10556 (Bernardes et al. 2010). Differences in antimicrobial activity on *S. sanguinis* ATCC 10556 strain, together with the high MIC values, comparing to the MIC values of selected EOs in our study, again implicate on hidden synergistic interactions of camphor with other constituents present in the oils.

Eugenol. Pure eugenol tested on clinical isolate of *S. sanguinis* achieved MIC = 250 μg mL^{-1}. In interaction with thymol or with carvacrol, it achieved stronger antimicrobial efficacy; the interactions were both times synergistic and reached the same MIC = 62.5 μg mL^{-1} (Didry et al. 1994). Its interactions when tested with some other oil constituents have to be further revealed, especially if we take in consideration that eugenol was a major constituent of *Ocimum sanctum* EO selected in this study due to its strong MIC = 0.25 μL mL^{-1} (Zomorodian et al. 2015), as well as in *Syzygium aromaticum* EO with MIC = 130 μg mL^{-1} (Nikolic 2015).

7.5 Conclusions

Study on essential oils with pronounced antimicrobial efficacy against target oral pathogens, *S. sanguinis* and *S. salivarius*, associated to standard endodontic procedure obstacles lead us to several statements we would like to outline.

The essential oils rich in *1,8*-cineole but lacking thymol are more efficient against *S. sanguinis* then *S. salivarius*, while *S. salivarius* proved to be more sensitive than *S. sanguinis* on thymol-rich oils lacking cineole.

Clinical isolates of both *Streptococcus* species generally show lower sensitivity to EOs than their corresponding referent strains.

Analysis of chemical composition of selected essential oils effective against the clinical isolates showed that chemical class to which constituents belong to do not influence antimicrobial activity. However, in case of referent strains, the class showed statistical difference between the MIC-strong and MIC-good essential oils.

The essential oils with predominating monoterpenoids content (monoterpene hydrocarbons and oxygenated monoterpenes) evenly contributed to both MIC groups of oils (the strong and the good efficacy EOs), while sesquiterpenoids (sesquiterpene hydrocarbons and oxygenated sesquiterpenes) predominated only in the good efficacy essential oils.

Analysis of data for MIC-strong EOs toward both referent *Streptococcus* species revealed that in the class of monoterpene hydrocarbons, one should look for the presence of myrcene, α-thujone, α-phellandrene, and *o*-cymene, while within the class of oxygenated monoterpenes, the target components have to be camphor, *1,8*-cineole, carvacrol, eugenol, and linalyl acetate. Compounds such as γ-terpinene, *p*-cymene, and thymol are common constituents in all selected EOs, regardless of whether they are MIC-strong or MIC-good; their interactions with the above-mentioned nine constituents should be subject of further investigation.

The antimicrobial compounds presented in this review could be valuable candidates for future studies of synergism, compatibility, and activity in dentistry processing systems. The great potential of essential oils to inhibit oral microorganisms may be used for prevention and treatment of oral and dental diseases, and connected disorders, in general as antibacterial agents in oral hygiene, and to replace conventional chemical at low and nontoxic concentrations.

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References

Abbaszadegan A, Sahebi S, Gholami A, Delroba A, Kiani A, Iraji A, Abott PV (2016) Time-dependent antibacterial effects of *Aloe vera* and *Zataria multiflora* plant essential oils compared to calcium hydroxide in teeth infected with *Enterococcus faecalis*. J Investig Clin Dent 7:93–101

Araya M, Morelli L, Reid G, Sanders M, Stanton C, Pineiro M, Ben Embarek P (2002) Guidelines for the evaluation of probiotics in food. London: Report of a Joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food

Bajaj JS (2016) Review article: potential mechanisms of action of rifaximin in the management of hepatic encephalopathy and other complications of cirrhosis. Aliment Pharmacol Ther 43:11–26

Barbour A, Philip K, Muniandy S (2013) Enhanced production, purification, characterization and mechanism of action of salivaricin 9 lantibiotic produced by *Streptococcus salivarius* NU10. PLoS One 8(10):e77751

Barretto C, Alvarez-Martin P, Foata F, Renault P, Berger B (2012) Genome sequence of the lantibiotic bacteriocin producer *Streptococcus salivarius* strain K12. J Bacteriol 194:5959–5960

Becerril R, Nerin C, Gomez-Lus R (2012) Evaluation of bacterial resistance to essential oils and antibiotics after exposure to oregano and cinnamon essential oils. Foodborne Pathog Dis 9:699–705

Berkiten M, Okar I, Berkiten R (2000) In vitro study of the penetration of *Streptococcus sanguis* and *Prevotella intermedia* strains into human dentinal tubules. J Endod 26:236–239

Bernardes WA, Lucarini R, Tozatti MG et al (2010) Antibacterial activity of the essential oil from *Rosmarinus officinalis* and its major components against oral pathogens. Z Naturforsch C 65:588–593

Bersan SMF, Galvao LCC, Goes VFF et al (2014) Action of essential oils from Brazilian native and exotic medicinal species on oral biofilms. BMC Complement Altern Med 14:451

Bogojevic J, Nikolic M, Markovic T, Ciric A, Markovic D (2016) Analysis of chemical composition of the most efficient essential oils towards *Enterococcus faecalis* referent strain ATCC 29212 and clinical isolates. Med Raw Mater 36:3–25

Borges MCL, Sesso MLT, Roberti LR, Oliveira M, Nogueira RD, Geraldo-Martins VR, Ferriani VP (2015) Salivary antibody response to *streptococci* in preterm and fullterm children: a prospective study. Arch Oral Biol 60:116–125

Burton JP, Drummond BK, Chilcott CN, Tagg JR, Thomson WM, Hale JDF, Wescombe PA (2013) Influence of the probiotic *Streptococcus salivarius* strain M18 on indices of dental health in children: a randomized double-blind, placebo-controlled trial. J Med Microbiol 62:875–884

Carlsson J, Edlund MB (1987) Pyruvate oxidase in *Streptococcus sanguis* under various growth conditions. Oral Microbiol Immunol 2:10–14

Carlsson J, Iwami Y, Yamada T (1983) Hydrogen peroxide excretion by oral *Streptococci* and effect of lactoperoxidase–thiocyanate–hydrogen peroxide. Infect Immun 40:70–80

Caufield PW, Dasanayake AP, Li Y, Pan Y, Hsu J, Hardin JM (2000) Natural history of *Streptococcus sanguinis* in the oral cavity of infants: evidence for a discrete window of infectivity. Infect Immun 68:4018–4023

Cha JD (2007) Chemical composition and antibacterial activity against oral bacteria by the essential oil of *Artemisia iwayomogi*. J Bacteriol Virol 37:129–136

Cha JD, Jung EK, Kil BS, Lee AY (2007) Chemical composition and antibacterial activity of essential oil from *Artemisia freddei*. J Microbiol Biotechnol 17:2061–2065

Chen YY, Clancy KA, Burne RA (1996) *Streptococcus salivarius* urease: genetic and biochemical characterization and expression in a dental plaque streptococcus. Infect Immun 64:585–592

CLSI (2013) Performance standards for antimicrobial susceptibility testing; twenty-third informational supplement. CLSI document M100-S23. Clinical and Laboratory Standards Institute, Wayne

Cosentino S, Tuberoso CI, Pisano B, Satta M, Mascia V, Arzedi E et al (1999) In-vitro antimicrobial activity and chemical composition of Sardinian *Thymus* essential oils. Lett Appl Microbiol 29:130–135

Crevelin EJ, Caixeta SC, Dias H, Groppo M, Cunha WR, Martins CHG, Crotti AEM (2015) Antimicrobial activity of the essential oil of *Plectranthus neochilus* against cariogenic bacteria. Evid Based Complement Alternat Med 2015:102317

Daniels S (2015) Health Canada approves BLIS K12 Probiotic. Nutraingredients-usa.com; http://www.nutraingredients-usa.com/Suppliers2/Health-Canada-approves-BLIS-K12-probiotic. 1 Sep 2017

Delgado B, Fernandez PS, Palop A, Periago PM (2004) Effect of thymol and cymene on *Bacillus cereus* vegetative cells evaluated through the use of frequency distributions. Food Microbiol 21:327–334

Di Filippo S, Delahaye F, Semiond B et al (2006) Current patterns of infective endocarditis in congenital heart disease. Heart 92:1490–1495

Di Pierro F, Colombo M, Zanvit A, Rottoli AS (2016) Positive clinical outcomes derived from using *Streptococcus salivarius* K12 to prevent *streptococcal* pharyngotonsillitis in children: a pilot investigation. Drug Healthc Patient Saf 8:77–81

Didry N, Dubreuil L, Pinkas M (1994) Activity of thymol, carvacrol, cinnamaldehyde and eugenol on oral bacteria. Pharm Acta Helv 69:25–28

Dorman HJD, Deans SG (2000) Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J Appl Microbiol 88:308–316

Fouad AF, Kum K-Y, Clawson ML, Barry J, Abenoja C, Zhu Q, Caimano M, Radolf JD (2003) Molecular characterization of the presence *of Eubacterium spp.* and *Streptococcus spp*. in endodontic infections. Oral Microb Immunol 18:249–255

Fujimura S, Nakamura T (1979) Sanguicin, a bacteriocin of oral *Streptococcus sanguis*. Antimicrob Agents Chemother 16:262–265

Gajan EB, Aghazadeh M, Abashov R, Milani AS, Moosavi Z (2009) Microbial flora of root canals of pulpally-infected teeth: *Enterococcus faecalis* a prevalent species. J Dent Res Dent Clin Dent Prospects 3:24–27

Ge Y, Caufield PW, Fisch GS, Li Y (2008) *Streptococcus mutans* and *Streptococcus sanguinis* colonization correlated with caries experience in children. Caries Res 42:444–448

Gomes B, Pinheiro ET, Gade-Neto CR et al (2004) Microbiological examination of infected dental root canals. Oral Microbiol Immunol 19:71–76

Gregori G, Righi O, Risso P et al (2016) Reduction of group A beta-hemolytic *streptococcus* pharyngo-tonsillar infections associated with use of the oral probiotic *Streptococcus salivarius* K12: a retrospective observational study. Ther Clin Risk Manag 12:87–92

Heng NC, Haji-Ishak NS, Kalyan A et al (2011) Genome sequence of the bacteriocin producing oral probiotic *Streptococcus salivarius* strain M18. J Bacteriol 193:6402–6403

Herrero ER, Slomka V, Bernaerts K et al (2016) Antimicrobial effects of commensal oral species are regulated by environmental factors. J Dent 47:23–33

Hyink O, Wescombe PA, Upton M, Ragland N, Burton JP, Tagg JR (2007) Salivaricin A2 and the novel lantibiotic salivaricin B are encoded at adjacent loci on a 190-kilobase transmissible megaplasmid in the oral probiotic strain *Streptococcus salivarius* K12. Appl Environ Microbiol 73:1107–1113

Jacinito RC, Gomes BP, Ferraz CC, Zaia AA, Filho FJ (2003) Microbiological analysis of infected root canal from symptomatic and asymptomatic teeth with periapical periodontitis and the antimicrobial susceptibility of some isolated anaerobic bacteria. Oral Microbiol Immunol 18:285–292

Juliano C, Mattana A, Usai M (2000) Composition and in vitro antimicrobial activity of the essential oil of *Thymus herba-barona Loisel* growing wild in Sardinia. J Essent Oil Res 12:516–522

Juven BJ, Kanner J, Schved F, Weisslowicz H (1994) Factors that interact with the antibacterial action of thyme essential oil and its active constituents. J Appl Bacteriol 76:626–631

Kaci G, Goudercourt D, Dennin V et al (2014) Anti-inflammatory properties of *Streptococcus salivarius*, a commensal bacterium of the oral cavity and digestive tract. Appl Environ Microbiol 80:928–934

Kadowaki M, Hashimoto M, Nakashima M, Fukata M, Odashiro K, Uchida Y, Shimono N (2013) Radial mycotic aneurysm complicated with infective endocarditis caused by *Streptococcus sanguinis*. Intern Med 52:2361–2365

Kaneko F, Togashi A, Saito S et al (2011) Behcet's disease (Adamantiades-Behcet's disease). Clin Dev Immunol 2011:681956

Kao YT, Shih CM, Tsao NW, Lin FY, Chang NC, Huang CY (2013) Subacute bacterial endocarditis presenting as left upper quadrant abdominal pain. J Chin Med Assoc 76:521–523

Kreth J, Merritt J, Shi W, Qi F (2005) Competition and coexistence between *Streptococcus mutans* and *Streptococcus sanguinis* in the dental biofilm. J Bacteriol 187:7193–7203

Kreth J, Zhang Y, Herzberg MC (2008) *Streptococcal* antagonism in oral biofilms: *Streptococcus sanguinis* and *Streptococcus gordonii* interference with *Streptococcus mutans*. J Bacteriol 190:4632–4640

Lambert RJW, Skandamis PN, Coote PJ, Nychas GJE (2001) A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J Appl Microbiol 91:453–462

Laura DM, Quinones A, Benenstein R et al (2014) Giant nonfamilial left atrial myxoma presenting with eye embolism and nonvalvular *Streptococcus sanguinis* endocarditis. J Am Coll Cardiol 63:2049

Lew HP, Quah SY, Lui JN, Bergenholtz G, Yu VSH, Tan KS (2015) Isolation of alkaline-tolerant bacteria from primary infected root canals. J Endod 41:451–456

Li J, Helmerhorst EJ, Leone CW, Troxler RF et al (2004) Identification of early microbial colonizers in human dental biofilm. J Appl Microbiol 97:1311–1318

Lysakowska ME, Ciebiada-Adamiec A, Sienkiewicz M, Sokolowski J, Banaszek K (2016) The cultivable microbiota of primary and secondary infected root canals, their susceptibility to antibiotics and association with the signs and symptoms of infection. Int Endod J 49:422–430

Mahboubi M, Kazempour N (2011) Chemical composition and antimicrobial activity of *Satureja hortensis* and *Trachyspermum copticum* essential oil. Iran J Microbiol 3:194–200

Mancl AK, Kirsner SR, Ajdic D (2013) Wound biofilms: lessons learned from oral biofilms. Wound Repair Regen 21:352–362

Marković T (2011) Etarska ulja i njihova bezbedna primena. Institut za proučavanje lekovitog bilja „dr Josif Pančić", Beograd (in Serbian)

Masdea L, Kulik EM, Hauser-Gerspach I, Ramseier AM, Filippi A, Waltimo T (2012) Antimicrobial activity of *Streptococcus salivarius* K12 on bacteria involved in oral malodour. Arch Oral Biol 57:1041–1047

Mashima I, Nakazawa F (2014) The influence of oral *Veillonella* species on biofilms formed by *Streptococcus* species. Anaerobe 28:54–61

Matsuo T, Shirakami T, Ozaki K, Nakanishi T, Yumoto H, Ebisu S (2003) An immunohistologic al study of the localization of bacteria invading root pulpal walls of teeth with periapical lesions. J Endod 29:194–200

McCarthy C, Snyder M, Parker RB (1965) The indigenous oral flora of man. The newborn to the 1-year old infant. Arch Oral Biol 10:61–70

Nikolić M (2015) Biological activity of selected essential oils towards *Staphylococcus, Streptococcus, Lactobacillus, Pseudomonas, Enterococcus* and *Candida* species isolated from human oral cavity. Ph.D. Thesis Dissertation, Faculty of Biology, University of Belgrade

Nikolic M, Glamoclija J, Ferreira I et al (2014) Chemical composition, antimicrobial, antioxidant and antitumor activity of *Thymus serpyllum* L., *Thymus algeriensis* Boiss. and Reut and *Thymus vulgaris* L. essential oils. Ind Crop Prod 52:183–190

Nikolić M, Marković T, Marković D, Glamočlija J, Ćirić A, Smiljković M, Soković M (2016) Antimicrobial activity of three *Lamiaceae* essential oils against common oral pathogens. Balk J Dent Med 20:160–167

Ohnishi Y, Kubo S, Ono Y et al (1995) Cloning and sequencing of the gene coding for dextranase from *Streptococcus salivarius*. Gene 156:93–96

Okahashi N, Nakata M, Sakurai A et al (2010) Pili of oral *Streptococcus sanguinis* bind to fibronectin and contribute to cell adhesion. Biochem Biophys Res Commun 391:1192–1196

Perez F, Calas P, de Falguerolles A, Maurette A (1993) Migration of a *Streptococcus sanguis* strain through the root dentinal tubules. J Endod 19:297–301

Power DA, Burton JP, Chilcott CN, Dawes PJ, Tagg JR (2008) Preliminary investigations of the colonization of upper respiratory tract tissues of infants using a pediatric formulation of the oral probiotic *Streptococcus salivarius* K12. Eur J Clin Microbiol Infect Dis 27:1261–1263

Provenzano JC, Rocas IN, Tavares LFD, Neves BC, Siqueira JF (2015) Short-chain fatty acids in infected root canals of teeth with apical periodontitis before and after treatment. J Endod 41:831–835

Rocas IN, Siqueira JF (2012) Characterization of microbiota of root canal-treated teeth with post-treatment disease. J Clin Microbiol 50:1721–1724

Rolph HJ, Lennon A, Riggio MP, Saunders WP, MacKenzie D, Coldero L, Bagg J (2001) Molecular identification of microorganisms from endodontic infections. J Clin Microbiol 39:3282–3289

Rosan B, Lamont RJ (2000) Dental plaque formation. Microbes Infect 2:1599–1607

Sakamoto M, Siqueira JF Jr, Rôças IN, Benno Y (2007) Bacterial reduction and persistence after endodontic treatment procedures. Oral Microbiol Immunol 22:19–23

Seow WK, Lam JHC, Tsang AKL, Holcombe T, Bird PS (2009) Oral *Streptococcus* species in preterm and full-term children—a longitudinal study. Int J Paediatr Dent 19:406–411

Shovelton DA (1959) Bacterial invasion of dentine around infected pulp canals: a preliminary report. Alabama Dent Rev 7:7–12

Siqueira JF Jr, Rocas IN (2008) Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod 34:1291–1301

Siqueira JF Jr, Rôças IN (2009) Diversity of endodontic microbiota revisited. J Dent Res 88:969–981

Siqueira JF, As I, Paiva SSM, Magalhaes KM, Guimaraes-Pinto T (2007) Cultivable bacteria in infected root canals as identified by 16S rRNA gene sequencing. Oral Microbiol Immunol 22:266–271

Stauffacher S, Lussi A, Nietzsche S, Neuhaus KW, Eick S (2017) Bacterial invasion into radicular dentine—an in vitro study. Clin Oral Investig 21:1743

Stingu CS, Eschrich K, Rodloff AC, Schaumann R, Jentsch H (2008) Periodontitis is associated with a loss of colonization by *Streptococcus sanguinis*. J Med Microbiol 57:495–499

Tagg JR (2008) *Streptococci* as effector organism for probiotic and replacement therapy. In: Versalovic J, Wilson M (eds) Therapeutic microbiology: probiotics and related strategies. ASM Press, Washington, DC, pp 61–81

Tagg JR, Dierksen KP (2003) Bacterial replacement therapy: adapting "germ warfare" to infection prevention. Trends Biotechnol 21:217–223

Tatikonda A, Sudheep N, Biswas KP, Gowtham K, Pujari S, Singh P (2017) Evaluation of bacteriological profile in the apical root segment of the patients with primary apical periodontitis. J Contemp Dent Pract 18:44–48

Thenisch NL, Bachmann LM, Imfeld T, Leisebach Minder T, Steurer J (2006) Are *mutans streptococci* detected in preschool children a reliable predictive factor for dental caries risk? A systematic review. Caries Res 40:366–374

Ultee A, Gorris LGM, Smid EJ (1998) Bactericidal activity of carvacrol towards the food-borne pathogen *Bacillus cereus*. J Appl Microbiol 85:211–218

Ultee A, Bennik MHJ, Moezelar R (2002) The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. Appl Environ Microbiol 68:1561–1568

Walker GV, Heng NCK, Carne A, Tagg JR, Wescombe PA (2016) Salivaricin E and abundant dextranase activity may contribute to the anti-cariogenic potential of the probiotic candidate *Streptococcus salivarius* JH. Microbiology 162:476–486

Wescombe PA, Hale JD, Heng NC et al (2012) Developing oral probiotics from *Streptococcus salivarius*. Future Microbiol 7:1355–1371

Wilson W, Taubert KA, Gewitz M et al (2008) Prevention of infective endocarditis: guidelines from the American Heart Association. J Am Dent Assoc 139:3S

Wisniewska-Spychala B, Sokalski J, Grajek S et al (2012) Dentigenous infectious foci – a risk factor of infective endocarditis. Med Sci Monit 18:CR93–CR104

Yang TS, Liou ML, Hu TF, Peng CW, Liu TT (2013) Antimicrobial activity of the essential oil of *Litsea cubeba* on cariogenic bacteria. J Essent Oil Res 25(2):120–128

Zheng LY, Itzek A, Chen ZY, Kreth J (2011) Oxygen dependent pyruvate oxidase expression and production in Streptococcus sanguinis. Int J Oral Sci 3:82–89

Zhu L, Kreth J (2010) Role of *Streptococcus mutans* eukaryotic-type serine/threonine protein kinase in interspecies interactions with *Streptococcus sanguinis*. Arch Oral Biol 55:385–390

Zhu L, Kreth J (2012) The role of hydrogen peroxide in environmental adaptation of oral microbial communities. Oxid Med Cell Longev 2012:717843

Zomorodian K, Ghadiri P, Saharkhiz MJ et al (2015) Antimicrobial activity of seven essential oils from iranian aromatic plants against common causes of oral infections. Jundishapur J Microbiol 8:17766e

Chapter 8
Chemical Diversity and Insecticidal and Anti-tick Properties of Essential Oils of Plants from Northeast Brazil

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8.1 Introduction

Northeast Brazil, the third largest region of the country, is made up of nine states: Alagoas (AL), Bahia (BA), Ceará (CE), Maranhão (MA), Paraíba (PB), Pernambuco (PE), Piauí (PI), Rio Grande do Norte (RN), and Sergipe (SE) (Fig. 8.1). Most of the Northeast is characterized by scarce rainfall, areas with pronounced spatial-temporal irregularity, and prolonged drought periods, where the annual accumulated rainfall is less than 500 mm. Conversely, the East Coast presents rainy climate, with annual accumulated rainfall greater than 1500 mm. The climatic variability and the diverse biomes of the region favor the occurrence of significant plant diversity, including medicinal and aromatic species. Part of this diversity is found in the main northeastern biome, the Caatinga, where several endemic species occur. The unfavorable climatic conditions, marked by water scarcity and high temperatures, might have favored the emergence of better-adapted species, which have improved their biosynthetic capacity in response to the environment over their evolutionary process. The essential oils of some species found in the Caatinga have a particularly different chemical composition from those plants found in regions with more favorable survival conditions. The availability of these plants, together with the need to obtain natural products to be used as alternatives to the toxic synthetic products widely applied to crops—which are harmful to man and the environment—has made them the subject of research on their biotechnological potentialities. In this sense, the Federal University of Sergipe (UFS) has been developing studies on

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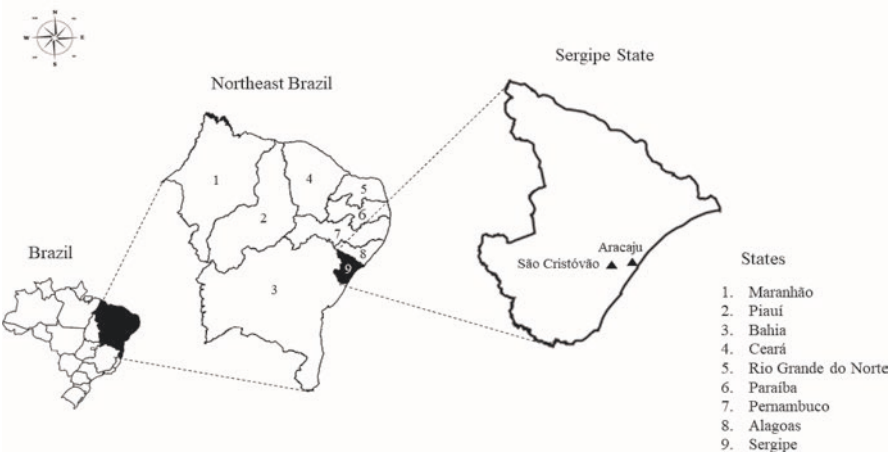

Fig. 8.1 Map of Northeast Brazil and its states, with emphasis on the state of Sergipe

aromatic species from Sergipe and other northeastern states, especially regarding the chemical diversity of essential oils and their biological activities. The essential oils are analyzed by a mass GC-MS/FID (QP2010 Ultra, Shimadzu Corporation, Kyoto, Japan), equipped with an autosampler AOC-20i (Shimadzu). The results enable developing technologies for the sustainable use of these resources in agriculture and livestock, focusing on natural pesticides and new cultivars of bioactive plants.

This chapter describes some knowledge about the chemical diversity of the essential oil of species from Northeast Brazil, their biotechnological potentialities, and the main factors that have already been described regarding their chemical diversity.

8.2 Chemical Diversity of Essential Oils of Plants from Northeast Brazil

Essential oils are substances derived from plants' secondary metabolites. In the past, they were believed to be primary metabolism by-products. Despite not being part of the essential units of cells, secondary metabolites perform very specific functions in plants, being advantageous to them for survival and adaptation to adverse conditions. These substances are synthesized in complex routes, involving energy expenditure and several chemical reactions using specific enzymes. Plants produce a true biosynthetic arsenal with a vast diversity of substances, varying both between and within the same species. Harborne (1988) states that the diversity of secondary metabolites is partly justified by the fact that plants cannot move nor respond to the environment as animals can.

Essential oils are constituted mostly by a set of volatile and low molecular weight substances—the terpenes (mono- and sesquiterpenes) and phenylpropanoids. They are lipophilic, liquid, and unstable in the presence of light and heat, with characteristic aroma. They usually present a predominant compound, denominated as major compound. Species are known to have different chemotypes when the essential oil of their plants contains different major compounds. The essential oil of some species is formed by ten or fewer compounds; in other cases, it can be formed by 60 or more. The literature review "Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants" by Degenhardt et al. (2009) reports that part of this diversity is due to the ability of monoterpene and sesquiterpene synthase enzymes to form multiple products. All these characteristics make essential oils the subject of studies focused on their chemical diversity and potentialities as a source of useful molecules to humans. Several plants are conserved in the collections maintained at the Active Germplasm Bank of Medicinal and Aromatic Plants of the Federal University of Sergipe (Table 8.1), located in the municipality of São Cristóvão, state of Sergipe (lat. 11°00′S, long. 37°12′W). The following topics describe a few species that occur in Northeast Brazil and their biotechnological potentialities.

8.2.1 Lippia gracilis *and* L. sidoides

Lippia gracilis (*alecrim de tabuleiro*) and *Lippia sidoides* (*alecrim de pimenta*) are shrubby plants endemic to the Caatinga, with quite aromatic leaves. *L. gracilis* is mainly found in the states of Bahia, Sergipe, and Piauí, while *L. sidoides* predominantly occurs in the states of Ceará, Rio Grande do Norte, Bahia, and Sergipe. Since 2008, the Federal University of Sergipe has carried out the conservation of genotypes of these species in field collections, with seven accessions of *L. gracilis* and ten accessions of *L. sidoides*. These plants have a high potential of use owing to their diverse biological activities and are a good example of native plants that could become a cultivated species once they receive the appropriate investments for research and technological development. The essential oils of these

Table 8.1 Species, number of accessions, and biological activities detected in aromatic medicinal plants maintained at the Active Germplasm Bank of Medicinal and Aromatic Plants of the Federal University of Sergipe, São Cristóvão, state of Sergipe, Brazil

Species	No. of accessions	Proven biological properties
Lippia gracilis Schauer	07	Insecticide, acaricide, and fungicide
Lippia sidoides Cham.	10	Insecticide, acaricide, and fungicide
Lippia alba (Mill.) N. E. Brown	48	Insecticide, acaricide, and fungicide
Varronia curassavica Jacq.	42	Antiprotozoal
Eplingiella fruticosa Salzm. ex Benth	23	Insecticide
Croton tetradenius Baill.	40	Bactericide and insecticide

species are rich in monoterpenes, especially thymol and carvacrol. These compounds are isomers, structurally differing only by the position of the hydroxyl group of the aromatic ring. Despite having the same molecular formula ($C_{10}H_{14}O$) and molecular weights (150.22 g.mol^{-1}) at room temperature, carvacrol is found in the liquid form while thymol is crystallized (Nostro and Papalia 2012; Holland et al. 2014). This chapter will further discuss the differences in their biological activities.

Six of the seven *L. gracilis* accessions maintained at the Active Germplasm Bank have carvacrol as major compound; the other one has thymol as major compound. Conversely, nine accessions of *L. sidoides* present thymol as the major compound; the remaining one presents carvacrol as major compound. Therefore, *L. gracilis* and *L. sidoides* contain two well-defined chemotypes—thymol and carvacrol.

Although genetic factors determine the ability to synthesize specific compounds, some quantitative and qualitative variations may occur due to environmental factors—such as geographic location and soil and climate characteristics, agricultural practices, development stage, seasonality, and insect attack—and post-harvest factors. Each of these factors or the interaction between them will act in a particular way depending on the species. Younger plants can produce essential oil at a higher quantity than older plants as they can perform the full biosynthetic activity. Conversely, older plants may, sometimes, produce more essential oils due to their mature, fully developed biosynthetic "machinery". Obtaining essential oils continuously and with standardized quantitative and qualitative characteristics is still a challenge. Thus, the knowledge about the effects of genetic and environmental factors on the essential oil characteristics is fundamental.

In *L. gracilis*, plant age is a critical factor in essential oil production and can cause quantitative and qualitative changes in this metabolite (Santos et al. 2016). Four-year-old plants produce on average twice the amount of essential oil produced by 1-year-old plants. Regarding the chemical composition, the contents of the major compounds thymol and carvacrol tend to decrease with plant aging, while the contents of secondary compounds—mainly myrcene, p-cymene, 1,8-cineole, and γ-terpinene—tend to increase. Other factors, such as seasonality and water availability, do not cause significant changes in the chemical composition of the essential oil of *L. gracilis*. However, a mean increment of 35% in essential oil yield (mL.plant^{-1}) is obtained when plants are harvested in the dry season. A significant advantage of this species is the stability of the chemical compounds of their essential oil, regardless of the harvesting season or the use or not of artificial irrigation (Cruz et al. 2014).

Significant variations in the content and chemical composition may also occur in the essential oils of older plants of *L. sidoides* when compared with younger ones (Santos et al. 2015). Unlike *L. gracilis*, the essential oil content of *L. sidoides* reduces with plant aging. Eight-year-old plants produce, on average, 18% less essential oil than 2-year-old plants. Carvacrol content tends to increase while thymol content tends to decrease in older plants of *L. sidoides*. Plant age also interferes with the secondary compounds. The contents of terpinen-4-ol, thymol methyl ether, (*E*)-caryophyllene, and caryophyllene oxide may increase or remain the same in older plants. In older plants, the contents of *p*-cymene and myrcene may reduce or remain the same, while the content of γ-terpinene may reduce or increase.

The variations in the essential oil contents and the percentages of essential oil compounds are in fact phenotypic variations of the plant. The phenotypic variation is the result of the genetic variation between the plants, associated with the effects of non-genetic factors. Understanding the factors that affect essential oil production can help in obtaining products with preferable chemical characteristics and at the desired quantity. In *L. sidoides*, environmental factors have more influence on the essential oil content than genetic factors when comparing 2- and 8-year-old plants (Santos et al. 2015). Conversely, genetic factors act more strongly than environmental factors on thymol, carvacrol, thymol methyl ether, (*E*)-caryophyllene, and caryophyllene oxide.

Although the different factors mentioned above may have a greater or lesser influence on the chemical content and essential oil composition of *L. gracilis* and *L. sidoides*, the chemotypes of these species do not vary. Regardless of the culture conditions, both species will always present the chemotypes thymol and carvacrol.

8.2.2 Lippia alba

L. alba, popularly known as *erva-cidreira-brasileira* or lemon balm, is widely distributed throughout Brazil. It is a heavily branched, perennial, shrub species and can reach up to 2 m in height. The species is widely used in folk medicine, owing to its soothing, spasmolytic, analgesic, sedative, anxiolytic, and expectorant properties. The *L. alba* collection maintained at the Active Germplasm Bank of the Federal University of Sergipe consists of 48 accessions from all over Brazil, of which 21 were collected specifically in northeastern states (Ceará, Alagoas, Sergipe, and Bahia). The essential oils of these plants have a variable chemical composition, with marked differences between major compounds. The major compounds most commonly identified are 1,8-cineole, linalool, myrcene, limonene, carvone, geranial, and neral. Neral and geranial are two isomeric acyclic monoterpene aldehydes which together generate citral. The Active Germplasm Bank of *L. alba* is characterized by six chemotypes: (1) linalool + 1,8-cineole + caryophyllene oxide, (2) linalool + citral + 1,8-cineole + caryophyllene oxide, (3) limonene + carvone + sabinene, (4) carvone + limonene + γ-muurolene + myrcene, (5) citral + caryophyllene oxide, and (6) citral + *o*-cymene + limonene + caryophyllene oxide (Blank et al. 2015). Environmental or genetic effects can explain the chemical diversity of the essential oil of *L. alba*. Considering that the species occurs all over the country, in regions with peculiar climatic and geological characteristics, it must have developed strategies for better adaptation. This phenomenon happened in the course of an evolutionary process, where, at a certain moment, given the pressures continually imposed by the environment, a plant underwent genetic changes, allowing the synthesis of certain substances that could favor its permanence and perpetuation.

L. alba chemotypes are a valuable source due to their different biological activities, which will be further addressed in this chapter, besides other potentialities to be discovered. *L. alba* has excellent potential as raw material or as a model for the development of new molecules that can be used in a sustainable agriculture system.

8.2.3 Myrcia lundiana *and* Myrcia ovata

The Myrtaceae family is found in Australia, Asia, Africa, and America and consists of a large number of species, distributed within 132 genera. Among the 985 species of the 23 genera that occur in Brazil, 744 are endemic (Sobral et al. 2012). *M. lundiana* and *M. ovata* restrictively occur in only two regions—Northeast and Southeast. In the state of Sergipe, *M. lundiana* can be found in the Atlantic forest, shrub *restinga*, high *restinga*, and white sand *restinga*. Several plants of this species occur mainly in the National Park of Serra de Itabaiana (municipality of Areia Branca). *M. ovata* is found in the municipality of Japaratuba, in a sandy vegetation area with intense anthropic activity. Plants of both species are visually similar, and most of the times their identification requires a taxonomist specialized in this botanical family.

These two forest species are not conserved at the Active Germplasm Bank of Medicinal and Aromatic Plants of the Federal University of Sergipe due to their difficult vegetative propagation. Also, seeds and seedlings do not easily develop when removed from their natural habitat. However, much information has been obtained from the study on natural populations of these species. Both plants have essential oils with high antimicrobial potential owing to their compounds. Some compounds are present in both *M. lundiana* and *M. ovata*.

The essential oils of *M. lundiana,* found in the National Park of Itabaiana, and *M. ovata,* found in the municipality of Japaratuba, have a mean content of 1.10% and 1.76%, respectively, considering the volume of oil obtained from leaves dried at 40 °C for 5 days. The essential oil of *M. lundiana* is classified into three chemotypes, based on the most abundant compounds: (1) chemotype nerolic acid + 1,8-cineole; (2) chemotype 1,8-cineole + citral (neral + geranial); and (3) chemotype 1,8-cineole + isopulegol and/or iso-isopulegol. Conversely, with a higher variability, the essential oil of *M. ovata* is classified into six chemotypes: (1) nerolic acid, (2) linalool + nerolic acid, (3) geraniol, (4) citral (neral + geranial), (5) nerolic acid + *E*-nerolidol, and (6) linalool + isopulegol + nerolic acid (Alves et al. 2016; Sampaio et al. 2016).

Among the major compounds identified in the essential oil of these two species, nerolic acid [*(Z)*-3,7-dimethyl-2,6-octadienoic acid] stands out for being a very rare compound, reported in few aromatic species, whose content ranges from 0.8% to 7.7%. Nevertheless, some plants from natural populations of *M. lundiana* and *M. ovata* from the state of Sergipe have been reported to present 33.75% and 72.11% of nerolic acid, respectively, being the first report of this chemical compound in the genus *Myrcia*. Both species have great potential to control phytopathogenic fungi, especially those responsible for the post-harvest deterioration in tropical fruits. Between 2013 and 2015, three patents of antimicrobial products and formulations developed with the use of the essential oil of *M. lundiana* and *M. ovata* were deposited by the Federal University of Sergipe at the National Institute of Industrial Property of Brazil. Despite the need for basic studies on the propagation of these species, both for conservation and large-scale production, the potential of these species to generate safer plant-based antimicrobial products to be used in freshly consumed foods is evident.

8.2.4 Varronia curassavica

Varronia curassavica (ex-*Cordia verbenacea*), commonly known as *erva-baleeira*, is a medicinal and aromatic species widely distributed in Brazil. Besides the antimicrobial and antiparasitic activities, the essential oil has anti-inflammatory properties and is therefore used by a pharmaceutical corporation as an ingredient for herbal medicine for topical use. Despite not being the major compound, the sesquiterpene α-humulene is responsible for the anti-inflammatory property of the essential oil of *V. curassavica* (Passos et al. 2007).

In Sergipe, this species occurs in several municipalities and can be found both in coastal and in pasture areas, roadsides, and at the banks of small forests, which are usually sunny locations with well-drained soils. The essential oil of *V. curassavica* contains a wide variety of chemical compounds. Samples of this species analyzed by gas chromatography/mass spectrometer exhibit chromatograms with a lot of peaks, usually 30 or more. Some of these peaks are not identified by the libraries stored in the computer system of the equipment, nor are they found in spectra available in the printed and virtual literature. The essential oils of plants from Southeast Brazil usually present α-pinene, (*E*)-caryophyllene, bicyclogermacrene, alloaromadendrene, or α-santalene as major compounds. Plants from Sergipe—Northeast Brazil—also present camphene, tricyclene, sabinene, *β*-phellandrene, *α*-zingiberene, germacrene D-4-ol, ar-turmerone, and viridiflorol as major compounds (Nizio et al. 2015). Some plants synthesize both mono- and sesquiterpenes, while others synthesize only sesquiterpenes. *V. curassavica* presents up to 10% of *α*-humulene content, considered as the species chemical marker. A particular compound, isolated and identified by the Department of Chemistry of the Federal University of Sergipe in partnership with other institutions, 7-ciclodecen-1-ona, 7-methyl-3-methylene-10-(1-propyl) (Anjos 2014), is the major compound of the essential oil of several plants that occur in the state of Sergipe.

The vast chemical diversity of the essential oils of *V. curassavica* is undoubtedly the result of genotype vs. environment interaction. However, researchers believe in a strong influence of genetic factors since the essential oils of plants that occur in similar and nearby sites have different major compounds. This probable genetic variability can be attributed, at least in part, to the reproductive system of the species, which is considered as facultative allogamous, i.e., it can reproduce both by self- and cross-pollination (Brandão et al. 2015). Its reproductive system favors the maintenance of a high evolutionary capacity, which in practice results in easier plant adaptation and colonization of new areas by gene recombination.

The knowledge of the chemical diversity of the essential oil of *V. curassavica* helps in the elaboration of breeding and conservation strategies. Currently, more than 40 accessions have been conserved in a field collection at the Active Germplasm Bank of the Federal University of Sergipe.

Besides the genetic factors, the extraction method can also influence the variation in essential oils' chemical composition. Conventional methods that use high temperatures and large volumes of water may change the qualitative and quantitative characteristics of essential oils. Nevertheless, recently developed methods involving

lower water and energy consumption are more expensive and require that several parameters be established and standardized for each species, such as power and time in microwave-assisted extraction. In *V. curassavica*, lower water volume and longer time lead to higher essential oil contents extracted by hydrodistillation. Conversely, in microwave-assisted extraction, the time and power must be pre-adjusted to obtain higher essential oil contents. Also, water is not necessary when using fresh leaves. In both methods, the sesquiterpene hydrocarbon compounds tend to decrease—e.g., α-humulene and (*E*)-caryophyllene—when using long extraction times and/or high water volumes. Oxygenated compounds, however, tend to increase under these conditions (Nizio et al. 2018).

The knowledge about the effects of different factors, whether involved or not in the processing stages, on the chemical compounds of the essential oils of a species is fundamental since they influence the biological activity of the metabolite. The essential oil of *V. curassavica* must contain at least 2.3% α-humulene to be used by the pharmaceutical industry in the production of herbal medicine for inflammation treatment (Quispe-Condori et al. 2008).

For being commercially exploited, *V. curassavica* plants from Southeast and southern Brazil are well known. However, those from Northeast Brazil might present other potentialities still to be discovered and exploited, whether for medicinal or agricultural use. A recent study has confirmed the antiprotozoal potential of the essential oil of *V. curassavica* to control fish parasites (Nizio et al. 2017). Several studies on the insecticide activity of the essential oil of this species have been conducted, and some of them have shown substantial evidence of its potential to control insect-pests.

8.2.5 Eplingiella fruticosa

Popularly known as *alecrim-de-vaqueiro*, *E. fruticosa* is a native species to Brazil, with high occurrence in the northeast region. This plant used to be denominated as *Hyptis fruticosa*; however, after a taxonomic reclassification, species of the genus *Hyptis* originated other genera, such as the genus *Eplingiella*, which is formed by three species, all endemic to Brazil. In folk medicine, the plant is used as analgesic and anti-inflammatory. Its essential oil has antitumor, antinociceptive, and larvicidal properties (Menezes et al. 2007; Silva et al. 2008).

Plants of native populations of *E. fruticosa* are found from north to south of the state of Sergipe. They have a yellow-translucent essential oil with a mean content of 1.08% (volume, mass) and a large number of compounds. None of the major compounds reach 20%, and several secondary compounds present less than 1% of content. The major compounds of the essential oils of *E. fruticosa* are 1,8-cineole, α-pinene, β-pinene, (*E*)-caryophyllene, bicyclogermacrene, camphor, spathulenol, and caryophyllene oxide (Silva et al. 2018). In general, *E. fruticosa* plants are divided into two chemical groups, based on the major compounds of their essential oils. The first group presents bicyclogermacrene, spathulenol, (*E*)-caryophyllene,

and caryophyllene oxide as major compounds. The second group has 1,8-cineole, α-pinene, and camphor as major compounds, in addition to those of the first group, but at lower contents. The insecticidal potential to control leaf-cutting ants was recently confirmed in a study on the essential oils of plants of the second group (Silva 2017).

Despite its popular use, scientific studies on *E. fruticosa* are still scarce. The species has been maintained in a field collection of the Federal University of Sergipe, which enables the study on factors that interfere with the characteristics of its essential oils and the development of conservation strategies, as the species occurs in environments with intense anthropic activity. *E. fruticosa* has excellent potential to combat insect-pests and be used in the production of human medicine.

8.2.6 Hyptis pectinata

Hyptis pectinata is widely distributed in Northeast Brazil, where it is popularly known as *sambaicatá* or *canudinho*. The plant is used in folk medicine, owing to its therapeutic properties (Raymundo et al. 2011). It is commonly found on roadside, vacant lots, and backyards, mainly in the states of Alagoas and Sergipe. In Sergipe, the species is found from the coast to the *sertão* region of the state. Similar to *E. fruticosa*, *H. pectinata* belongs to the Lamiaceae family and is traditionally used by communities in the treatment of infections and inflammation. *H. pectinata*, as well as other species of the genus *Hyptis*, are known for the anti-inflammatory, antimicrobial, and antiparasitic properties of their essential oil.

H. pectinata plants of the state of Sergipe dried in a forced-air circulation oven have a mean essential oil content of 0.58%. Their major compounds are calamusenone, (*E*)-caryophyllene, caryophyllene oxide, germacrene-D, (*E*)-elemene, and (*Z*)-*β*-guaene. *H. pectinata* plants usually have calamusenone and (*E*)-caryophyllene or (*E*)-caryophyllene and caryophyllene oxide as major compounds (first and second, respectively). The compounds α-copaene and α-humulene are also commonly observed in *H. pectinata* plants from the state of Sergipe, although at lower concentrations (<6.0%) (Feitosa-Alcântara 2017).

Abiotic factors related to the post-harvest stages—such as leaves' drying and storage—influence the chemical composition of essential oils. The storage temperature may also change the chemical composition of the essential oil. In *H. pectinata*, the contents of *β*-elemene, *α*-copaene, germacrene D, caryophyllene oxide, and (*E*,*E*)-*α*-farnesene may increase in essential oils stored at warmer temperatures (±32 °C) for 1 year. Conversely, the contents of α-humulene and (*E*)-caryophyllene can decrease in essential oils stored at warmer temperatures when compared with those stored at lower temperatures (−20 °C) (Jesus et al. 2016). Higher temperatures favor chemical reactions, degrading some compounds and forming others. In contrast, lower temperatures ensure greater stability of the essential oil chemical composition over time.

Understanding the chemical diversity of the essential oils of species already used in folk medicine is fundamental as they might present several other potentialities. Besides providing information on the establishment of genetic conservation and breeding strategies, the knowledge about the essential oil compounds will be useful in directing further studies. This chapter will report the insecticidal potential of the essential oil of *H. pectinata* to control leaf-cutting ants.

8.2.7 Croton tetradenius

Croton tetradenius (Euphorbiaceae) is a shrubby plant whose leaves have a strong and characteristic aroma. It is an endemic species to Northeast Brazil, widely found in the states of that region—except for Piauí and Maranhão—mainly in areas of sandy or rocky soil (Silva 2009). Little is known about this plant, even regarding its popular use. However, species of the same genus are popularly used in the treatment of hypercholesterolemia, obesity, colic, intestinal inflammation, and rheumatism. Therefore, the essential oil of *C. tetradenius* is believed to present a high potential of use, which is still being investigated. In 2017, the Federal University of Sergipe applied for a patent deposit at the National Institute of Industrial Property of Brazil for a fungicide formulation based on the essential oil of *C. tetradenius* to control a phytopathogenic fungus.

Natural population plants from the state of Sergipe, especially from the municipalities of Lagarto, Poço Redondo, and Porto da Folha, produce higher essential oil contents than other species. The leaves present mean essential oil contents of 3.65% when obtained by hydrodistillation in a modified Clevenger apparatus. The major compounds of these plants are α-pinene, α-terpinene, *p*-cimene, 1,8-cineole, *(E)*-pinocarveol, camphor, pinocarvone, *(Z)*-ascaridole, and *(E)*-ascaridole. The compounds α-tujene, α-pinene, camphene, sabinene, β-pinene, myrcene, α-phellandrene, α-terpinene, *p*-cymene, limonene, 1,8-cineole, γ-terpinene, linalool, camphor, terpinen-4-ol, α-terpineol, (*Z*)-ascaridole, isobornyl acetate, bornyl acetate, and (*E*)-ascaridole are found in the essential oils of all plants of these populations. In general, what chemically differs one plant from another is that some of them also present (*E*)-pinocarveol, pinocarvone, myrtenyl acetate, α-humulene, and caryophyllene oxide, which are absent in others (Almeida-Pereira 2017).

Given the chemical diversity of the essential oil of *C. tetradenius*, several biological activities might be soon unveiled. A field collection of this species was implemented in June 2018, where 40 accessions from the state of Sergipe were conserved. This collection will enable several studies related to the factors that influence the chemical composition of the essential oil of this species.

8.3 Insecticidal Activity

After World War II, more precisely with the emergence of the technologies of the "Green Revolution," countless synthetic chemicals were used in agriculture. The development and use of organoclorine, organophosphorus, and carbamate insecticide stand out among the novelties of that time. Such products, together with other technologies, have leapfrogged food production in the face of the high demand of the postwar period. However, these products have had significant negative impacts on the environment and human health.

Although several of these active principles have been banned, until today, the principal control method for various insect-pests is based on synthetic chemicals. Despite the efficiency and reduction of agricultural damage, the use of these products excessively and indiscriminately continues to damage the ecosystems. Therefore, studies on plant-based products aimed at alternative pest-control methods and the sustainable use of natural resources have gained prominence for being environmentally safer and less toxic to non-target organisms, including humans.

The essential oils are among the plant metabolites with high potential for use in agriculture. In addition to medicinal, antimicrobial, antiparasitic, and acaricidal properties, they also have insecticidal properties, which can cause insect deaths or affect their development, behavior and reproduction.

The following topics address the activities of some essential oils of plants that occur in Northeast Brazil on ants, termites, and stored-grain insects.

8.3.1 Formicidal Activity

Ants play an important role in ecosystems, as they act in the processes of nutrient cycling, soil aeration, and seed dissemination (Galitzki et al. 2013; Leal et al. 2015). Nevertheless, due to the increased use of areas for cultivation and several other environmental changes caused by anthropic action, ant species have been considered as "pests" due to the significant damage caused to crops. Leaf-cutting ants, mainly those of the genus *Atta* and *Acromyrmex*, are considered as pests. These ants cause direct damage to agricultural and forestry crops mainly for their intense leaf-cutting activity at any stage of plant development, leading to considerable economic losses. All the plant material is taken to the subterranean nest to serve as a substrate for the cultivation of fungi with which they maintain a symbiotic relationship (Buratto et al. 2012).

Chemical control is the most efficient and commonly used method against leaf-cutting ants. Moreover, certifying entities are abolishing the use of these products in pest control of planted forests, aiming at economic viability, environmental balance, and social safety (Jung et al. 2013; FSC 2016).

Essential oils and their major compounds have been studied to understand their potential to control leaf-cutting ants. These substances can be directly used as active ingredients in formulations, mixed with other active ingredients, or serve as a model for the synthesis of new molecules, as an alternative to the few commercially available active principles. Several advantages are attributed to the use of plant-based products as bioinsecticides when compared with synthetic products. For instance, they originate from renewable resources, present less or no residual effect on foods—as they are biodegradable—and have minor harmful effects on non-target organisms. Moreover, for being composed of complex mixtures of compounds, the possibility of the emergence of resistant individuals is much lower, which enables the use of the essential oil for a longer period (Gonzalez et al. 2014).

Studies on the essential oils of medicinal and aromatic species from Northeast Brazil have been carried out, aiming at knowing the toxicity against species of leaf-cutting ants. A recent study revealed that the exposure pathway is crucial when evaluating the toxicity of essential oils. The sensitivity to essential oils also varies between species of leaf-cutting ants. Considering the essential oil of *H. pectinata*, the chemotype calamusenone + caryophyllene oxide was more toxic to *Acromyrmex balzani* than the chemotype (*E*)-caryophyllene + caryophyllene oxide (Feitosa-Alcantara et al. 2017). The doses required to kill 90% of the ants (lethal doses, LD_{90}) were 9.84 and 31.16 $\mu g.mg^{-1}$, respectively, by contact. The opposite was observed for the species *Atta sexdens rubropilosa,* where the chemotype (*E*)-caryophyllene + caryophyllene oxide was more toxic than the chemotype calamusenone + caryophyllene oxide, with LD_{90} of 11.67 and 25.80 $\mu g.mg^{-1}$, respectively, considering the same exposure pathway. When testing the toxicity of the same chemotypes by fumigation, for both species, the chemotype calamusenone + caryophyllene oxide was more toxic, with LC_{90} of 1.35 and 3.52 $\mu L.L^{-1}$ for *A. balzani* and *A. sexdens*, respectively. The chemotype (*E*)-caryophyllene + caryophyllene oxide had an LC_{90} of 6.34 and 6.15 $\mu L.L^{-1}$, respectively. The major compounds (*E*)-caryophyllene, calamusenone, and caryophyllene oxide tested individually showed lower toxicity levels against species of leaf-cutting ants than the essential oils tested by contact. These compounds were not toxic to the ants when applied by fumigation.

The essential oils of *E. fruticosa* are also toxic to *A. balzani* when applied by fumigation, with LC_{90} values similar to those of the essential oils of *H. pectinata*. In addition to insect deaths, the essential oils and major compounds of *E. fruticosa* also change the behavior of *A. balzani* workers (Silva 2017).

H. pectinata and *E. fruticosa* are potential raw material sources for the development of formulations and bioproducts to be used in the management of leaf-cutting ants as an alternative to synthetic insecticides.

8.3.2 *Anti-termite Activity*

In natural environments, termites have critical ecological functions, mainly linked to wood decomposition. Nonetheless, due to their food habit, which is based on cellulosic resources, they are considered as important pests in agricultural and urban environments. The irreversible damage caused to wooden structures has required attention to the control of these insects. Moreover, termites are responsible for priceless losses to historic buildings, museums, and libraries (Albuquerque et al. 2012). Unlike other pests, the damage caused by termites is usually inestimable for they are found inside wood pieces, hindering the visualization. Therefore, for the most part, the criterion for the use of control measures is based solely on the presence of termites. This measure leads to the indiscriminate use of insecticides, with doses higher than that recommended (Santos et al. 2017), causing severe damage to the environment and human health.

Nasutitermes corniger (Termitidae) and *Cryptotermes brevis* (Kalotermitidae) are termite species that occur in South America. Despite the several strategies to control these species in agricultural and urban environments, insecticides are the most commonly used control method, either by directly applying it to the material attacked by the insect or by using baits or fumigant agents (Verma et al. 2009; Evans and Iqbal 2015). Studies have been looking for new, environmentally friendly alternatives of termite control that are less toxic to non-target species. In this sense, essential oils have been identified as alternative substances for termite-pest management (Bacci et al. 2015). Works on essential oils of species conserved in the Active Germplasm Bank of Medicinal and Aromatic Plants of the Federal University of Sergipe have reported toxicity to *N. corniger* and *C. brevis*.

The doses required to kill 50% and 90% of *N. corniger* workers (LD_{50} and LD_{90}) exposed by contact to the essential oils of *Lippia alba* (chemotype carvone + limonene), *Lippia gracilis* (chemotype thymol), and *Lippia sidoides* (chemotype thymol) were 2.15 and 13.52 $\mu g.mg^{-1}$; 1.57 and 3.06 $\mu g.mg^{-1}$, and 0.27 and 1.00 $\mu g.mg^{-1}$, respectively (Lima et al. 2013). Of the three species, *L. sidoides* proved to be the most toxic against the termite. Despite having the same compounds, an additive or synergistic activity of thymol with p-cymene (25.25%) and thymol methyl ether (16.87%) may explain the higher toxicity of the essential oil of *L. sidoides* in relation to *L. gracilis*, which also has large amounts of thymol but lower concentrations of the other compounds.

C. brevis colonies are also divided into castes: the reproductives, the soldiers, and the pseudergates. The latter behave as workers, but they may develop into substitute soldiers and reproductives. A study has recently confirmed that the essential oil of *L. sidoides* and their major compounds are toxic to *C. brevis* (Santos et al. 2017). Nevertheless, the essential oil was more toxic than the compounds thymol, *p*-cymene, and (*E*)-caryophyllene when applied by contact, with LD_{90} of 14.0, 29.0, 28.9, and 38.0 $\mu g.mg^{-1}$, respectively. When applied by fumigation to pseudergates, the essential oil and the compound thymol were more toxic than the other compounds, with LC_{90} of 23.4 and 32.4 $\mu L.L^{-1}$. The compound p-cymene was

less toxic (LC_{90} of 592 μL.L^{-1}) while (*E*)-caryophyllene showed no toxicity. Conversely, LT_{50} was lower for thymol by both exposure pathways (2.89 and 16.0 h). The LT_{50} of the essential oil was 110 h when applied by contact and 29.3 h when applied by fumigation. The test used doses and concentrations required to kill 50% of the termites.

Besides the lethal effect, essential oils and compounds can also change insects' behavior. This phenomenon is called sub-lethal effect. The knowledge about the sub-lethal effects caused by these substances is essential for the establishment of different control strategies. Social insects, such as ants and termites, have a very complex communication system, which, under normal conditions, are fundamental to maintaining colony safety and functioning. The application of products that change individuals' behavior, such as repellent substances, can lead to aggressive behavior and a high level of non-recognition, contributing to the loss of colony cohesion (Bacci et al. 2015).

The essential oil of *L. sidoides* can also change termite behavior by reducing allogrooming. Conversely, pseudergates of *C. brevis* treated with the major compounds of this plant—thymol, *p*-cimene, and *(E)*-caryophyllene—performed more butting. Changes in collective behavior have also been reported. Untreated *C. brevis* pseudergates reduced the contact with treated termites by decreasing the grooming and antennation. Regarding the individual behavior, termites treated with the essential oils of *L. sidoides* and thymol decreased the displacement and walking speed when compared with untreated individuals (control). In contrast, individuals treated with (*E*)-caryophyllene increased displacement and walking speed. In collective trials, both the essential oil and the compounds increased displacement and walking speed. Individual and collective observations were performed with half-treated half-untreated arenas. In the individual bioassays, pseudergates remained longer in the untreated side of the arena, considering all treatments. However, in collective bioassays, *C. brevis* pseudergates remained longer in the (*E*)-caryophyllene-treated side than in the untreated side, confirming the attractive effect of this compound. The time spent in the sides treated with thymol and essential oil was short. On the other hand, *C. brevis* pseudergates showed no preference when given a choice between the ρ-cymene-treated side and untreated side.

The essential oils of plants from Northeast Brazil have great potential for use in the development of bioinsecticides to control *N. corniger* and *C. brevis* due to the lethal and sub-lethal effects they cause to these termite-pests.

8.3.3 Activity on Stored Grain Pests

The world population's diet is primarily based on the consumption of seeds and grains. Faced with the high demand, billions of tons of cereals, oilseeds, and legumes are produced worldwide. Nevertheless, significant losses have been recorded, owing to insects' attacks to stored grains. These insect-pests feed on the grains, resulting in grain weight loss, low germination, and increased temperature and moisture in

the grain mass. Also, they can contaminate the grains by feces residues and body fragments and obstruct agricultural machinery (Negrisoli et al. 2013).

These insect-pests are mainly controlled by the use synthetic chemicals applied by contact, fumigation, or spraying with organophosphates, pyrethroids, and growth regulators. Despite their effectiveness, these products have led to the selection of resistant populations after their continuous use, requiring higher doses to achieve the same efficiency. Thus, in addition to the damage caused to the environment and the applicators' health, residues of these products have been found in foods at levels beyond the limits acceptable for consumption (Li et al. 2013; Wei et al. 2014).

Sitophilus zeamais (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae) are two of the main stored grain pests. *S. zeamais*, known as maize weevil, can damage grains both in the field and after storage. This insect-pest decreases grain weight by over 15%, and losses may reach 60% in 6 months (Sousa and Conte 2013). *T. castaneum*, known as red flour beetle, is a cosmopolitan species that usually appears after the occurrence of primary pests. They can infest peanuts, coffee, cocoa, soybeans, dried fruits, nuts, spices, cottonseed, and all types of ground cereals, such as bran, animal feed, flour, and meal (Gallo et al. 2002), causing substantial losses.

The essential oils of *L. alba* (chemotypes citral and carvone) maintained at the Active Germplasm Bank of the Federal University of Sergipe and the major compounds (carvone and citral) are toxic to the insect-pests *S. zeamais* and *T. castaneum* (Peixoto et al. 2015a). For these two species, the essential oils of carvone chemotype were more toxic than those of citral chemotype. The mean doses of citral and carvone required to kill 90% of *S. zeamais* were 29.65 and 141.5 $\mu L.L^{-1}$, respectively. *T. castaneum* showed to be more tolerant, especially to the essential oils of the citral chemotype, whose mean LC_{90} value was 299.0 $\mu L.L^{-1}$. For this species, the essential oils of carvone chemotypes had a mean LC_{90} of 33.65 $\mu L.L^{-1}$. Similarly, both for *S. zeamais* and *T. castaneum*, the major compound carvone was more toxic (LC_{90} of 24.0 and 22.8 $\mu L.L^{-1}$) than the compound citral (LC_{90} 38.0 and 40.5 $\mu L.L^{-1}$). Besides being toxic, the essential oil of the carvone chemotype has a repellent effect on these two species. Conversely, the major compound carvone has a repellency effect only on *T. castaneum*.

Studies have reported the resistance of *S. zeamais* populations to synthetic insecticides. Depending on the insect origin, it might be more or less resistant to these products. Likewise, different levels of sensitivity to essential oils may occur within the species. This fact has been recently confirmed in a study developed by the Federal University of Sergipe, using the essential oil of *L. sidoides* (Oliveira et al. 2017) and *S. zeamais* populations from different states of Brazil. The toxicity of the essential oil of *L. sidoides* and its major compound thymol varied according to the *S. zeamais* population. Lethal doses (LD_{90}) ranged from 26.88 to 79.16 $\mu g.mg^{-1}$ for the essential oil and from 41.12 to 172.78 $\mu g.mg^{-1}$ for thymol. The time required to kill 50% of the insects (LT_{50}) ranged from 6.8 to 32.4 h and from 26.3 to 46.4 h for the essential oil and thymol, respectively. These results evidenced the different sensitivity levels of *S. zeamais* populations to these metabolites.

Essential oils have lipophilic characteristics (low water solubility) and are formed by volatile substances (low molecular weight). Therefore, some obstacles to the direct application of these plant-based products still have to be overcome. Some formulations or "prototypes" have been proposed. However, these formulations must ensure that the essential oils maintain the toxicity level against the target organism, even in the presence of other products. Nanoformulations obtained from the essential oil of *L. sidoides* presented to be toxic to *S. zeamais* populations. The LD_{90} ranged from 81.72 to 114.23 $\mu g.mg^{-1}$ and from 40.55 to 61.04 $\mu g.mg^{-1}$ for the nanoformulations of essential oil and thymol, respectively (Oliveira et al. 2017). The LD_{90} values for the nanoformulation of the essential oil were higher than those previously described. Nonetheless, the nanoformulation has only 18% of essential oil in its composition. The LD_{90} values of the nanoformulation of thymol were lower than that of pure thymol. This fact is explained by the nanoparticles of essential oil and thymol, which are formed during the obtainment of the nanoformulations, increasing the contact surface of these substances and consequently potentiating their toxicity to insects.

The essential oils of *L. alba*, *L. gracilis*, and *L. sidoides*—which occur in Northeast Brazil and are maintained at the Active Germplasm Bank of Medicinal and Aromatic Plants of the Federal University of Sergipe—are promising sources for the development of bioproducts to be used in the management of stored grain insect-pests.

8.4 Anti-tick Activity

Ticks are hematophagous ectoparasites of vertebrates and present great relevance for animal health and production and public health for they are the vectors of several diseases, such as babesiosis, ehrlichiosis, spotted fever, and Lyme disease (Massard and Fonseca 2004; Araújo et al. 2016). Tick control is performed by applying synthetic products inappropriately and indiscriminately, causing severe environmental contamination and leading to resistant populations (Fig. 8.2) (Tabari et al. 2017). Acaricidal formulations produced with essential oils are an alternative for tick control (Apel et al. 2009; Borges et al. 2011; Lebouvier et al. 2013) for being natural products that rapidly degrade in the environment and for presenting low risk of meat and milk contamination by residues (Hernandez et al. 1987; Gupta et al. 2000; Borges et al. 2003; Hu and Coats 2008).

The essential oils of *L. sidoides* have demonstrated activity on several tick species, such as *Dermacentor nitens* (Gomes et al. 2012), *Rhipicephalus sanguineus* (Gomes et al. 2014), *Rhipicephalus microplus* (Gomes et al. 2012; Chagas et al. 2016), and *Amblyomma cajennense* (Gomes et al. 2014). The synergistic power of the association between the essential oil of *L. sidoides* and entomopathogenic nematodes to control the tick species *R. microplus* (Monteiro et al. 2014) has also been reported (Monteiro et al. 2014). Both *L. sidoides* chemotypes, thymol and carvacrol, have shown high activity against engorged females and larvae of *R.*

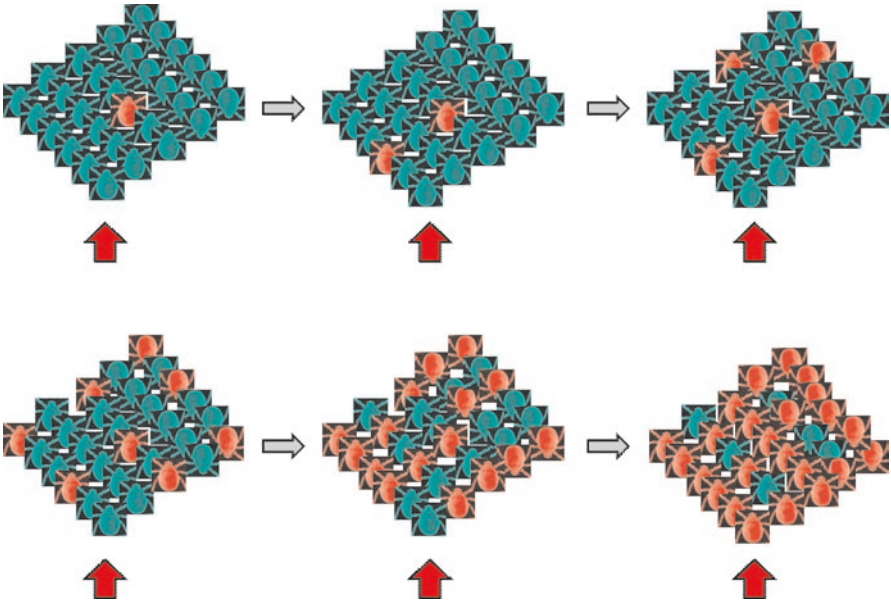

Fig. 8.2 Selection of resistant ticks (red ticks) population after sequential acaricide use (red arrow)

microplus but with some variations in the activity levels, based on the genotype used (Monteiro et al. 2014; Soares et al. 2016). Isolated carvacrol has a higher activity on *R. microplus* larvae resistant to pyrethroid and amidinic synthetic compounds than its isomer thymol (Cruz et al. 2013). Even with the higher activity of isolated carvacrol when compared with isolated thymol, the genotypes with higher carvacrol contents are not more active than those with higher thymol content. Therefore, the major compounds of the different genotypes are not correlated with acaricide activity (Soares et al. 2016).

The essential oil of *L. gracilis* has also been reported to present activity on *R. microplus* (Cruz et al. 2013; Chagas et al. 2016). *R. microplus*-resistant strains to organophosphates are more susceptible to the essential oils of *L. gracilis* when compared with ticks sensitive to synthetic compounds. This fact is independent of the *L. gracilis* genotype and its major compound thymol or carvacrol (Fig. 8.3) (Costa-Junior et al. 2016). The isolated monoterpene thymol shows no difference in the acaricidal activity, according to the susceptibility of the *R. microplus* population to the synthetic compounds. However, when exposed to isolated carvacrol, organophosphate-resistant ticks are 3.2 times more susceptible than organophosphate-sensitive ticks (Costa-Junior et al. 2016).

One of the greatest challenges to the use of essential oils or monoterpenes to control parasites is their high volatility of these substances, resulting in the low residual power of the antiparasitic activity. Pharmaceutical formulations reduce the volatility of essential oils and terpenes (Pham-Hoang et al. 2013), allowing the

Genotypes	Ticks train	LC_{50} (mg mL^{-1})*	95% CI	R^2
L. gracillis 106		1.02[b]	0.98 – 1.06	0.99
		0.84[a]	0.81 – 0.87	0.99
L. gracillis 201		1.03[b]	0.96 – 1.11	0.96
		0.65[a]	0.47 – 0.88	0.99
Thymol		1.94[c]	1.75 – 2.16	0.98
		1.70[c]	1.45 – 1.94	0.99
Carvacrol		2.56[d]	2.40 – 2.74	0.99
		0.80[a]	0.74 – 0.88	0.99

LC_{50}: concentration (mg mL^{-1}) at which 50 % of the *R. microplus* larvae died; CL95 %:confidence limits at 95 % probability; R^2: coefficient of determination.
* LC_{50} values with the same letter between susceptible and organophosphate (OP)-resistant tick strains across treatments (*L. gracillis* 106 and 201, and thymol and carvacrol) are equivalent.

Fig. 8.3 Efficacy of essential oils of *Lippia gracilis* genotypes 106 and 201 and their major compounds, thymol and carvacrol, on susceptible (green ticks) and organophosphate-resistant larvae (red ticks) of *Rhipicephalus microplus*. (Adapted from Costa-Junior et al. 2016)

residual effect of these substances. Yeast cell wall, a by-product of the alcohol industry, has been used to reduce the volatility of essential oils and monoterpenes (Martins 2009). Works have demonstrated the efficiency of the encapsulation of hydrophobic compounds, such as essential oils and monoterpenes (Pannell 1990; Normand et al. 2005; Shi et al. 2008; Paramera et al. 2011). Carvacrol, the major monoterpene of the essential oils of several *L. gracilis* and *L. sidoides* genotypes, showed higher acaricidal activity on *R. microplus* when encapsulated in yeast cell wall. This formulation had acaricidal activity up to 50 h after application, whereas the effect of unencapsulated carvacrol lasted only up to 5 h (Fig. 8.4) (Lima et al. 2017).

As previously described, the essential oils of *Lippia alba* are formed by several chemotypes, including carvone and citral. The chemotype citral of the essential oils of *L. alba* has higher activity on *R. microplus* larvae. However, they have no activity on engorged females (Peixoto et al. 2015b). The chemotype carvone of the essential oil of *L. alba* shows no acaricidal activity. The isolated monoterpenes citral and carvone have acaricidal activity on the larvae, with no difference between them; however, only citral has efficient acaricidal activity on engorged females of *R. microplus* (Peixoto et al. 2015b).

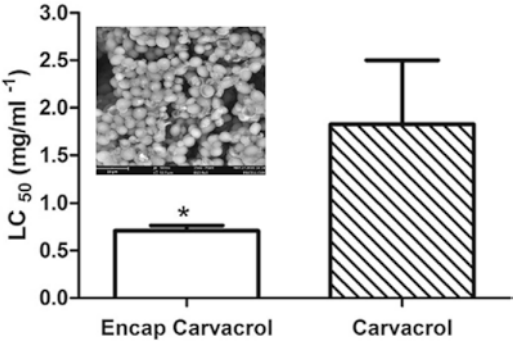

Fig. 8.4 Lethal concentration (LC_{50}) and the scanning electron image of carvacrol encapsulated with yeast cell walls (encap carvacrol) and unencapsulated carvacrol against *Rhipicephalus microplus* larvae. *Encapsulated carvacrol was significantly different from unencapsulated carvacrol at $p < 0.05$. (Adapted from Lima et al. 2017)

Even the chemotypes that have no acaricidal activity may be useful for tick control, owing to their repellency power (Jaenson et al. 2005). Essential oils with repellent power may be used to increase the residual period of natural or synthetic compounds that have only the knock-down effect (Novelino et al. 2007). The carvone chemotype of the essential oils of *L. alba*, which presents no activity on ticks, demonstrated repellency power over *R. microplus* larvae. Nevertheless, the repellency power varies among essential oils, and slight differences in the chemical composition interfere with the repellency activity (Lima et al. 2017).

These results evidence the importance of standardizing the entire essential oil production chain, from cultivation to post-harvest techniques. Further studies on the biotechnological bias must be developed by testing formulations for the most diverse activity to control ticks, aiming at producing a commercial formulation for tick control.

8.5 Conclusions

Contrary to most people's beliefs, Northeast Brazil is rich in plant biodiversity. The vegetation of the Caatinga biome is not as lush as that of other Brazilian biomes. However, a closer look reveals the biological relevance and peculiar beauty of the species that occur in the northeastern region. Faced with the adverse conditions imposed by the semi-arid climate, plants had to develop evolutionary strategies to overcome these unfavorable circumstances. Perhaps these challenges have made these species even more fascinating. The medicinal and aromatic species exposed in this chapter represent a small part of the plant richness found in this region. These plant resources deserve attention for their relevance and potential of use, especially of their essential oils. Owing to their biological activities, essential oils of plant

species of Northeast Brazil appear as alternative substances to be used against insect-pests and parasites that damage agriculture and livestock. Although promising results have been increasingly reported, only a few bioinsecticides made of essential oils are commercially available. In the near future, with more government support and partnerships between universities and research institutions and public and private corporations, more essential oil-based products should be developed in Brazil, which will be used in a sustainable agricultural system, both at an environmental and an economic point of view.

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References

Albuquerque AC, Matias GRRS, Couto AAVO, Oliveira MAP, Vasconcelos A (2012) Urban termites of Recife, Northeast Brazil (Isoptera). Sociobiology 59:183–188

Almeida-Pereira CS (2017) Diversidade química, genética e atividade antimicrobiana do óleo essencial de *Croton tetradenius* Baill. Thesis, Universidade Federal de Sergipe

Alves MF, Nizio DAC, Sampaio TS, Nascimento-Junior AF, Brito FA, Melo JO, Arrigoni-Blank MF, Gagliardi PR, Machado SMF, Blank AF (2016) *Myrcia lundiana* Kiaersk native populations have different essential oil composition and antifungal activity against *Lasiodiplodia theobromae*. Ind Crop Prod 85:266–273

Anjos CS (2014) Contribuicão química para os voláteis de acessos e extratos por fluido supercrítico de *Cordia verbenaceae* DC. Dissertation, Universidade Federal de Sergipe

Apel MA, Ribeiro VLS, Bordignon SAL, Henriques AT, Poser GV (2009) Chemical composition and toxicity of the essential oils from *Cunila* species (Lamiaceae) on the cattle tick *Rhipicephalus (Boophilus) microplus*. Parasitol Res 105:863–868

Araújo LX, Novato TPL, Zeringota V, Maturano R, Melo D, Silva BC, Daemon E, Carvalho MG, Monteiro CMO (2016) Synergism of thymol, carvacrol and eugenol in larvae of the cattle tick, *Rhipicephalus microplus*, and brown dog tick, *Rhipicephalus sanguineus*. Med Vet Entomol 30:377–382

Bacci L, Lima JKA, Araújo APA, Blank AF, Silva IMA, Santos AA, Santos ACC, Alves PB, Picanço MC (2015) Toxicity, behavior impairment, and repellence of essential oils from pepper-rosmarin and patchouli to termites. Entomol Exp Appl 156:66–76

Blank AF, Camêlo LCA, Arrigoni-Blank MF, Pinheiro JB, Andrade TM, Niculau ES, Alves PB (2015) Chemical diversity in *Lippia alba* (Mill.) N. E. Brown germplasm. Sci World J 2015:1–11

Borges LMF, Ferri PH, Silva WJ, Silva WC, Silva JC (2003) In vitro efficacy of extracts of *Melia azedarach* against the tick *Boophilus microplus*. Med Vet Entomol 172:28–31

Borges LMF, Sousa LAD, Barbosa CS (2011) Perspectives for the use of plant extracts to control the cattle tick *Rhipicephalus (Boophilus) microplus*. Rev Bras Parasitol Vet 20:89–96

Brandão DS, Mendes ADR, Santos RR, Rocha SMG, Leite GLD, Martins ER (2015) Biologia floral e sistema reprodutivo da erva-baleeira (*Varronia curassavica* Jacq.). Rev Bras Plantas Med 17:562–569

Buratto DA, Cardoso JT, Rolim FA, Filho WR (2012) Avaliação dos danos causados por formigas cortadeiras do gênero *Acromyrmex* (Hymenoptera) aos plantios de *Pinus taeda* no planalto Sul-Catarinense. Floresta 2:683–690

Chagas AC, Oliveira MC, Giglioti R, Santana RC, Bizzo HR, Gama PE, Chaves FC (2016) Efficacy of 11 Brazilian essential oils on lethality of the cattle tick *Rhipicephalus (Boophilus) microplus*. Ticks Tick Borne Dis 7:427–432

Costa-Júnior LM, Miller RJ, Alves PB, Blank AF, Li AY, Pérez de León AA (2016) Acaricidal efficacies of *Lippia gracilis* essential oil and its phytochemicals against organophosphate-resistant and susceptible strains of *Rhipicephalus (Boophilus) microplus*. Vet Parasitol 228:60–64

Cruz EMO, Costa-Junior LM, Pinto JAO, Santos DA, Araujo SA, Arrigoni-Blank MF, Bacci L, Alves PB, Cavalcanti SCH, Blank AF (2013) Acaricidal activity of *Lippia gracilis* essential oil and its major constituents on the tick *Rhipicephalus (Boophilus) microplus*. Vet Parasitol 195:198–202

Cruz EMO, Pinto JAO, Fontes SS, Arrigoni-Blank MF, Bacci L, Jesus HCR, Santos DA, Alves PB, Blank AF (2014) Water deficit and seasonality study on essential oil constituents of *Lippia gracilis* Schauer germplasm. Sci World J 2014:1–9

Degenhardt J, Köllner TG, Gershenzon J (2009) Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants. Phytochemistry 70:1621–1637

Evans TA, Iqbal N (2015) Termite (order Blattodea, infraorder Isoptera) baiting 20 years after commercial release. Pest Manag Sci 7:897–906

Feitosa-Alcantara RB (2017) Conservação *in vitro*, diversidade genética, química e atividade formicida de *Hyptis pectinata* L. Poit. Thesis, Universidade Federal de Sergipe

Feitosa-Alcantara RB, Bacci L, Blank AF, Alves PB, Silva IMDA, Soares CA, Sampaio TS, Nogueira PCL, Arrigoni-Blank MF (2017) Essential oils of *Hyptis pectinata* chemotypes: isolation, binary mixtures and acute toxicity on leaf-cutting ants. Molecules 22:1–13

FSC-Forest Stewardship Council. Available online: https://br.fsc.org/pt-br. Accessed 9 Dec 2016.

Galitzki EL, Cereto CE, Scherer KZ (2013) Formigas visitantes de inflorescências de *Actinocephalus polyanthus* (Bong.) Sano (Eriocaulaceae). Biotemas 26:75–83

Gallo D, Nakano O, Silveira-Neto S, Carvalho RPL, Batista GC, Berti-Filho E, Parra JRP, Zucchi RA, Alves SB, Vendramim JD, Marchini LC, Lopes JRS, Omoto C (2002) Entomologia agrícola. FEALQ, Piracicaba

Gomes GA, Monteiro CMO, Senra TO, Zeringota V, Calmon F, Matos RS, Daemon E, Gois RW, Santiago GM, De Carvalho MG (2012) Chemical composition and acaricidal activity of essential oil from *Lippia sidoides* on larvae of *Dermacentor nitens* (Acari: Ixodidae) and larvae and engorged females of *Rhipicephalus microplus* (Acari: Ixodidae). Parasitol Res 111:2423–2430

Gomes GA, Monteiro CMO, Julião LS, Maturano R, Senra TO, Zeringota V, Calmon F, Matos RS, Daemon E, De Carvalho MG (2014) Acaricidal activity of essential oil from *Lippia sidoides* on unengorged larvae and nymphs of *Rhipicephalus sanguineus* (Acari: Ixodidae) and *Amblyomma cajennense* (Acari: Ixodidae). Exp Parasitol 137:41–45

Gonzalez MS, Lima BG, Oliveira AFR, Nunes DD, Fernandes CP, Santos MG, Tietbohl LAC, Mello CB, Rocha L, Feder D (2014) Effects of essential oil from leaves of *Eugenia sulcataon* the development of agricultural pest insects. Rev Bras Farmacogn 24:413–418

Gupta A, Agarwal AK, Shukla GS (2000) Effect of quinalphos and cypermethrin exposure on developing blood-brain barrier: role of nitric oxide. Environ Toxicol Pharmacol 8:73–78

Harborne JB (1988) Introduction to ecology biochemistry. Academic, London

Hernandez LE, Parra DG, Marin AC (1987) Accion repelente y acaricida del *Melinis minutiflora* sobre el *Boophilus microplus*. Rev Colomb Cienc Quim Farm 16:17–21

Holland RD, Wilkes JG, Cooper WM, Alusta P, Williams A, Pearce B, Beaudoin M, Buzatu D (2014) Thymol treatment of bacteria prior to matrix-assisted laser desorption/ionization time-of-flight mass spectrometric analysis aids in identifying certain bacteria at the subspecies level. Rapid Commun Mass Spectrom 28:2617–2626

Hu D, Coats J (2008) Evaluation of the environmental fate of thymol and phenethyl propionate in the laboratory. Pest Manag Sci 64:775–779

Jaenson TGT, Palsson K, Borg-Karlson AK (2005) Evaluation of extracts and oils of tick-repellent plants from Sweden. Med Vet Entomol 19:345–352

Jesus AS, Blank AF, Alves MF, Arrigoni-Blank MF, Lima RN, Alves PB (2016) Influence of storage time and temperature on the chemical composition of the essential oil of *Hyptis pectinata* L. Poit. Rev Bras Plantas Med 18:336–340

Jung PH, Silveira AC, Nieri EM, Potrich M, Silva ERL, Refatti M (2013) Atividade Inseticida de *Eugenia uniflora* L. e *Melia azedarach* L. sobre *Atta laevigata* Smith. Floresta Ambient 20:191–196

Leal IR, Leal LC, Andersen AN (2015) The benefits of myrmecochory: a matter of stature. Biotropica 47:281–285

Lebouvier N, Hue T, Hnawia E, Lesaffre L, Menut C, Nour M (2013) Acaricidal activity of essential oils from five endemic conifers of New Caledonia on the cattle tick *Rhipicephalus (Boophilus) microplus*. Parasitol Res 112:1379–1384

Li SG, Li MY, Huang YZ, Hua RM, Lin HF, He YJ, Wei LL, Liu ZQ (2013) Fumigant activity of *Illicium verum* fruit extracts and their effects on the acetylcholinesterase and glutathione S -transferase activities in adult *Sitophilus zeamais*. J Pest Sci 86:677–683

Lima JKA, Albuquerque ELD, Santos ACC, Oliveira AP, Araújo APA, Blank AF, Arrigoni-Blank MF, Alves PB, Santos DA, Bacci L (2013) Biotoxicity of some plant essential oils against the termite *Nasutitermes corniger* (Isoptera: Termitidae). Ind Crop Prod 47:246–251

Lima AS, Maciel AP, Mendonça CJS, Costa-Junior LM (2017) Use of encapsulated carvacrol with yeast cell walls to control resistant strains of *Rhipicephalus microplus* (Acari: Ixodidae). Ind Crop Prod 108:190–194

Martins MS (2009) Leveduras de cerveja e Cana-de-Açúcar (*Saccharomyces cerevisiae*), autolisada e íntegra, na dieta de cães. Dissertation, Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias

Massard CL, Fonseca AH (2004) Carrapatos e doenças transmitidas, comuns ao homem e aos animais. A Hora Veterinária 135:15–23

Menezes IAC, Marque MS, Santos TC, Dias KS, Silva ABL, Mello ICM, Lisboa ACCD, Alves PB, Cavalcanti SCH, Marçal RM, Antoniolli AR (2007) Antinociceptive effect and acute toxicity of the essential oil of *Hyptis fruticosa* in mice. Fitoterapia 78:192–195

Monteiro CM, Araújo LX, Gomes GA, Senra TO, Calmon F, Daemon E, de Carvalho MG, Bittencourt VR, Furlong J, Prata MC (2014) Entomopathogenic nematodes associated with essential oil of *Lippia sidoides* for control of *Rhipicephalus microplus* (Acari: Ixodidae). Parasitol Res 113:189–195

Negrisoli CRCB, Negrisoli AS Jr, Bernardi D, Garcia MS (2013) Activity of eight strains of entomopathogenic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae) against five stored product pests. Exp Parasitol 134:384–388

Nizio DAC, Brito FA, Sampaio TS, Melo JO, Silva FLS, Gagliardi PR, Arrigoni-Blank MF, Anjos CS, Alves PB, Wisniewski-Junior A, Blank AF (2015) Chemical diversity of native populations of *Varronia curassavica* Jacq. and antifungal activity against *Lasiodiplodia theobromae*. Ind Crop Prod 76:437–448

Nizio DAC, Fujimoto RY, Maria AN, Carneiro PCF, França CCS, Sousa NC, Brito FA, Sampaio TS, Arrigoni-Blank MF, Blank AF (2017) Essential oils of *Varronia curassavica* accessions have different activity against white spot disease in freshwater fish. Parasitol Res 117:97–105

Nizio DAC, Blank AF, Sampaio TS, Brito FA, Matos TM, Arrigoni-Blank MF, Maria AN (2018) Distillation methods affect the chemical composition of *Varronia curassavica* Jacq. essential oil? Biosci J 34:629–639

Normand V, Dardelle G, Bouquerand PE, Nicolas L, Johnston DJ (2005) Flavor encapsulation in yeasts: limonene used as a model system for characterization of the release mechanism. J Agric Food Chem 53:7532–7543

Nostro A, Papalia T (2012) Antimicrobial activity of carvacrol: current progress and future prospectives. Recent Pat Antiinfect Drug Discov 7:28–35

Novelino MAS, Daemon E, Soares GLG (2007) Avaliação da atividade repelente do timol, mentol, salicilato de metila e ácido salicilico sobre larvas de *Boophilus microplus* (Canestrini, 1887) (Acari: Ixodidae). Arq Bras Med Vet Zootec 59:700–704

Oliveira AP, Santana AS, Santana EDR, Lima APS, Faro RRN, Nunesb RS, Lima AD, Blank AF, Araújo APA, Paulo CF, Bacci L (2017) Nanoformulation prototype of the essential oil of *Lippia sidoides* and thymol to population management of *Sitophilus zeamais* (Coleoptera: Curculionidae). Ind Crop Prod 107:198–205

Pannell NA (1990) Microencapsulation in microorganisms. European Patent EP 0242 135 B1

Paramera EI, Konteles SJ, Karathanos VT (2011) Microencapsulation of curcumin in cells of *Saccharomyces cerevisiae*. Food Chem 125:892–902

Passos GF, Fernandes ES, Da Cunha FM, Ferreira J, Pianowski LF, Campos MM, Calixto JB (2007) Anti-inflammatory and anti-allergic properties of theessential oil and active compounds from *Cordia verbenacea*. J Ethnopharmacol 110:323–333

Peixoto MG, Bacci L, Blank AF, Araújo APA, Alves PB, Silva JHS, Santos AA, Oliveira AP, Costa AS, Arrigoni-Blank MF (2015a) Toxicity and repellency of essential oils of *Lippia alba* chemotypes and their major monoterpenes against stored grain insects. Ind Crop Prod 71:1–36

Peixoto MG, Costa-Júnior LM, Blank AF, Lima ADS, Menezes TS, Santos DA, Alves PB, Cavalcanti SC, Bacci L, Arrigoni-Blank MF (2015b) Acaricidal activity of essential oils from *Lippia alba* genotypes and its major components carvone, limonene, and citral against *Rhipicephalus microplus*. Vet Parasitol 210:118–122

Pham-Hoang BN, Romero-Guido C, Phan-Thi H, Waché Y (2013) Encapsulation in a natural, preformed, multi-component and complex capsule: yeast cells. Appl Microbiol Biotechnol 97:6635–6645

Quispe-Condori S, Foglio MA, Rosa PTV, Meireles MAA (2008) Obtaining β-caryophyllene from *Cordia verbenacea* de Candolle by supercritical fluid extraction. J Supercrit Fluids 46:27–32

Raymundo LJRP, Guilhon CC, Alviano DS, Matheus ME, Antoniolli AR, Cavalcanti SCH, Alves PB, Alviano CS, Fernandes PD (2011) Characterization of the anti-inflammatory and antinociceptive activities of the *Hyptis pectinata* (L.) Poit essential oil. J Ethnopharmacol 134:725–732

Sampaio TS, Nizio DAC, White LAS, Melo JO, Almeida CS, Alves MF, Gagliardi PR, Arrigoni-Blank MF, Wisniewski-Junior A, Sobral MEG, Blank AF (2016) Chemical diversity of a wild population of *Myrcia ovata* Cambessedes and antifungal activity against *Fusarium solani*. Ind Crop Prod 86:196–209

Santos CP, Oliveira TC, Pinto JAO, Fontes SS, Cruz EMO, Arrigoni-Blank MF, Andrade TM, Matos IL, Alves PB, Innecco R, Blank AF (2015) Chemical diversity and influence of plant age on the essential oil from *Lippia sidoides* Cham. germplasm. Ind Crop Prod 76:416–421

Santos CP, Pinto JAO, Santos CA, Cruz EMO, Arrigoni-Blank MF, Andrade TM, Santos DA, Alves PB, Blank AF (2016) Harvest time and geographical origin affect the essential oil of *Lippia gracilis* Schauer. Ind Crop Prod 79:205–210

Santos AA, Oliveira BMS, Melo CR, Lima APS, Santana EDR, Blank AF, Picanço MC, Araújo APA, Cristaldo PF, Bacci L (2017) Sub-lethal effects of essential oil of *Lippia sidoides* on drywood termite *Cryptotermes brevis* (Blattodea: Termitoidea). Ecotoxicol Environ Saf 145:436–441

Shi G, Rao L, Yu H, Xiang H, Yang H, Ji R (2008) Stabilization of photosensitive resveratrol within yeast cell. Int J Pharm 349:83–93

Silva JS (2009) O gênero *Croton* L. (Euphorbiaceae) em Pernambuco, com ênfase nas espécies da microrregião do Vale do Ipanema. Dissertation, Universidade Federal Rural de Pernambuco

Silva DC (2017) Diversidade química, genética e estudo do potencial formicida do óleo essencial de *Eplingiella fruticosa* (Salzm. ex Benth.) Harley & J.F.B. Pastore. Thesis, Universidade Federal de Sergipe

Silva WJ, Dória GAA, Maia RT, Nunes RS, Carvalho GA, Blank AF, Alves PB, Marçal RM, Cavalcanti SCH (2008) Effects of essential oils on *Aedes aegypti* larvae: alternatives to environmentally safe insecticides. Bioresour Technol 99:3251–3255

Silva DC, Blank AF, Nizio DAC, Sampaio TS, Nogueira PCL, Arrigoni-Blank MF (2018) Chemical diversity of essential oils from native populations of *Eplingiella fruticosa*. Crop Breed Appl Biotechnol 18:205–214

Soares AM, Penha TA, Araújo SA, Cruz EMO, Blank AF, Costa-Junior LM (2016) Assessment of different *Lippia sidoides* genotypes regarding their acaricidal activity against *Rhipicephalus (Boophilus) microplus*. Rev Bras Parasitol Vet 25:401–406

Sobral M, Grippa CR, Souza MC, Aguiar OT, Bertoncello R, Guimarães TB (2012) Fourteen new species and two taxonomic notes on Brazilian Myrtaceae. Phytotaxa 50:19–50

Sousa G, Conte H (2013) Midgut morphophysiology in *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae). Micron 51:1–8

Tabari MA, Youssefi MR, Maggi F, Benelli G (2017) Toxic and repellent activity of selected monoterpenoids (thymol, carvacrol and linalool) against the castor bean tick, *Ixodes ricinus* (Acari: Ixodidae). Vet Parasitol 245:86–91

Verma M, Sharma S, Prasad R (2009) Biological alternatives for termite control: a review. Int Biodeterior Biodegradation 63:959–972

Wei L, Hua R, Li M, Huang Y, Li S, He Y, Shen Z (2014) Chemical Composition and biological activity of star anise Illicium verum extracts against maize weevil, Sitophilus zeamais adults. J Insect Sci 14:1–13

Part III
Extraction and Bioanalytical Techniques

Chapter 9
Adulteration Analysis in Essential Oils

Prabodh Satyal and William N. Setzer

9.1 Essential Oils and Their Uses

According to Harrewijn et al. (2000), essential oils are complex mixtures of volatile compounds that are produced by living organisms and isolated by physical means, such as pressing and distillation from a whole plant or plant portion of known taxonomic origin. Controversy surrounds the definition of essential oils from the point of view of distillation (Sadgrove and Jones 2015). Therefore, the ISO (International Organization for Standardization) has defined a universally accepted definition for essential oil as "Products obtained from natural raw material, either by distillation with water and steam, or from the epicarp of citrus fruits by mechanical processing, or by dry distillation" (Schnaubelt 1999; International Organization for Standardization 2013).

Essential oils are stored in specialized cells/glandular cells or organelles within any plant tissue. The first recorded method of extracting essential oils was written by Andalusian physician and chemist Ibn al-Baitar in the thirteenth century (Firenzuoli et al. 2014). Modern techniques rely on pressing, rubbing, or heating particular regions to rupture the cells and release the aromatic compounds. Therefore, essential oils are mostly obtained by cold pressing, steam distillation, and hydrodistillation.

The essential oil components are mainly produced through four major biosynthetic pathways: (1) the mevalonate pathway leading to sesquiterpenes and triterpenes, (2) the methyl-erythritol pathway leading to mono- and diterpenes, (3) the shikimic acid pathway yielding phenylpropanoids, and (4) the acetate pathway for fatty acid-derived compounds. With only these pathways, there are still large num-

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bers of compounds with tremendous variation within the composition of essential oils (Kubeczka 2010).

Essential oils (EOs) have a rich history and are of great importance in traditional medicine all around the world. The World Health Organization (WHO) defined traditional medicine as "the sum total of the knowledge, skills, and practices based on the theories, beliefs and experiences indigenous to different cultures whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness" (World Health Organization). On the basis of this definition, one can appreciate the vast importance of traditional medical systems globally. In the Indian subcontinent, essential oils have a profound impact on the Ayurvedic "Gandhshastra"—the science that deals with flavor and fragrance (NIIR Board 2004). According to the Egyptian Ebers Papyrus manuscript from around 1550 BC, ancient Egyptians were the first to identify the therapeutic potential of botanical extracts (Brumer 2014). They also used these extracts for flavor and fragrance during religious ceremonies in temples and pyramids. In the Chinese herbal tradition, dating back 4000 years, aromatic materials were used as therapeutic agents (Tang et al. 2009). Aromatic botanical materials have also repeatedly been mentioned in many Judeo-Christian and Muslim religious texts (Singh and Hamal 2013).

9.2 Adulteration of Essential Oils

According to the *Merriam-Webster Dictionary*, to adulterate something means "to corrupt, debase, or make impure by the addition of an inferior substance or element, especially to prepare for sale by replacing more valuable with less valuable or inert ingredients" (Merriam-Webster.com). Approximately 17,500 aromatic plants have been investigated (Lawrence 2002); however, only 300 commercial essential oils are in use, with an estimated bulk value of $1 billion in 2013 (Tisserand and Young 2014). Overall, 50% of raw materials for commercial essential oils originate from wild sources, while 50% are from cultivated sources (Lawrence 2002). Typically, the adulteration of essential oils occurs through the addition of synthetic and natural compounds, those related and unrelated to the oil's composition, in order to increase profits or meet some established ISO requirement. So adulteration is also defined as economically motivated adulteration. There are about 60,000 articles on *PubMed* for food adulteration (Everstine et al. 2013). Adulteration of herbal medicines with synthetic drugs has been reported in detail (Ernst 2002), and studies carried out by Huang et al. in 1997, which presented the trends of Chinese medicine adulteration, found that 24% of 2609 samples were adulterated by at least one type of adulterant (Huang et al. 1997).

9.3 Commonly Used Instruments in Adulteration Detection

Several analytical instruments can detect adulteration in essential oils (Lawrence 2002; Do et al. 2015). The most commonly used analytical tools are standard gas chromatography with flame ionization detection (GC-FID), gas chromatography–mass spectrometry (GC-MS) (Marriott et al. 2001), gas chromatography–isotope-ratio mass spectrometry (GC-IRMS) (Schipilliti et al. 2012), site-specific natural isotope fractionation NMR (SNIF-NMR) (Remaud et al. 1997), enantioselective chiral GC-MS (Tranchida et al. 2012), and components' ratio quantitation (Shu and Lawrence 1997). GC-IRMS is often used to detect the authenticity of the essential oil's origin via the isotopic ratio measurement; however this technique has several limitations. The first limitation is that the ^{14}C activity of an essential oil can be manipulated with the addition of ^{14}C-labeled compounds. Second, natural precursor-based synthetic compounds are not detected by GC-IRMS. Thus, GC-IRMS is not a completely reliable source of essential oil authentication (Culp and Noakes 1990). Enantioselective or chiral GC-MS also has specific limitations, as it is only applicable to chiral molecules and the enantiomeric ratio of chiral compounds varies from origin to origin. SNIF-NMR is only useful for small molecules (such as monoterpenes) to authenticate their origin via the deuterium ratio, but essential oils are also composed of sesquiterpenes and diterpenes. Likewise, this method requires pure isolated compounds (Lawrence 2002). Minor components and their quantities (with respect to major components) can also indicate adulteration in an essential oil. Every adulterant, whether synthetic or natural, has some kind of marker or impurity. Identifying the unique markers for each specific adulterant is a more effective way of detecting essential oil adulteration (Krock et al. 1994; Aprotosoaie et al. 2014; Schmidt 2016).

9.4 Synthetic Markers for Adulteration Detection

Impurities (markers) of a synthetic compound originate from starting material by-products, degradation products, and oxidation or reduction reactions of reactants (or decarboxylation reactions) so that the formation of impurities from synthetic materials is inevitable (Roy 2002; Rao et al. 2010). Synthetic volatile compounds are typically synthesized from petrochemicals or natural, plant-derived precursors. According to Ernest Guenther (1992), in 1949, worldwide production of essential oils included approximately 4000 tons of citronella oil, which was used to synthesize geraniol, citronellol, hydroxy citronellol, and l-menthol; 100 tons of rose wood oil, which was used to make linalool and linalyl acetate; and 600 tons of lemongrass oil, which was processed to make citral, ionones, and vitamins. Obviously things have become much more sophisticated since Guenther's time, with tremendous advancements in synthetic and analytical techniques.

9.5 Types of Synthetic Markers

The formation of synthetic markers can be caused by oxidation (e.g., neric acid as a synthetic marker for neral), reduction (e.g., dihydro linalool as a synthetic marker of linalool, dihydro linalyl acetate as a synthetic marker of linalyl acetate (Frey 1988) (see below)), thermal rearrangement (e.g., citrollene, 2,6-dimethyloctane, and isolimonene for monoterpene hydrocarbons (Stolle et al. 2009); plinol as a synthetic marker of linalool and plinyl acetate as a synthetic marker of linalyl acetate, derived from α-pinene as a natural precursor (see below) (Sell 2003; Leiner et al. 2013)), intermediate isomerization (e.g., α-linalool (see below) (Radulović et al. 2013)), acetylated markers (e.g., terpin diacetate as a synthetic marker of terpinyl acetate (Suga et al. 1983)), and product–reactant interaction markers (e.g., phenylpentadienal as a synthetic marker of (*E*)-cinnamaldehyde (see below) (Frey 1988)).

Many individual essential oil components are synthetically accessible with modern synthetic techniques, but the cost of mass production and the purity of the final product dictate the selection of synthetic adulterants based on economic viability. In fact, few sesquiterpenes are synthesized from natural or synthetic precursors. Thus, despite having large numbers of sesquiterpenoids, adulteration of essential oils with synthetic or semisynthetic sesquiterpenoids is not generally a problem, so monitoring synthetic markers has not been pursued. However, emerging genetically engineered biosynthetic chemicals are in the process of being industrially produced (Diaz-Chavez et al. 2013), and thus this will introduce an added level of concern in the years to come as these processes become more refined and cost effective.

In almost all of the literature reports, the discovered synthetic markers were found in very minor quantities (<0.5%) in the oils they were used to adulterate. Therefore, the selected ion monitoring analysis (SIM) method (Frey 1988) should be used for efficiently detecting synthetic markers in complex adulterated essential oils.

9.6 Synthetic Linalool and Linalyl Acetate

Linalool and linalyl acetate are the main constituents of several commercial essential oils, such as coriander (*Coriandrum sativum*) leaf and seed, ho wood (*Cinnamomum camphora*), rosewood (*Aniba rosaeodora*), lavender (*Lavandula angustifolia*), bergamot (*Citrus bergamia*), lavandin (*Lavandula* spp.), and clary sage (*Salvia sclarea*). Synthetic linalool contains α-linalool, which is produced by the incomplete acid-catalyzed isomerization of 6-methyl-6-hepten-2-one to 6-methyl-5-hepten-2-one, followed by the base-catalyzed ethynylation and selective hydrogenation of the resulting mixture, resulting in α-linalool, a synthetic marker, and β-linalool, the target compound (Fig. 9.1) (Radulović et al. 2013). Similarly, over-hydrogenation of linalool or linalyl acetate gives rise to dihydro linalool or dihydro linalyl acetate as synthetic impurities (Fig. 9.1). Synthetic

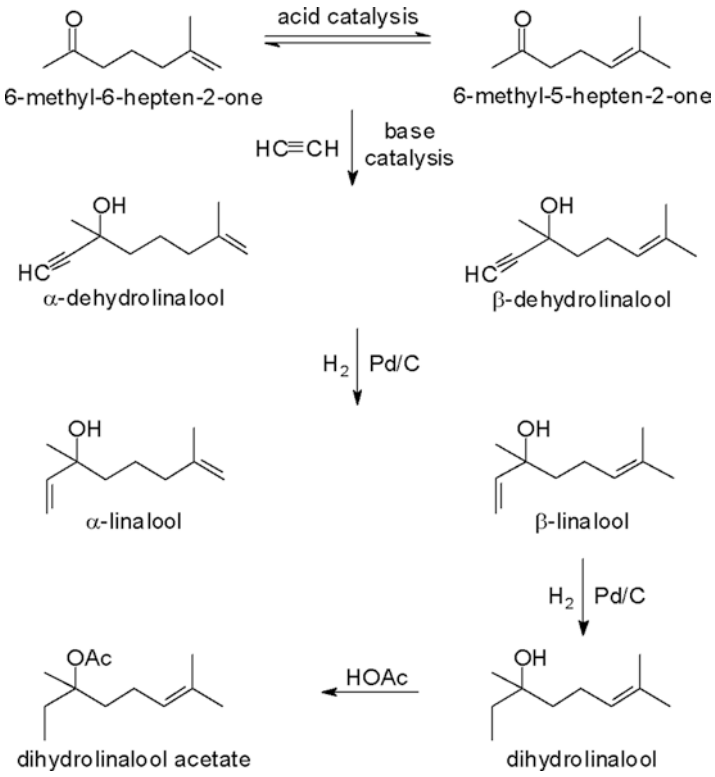

Fig. 9.1 Generation of the synthetic marker of linalool and linalyl acetate, following a petrochemical-based precursor, 6-methyl-5-hepten-2-one

linalool has also been prepared from α-pinene by pyrolysis of the intermediate pinane hydrate (Fig. 9.2). Intramolecular ene reaction leads to a mixture of plinols as synthetic markers, which can be converted to the corresponding plynyl acetates.

9.7 Synthetic Carvone and Carvacrol

Carvone is the major component of spearmint (*Mentha spicata*, (*R*)-(−)-carvone) and caraway seed (*Carum carvi*, (*S*)-(+)-carvone) essential oils. Similarly, carvacrol is one of the major components of oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), and savory (*Satureja hortensis* and *S. montana*) essential oils. In fact, carvone and carvacrol are synthesized from limonene as a starting precursor (Bordenca and Allison 1951; Royals and Horne 1951; Sell 2003), which is shown in Fig. 9.3. During the synthesis of these two compounds, both of which are prepared via carvoxime (a nitrogen-containing intermediate); two nitrogen-containing synthetic markers are produced. The synthetic marker for synthetic carvone is

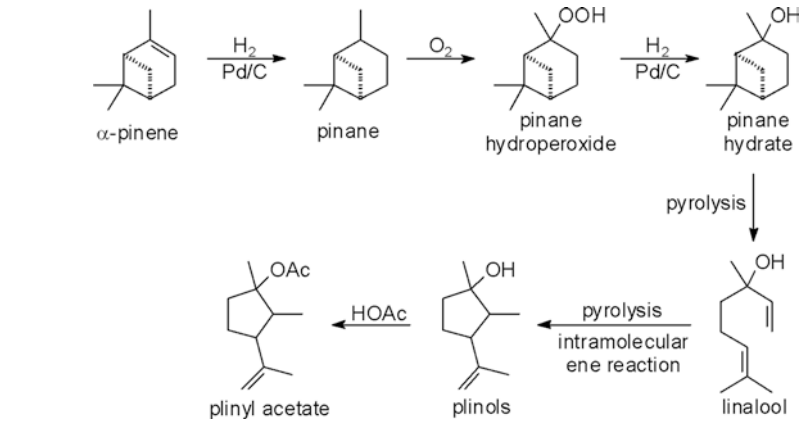

Fig. 9.2 Generation of the synthetic marker of linalool and linalyl acetate, following a natural precursor, α-pinene

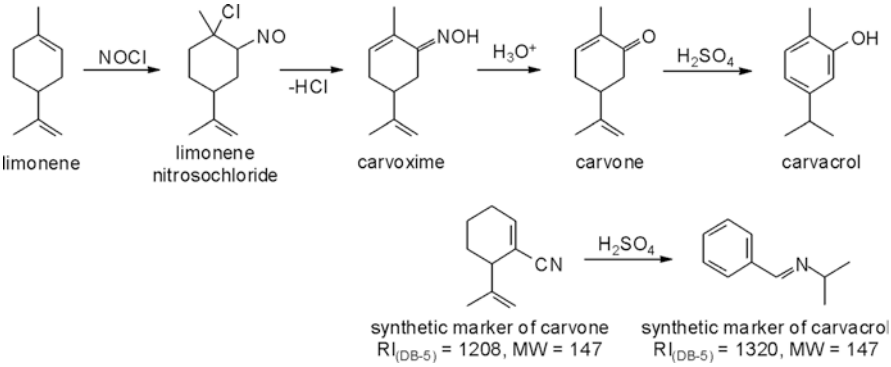

Fig. 9.3 Synthetic scheme of carvone/carvacrol from limonene as a natural precursor

2-cyano-3-isopropenyl-cyclohexene ($C_{10}H_{13}N$, MW 147, RI 1208 on a DB-5 column), while the marker for synthetic carvacrol is *N*-benzylideneisopropylamine ($C_{10}H_{13}N$, MW 147, RI 1320 on a DB-5 column).

9.8 Synthetic Thymol

The major component of thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*) essential oils is thymol. Thymol is industrially synthesized from *m*-cresol as the starting material by electrophilic aromatic substitution of *m*-cresol with propene (Fig. 9.4) to give 6-isopropyl-3-methylphenol (thymol) at 1290 RI on a DB-5 column as the major, thermodynamically favored, product (Biedermann et al. 1978; Wimmer et al. 1991). However, a less thermodynamically favored, minor component,

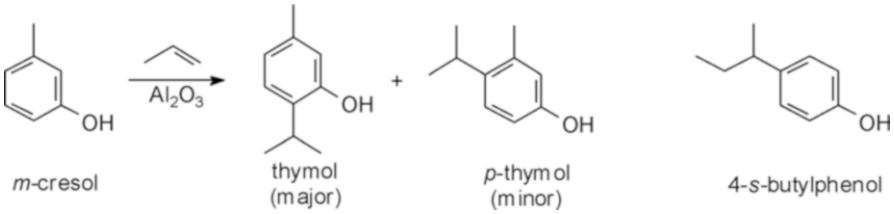

Fig. 9.4 Preparation of thymol from *m*-cresol, a petroleum-based precursor, as the starting material

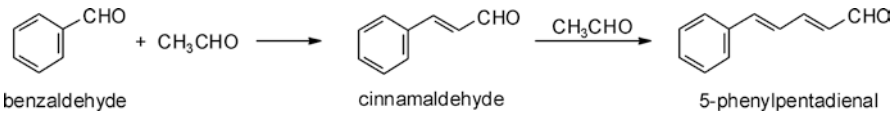

Fig. 9.5 Generation of cinnamaldehyde synthetic marker: crossed-aldol condensation of benzaldehyde and acetaldehyde gives *trans*-cinnamaldehyde (a major component of cassia and cinnamon bark essential oils). *trans*-Cinnamaldehyde further reacts with acetaldehyde to produce trace amounts of both (2*E*,4*Z*)-5-phenylpentadienal and (2*E*,4*E*)-5-phenylpentadienal (MW 158, $RI_{(DB\text{-}5)}$ = 1410 and 1565, respectively)

4-isopropyl-3-methylphenol (*p*-thymol, RI = 1282) as a synthetic marker of thymol, is also produced (Carpenter and Easter 1955; Biedermann et al. 1978). Thus, the presence of this marker would indicate adulteration in the tested thyme essential oils. Additionally, another unnatural impurity, 4-*s*-butylphenol, was also detected as an additional synthetic marker of thymol.

9.9 Synthetic Cinnamaldehyde

Cinnamon (*Cinnamomum zeylanicum*) bark and cassia (*C. cassia*) bark essential oils are often adulterated with synthetic *trans*-cinnamaldehyde. Synthetic *trans*-cinnamaldehyde is produced by the base-catalyzed aldol condensation of benzaldehyde and acetaldehyde (Fig. 9.5) (Richmond 1950). In this synthesis, a common impurity is 5-phenylpentadienal, which is confirmed by the presence of 129, 128, and 158 m/z peaks in the mass spectrum. 5-Phenylpentadienal is formed by the crossed-aldol reaction of cinnamaldehyde with acetaldehyde (Frey 1988).

9.10 Synthetic Methyl Salicylate

Wintergreen (*Gaultheria procumbens*) and birch (*Betula lenta*) essential oils are similarly adulterated by synthetic methyl salicylate, which can be characterized by the presence of phenol and an isomer of dimethyl-2-hydroxyterephthalate (Frey 1988). Methyl salicylate is prepared by Kolbe-Schmitt carboxylation of phenol

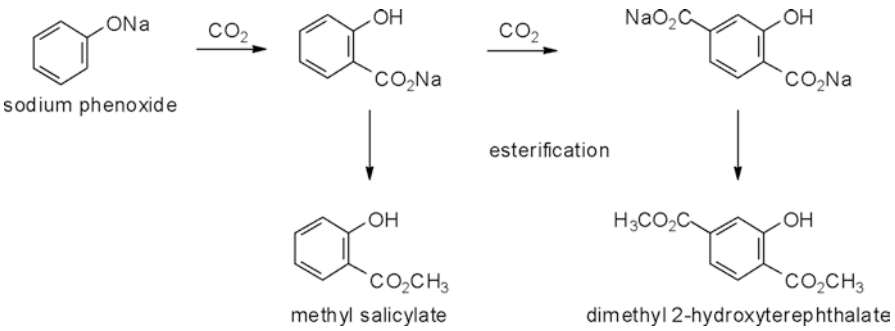

Fig. 9.6 Generation of methyl salicylate synthetic marker, dimethyl-2-hydroxyterephthalate, during synthesis of methyl salicylate. Methyl salicylate is the major (>99%) component present in wintergreen and birch wood essential oils

followed by esterification (Fig. 9.6). The synthetic marker is produced by the repeated Kolbe-Schmitt reaction of salicylate followed by esterification.

9.11 Synthetic Nerolidol

Nerolidol is a major component in the neroli (*Citrus auranthus* floral) and nerolina (*Melaleuca quinquenervia*) essential oils. Synthetic nerolidol has been prepared using linalool as a starting material (Fig. 9.7) (Surburg and Panten 2006; Chan et al. 2016). Linalool is reacted with ethyl acetoacetate; the acetoacetic ester intermediate undergoes thermal rearrangement with decarboxylation to give (*E*)- and (*Z*)-geranylacetone. Nucleophilic addition of acetylene to the geranylacetones gives the corresponding (*E*)- and (*Z*)-dehydronerolidols, which can be partially hydrogenated to give (*E*)-nerolidol and (*Z*)-nerolidol. Adulteration with synthetic nerolidol generally shows trace amounts of dehydronerolidol and geranylacetone.

9.12 Impact of Adulteration

One concern of synthetic adulteration is the toxicity of the synthetic markers themselves, impurities like carvoxime, 2-cyano-3-isopropenyl-cyclohexene, or *N*-benzylideneisopropylamine are always produced during the synthesis of carvone and carvacrol from limonene (see above). In addition to the concerns of marker toxicity, synthetic adulteration gives incorrect steroisomeric distributions of chiral components, which can also have additional adverse health effects (Patočka and Dvořák 2004). For example, (−)-α-thujone has been reported to be more toxic than the (+)-β-diastereomer (Höld et al. 2000); (−)-gossypol is significantly more toxic to nonruminant vertebrates than (+)-gossypol (Stipanovic et al. 2005). The

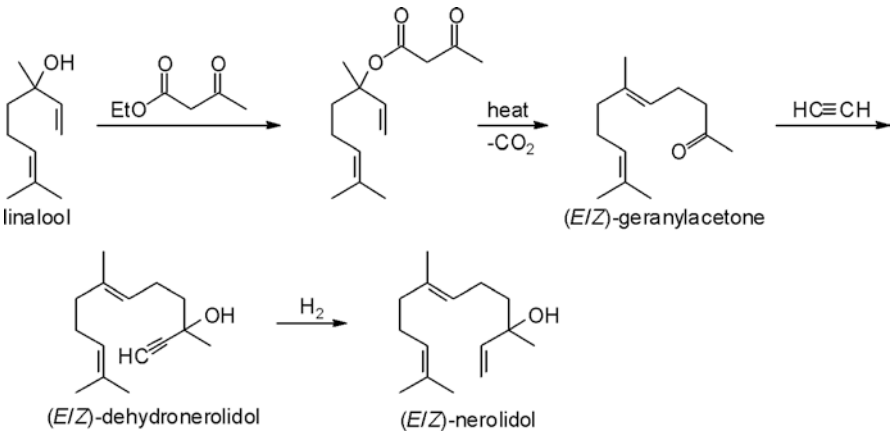

Fig. 9.7 Chemical synthesis of nerolidol using linalool as a starting material

therapeutic properties of pure essential oil components vary from component to component and vary enantiomerically as well. For example, inhaling (+)-limonene can increase systolic blood pressure and alter alertness and restlessness, whereas (−)-limonene only affects alertness. Similarly, (−)-carvone was reported to increase pulse rate, diastolic blood pressure, and restlessness, whereas (+)-carvone increased systolic and diastolic blood pressure (Heuberger et al. 2001). Likewise, (+)-rose oxide provides relaxing physiological properties, whereas (−)-rose oxide causes a significantly higher stimulative effect (Traynor 2001). Similarly, Sugawara found that racemic linalool has adverse effects on inhalation as compared to pure enantiomers, (+) or (−) (Sugawara et al. 2000). According to a review article (Ernst 2002), one fatal and six potentially life-threatening occurrences were reportedly caused by adulterations of Chinese herbal medicines. In addition to the adverse health considerations of synthetic adulteration, there are also the economic implications to consider. Most of the pure natural essential oils are distilled in developing countries; not only are individual farmers affected, but also the entire economies of the countries can be adversely affected by competition with adulterated essential oils.

9.13 Discovery of Synthetic Markers

In the synthesis of organic compounds, 100% pure desired yield is never achieved since there are always side reactions that occur yielding at least trace by-products. Identifying them is challenging since they are not thoroughly researched nor even incorporated into any commercial libraries. However, identifying and characterizing the peaks of these trace markers is perhaps the best overall strategy of all the currently available technologies for detecting essential oil adulteration. This work is

perhaps the first significant overview that has been published on the synthetic markers of essential oil components. Sometimes essential oil components are also degraded, or the wrong distillation technique is applied, which results in unusual essential oil components, but synthetic markers are most always distinct from those arising from unintended degradations (Turek and Stintzing 2013). As knowledge increases on the synthetic markers in essential oil adulteration, manufacturers of the various commercially available components are continually developing new ways to remove the identifying trace markers by using various purification techniques. Target synthesized compounds and trace markers have similar boiling points and retention properties, so they present a considerable challenge to current purification methodologies.

There are two types of precursor molecules used in synthesizing essential oil components: natural component-based and petrochemical-based. Sometimes, natural component-based synthesis produces enantioselective products (e.g., the synthesis of carvone from limonene (Fig. 9.3) (Surburg and Panten 2006)). As a result, it passes isotopic ratio testing and enantioselective testing. Petrochemical-based synthetic products, however, do not pass the isotopic ratio analysis. Petrochemical-based synthetics may also fail enantioselective measurements, but in some cases, there are no enantiomeric aspects to consider. For example, in the synthesis of thymol (Fig. 9.4), we see that the thymol molecule has no chiral centers and thus not amenable to enantioselective testing. With chiral GC-MS being of no value in this case, it leaves only the issue of isotopic ratios to be concerned about from an adulteration standpoint. To pass isotopic ratio testing, most of the so-called natural essential oil producers prefer to use natural precursor-based synthetic compounds rather than those derived from a petrochemical sources. While the naturally derived components can be more expensive than petrochemical-based compounds, oftentimes they are still considerably cheaper than the essential oils themselves, thus making them very attractive additives. Compounds like α-pinene, limonene, geraniol, 1,8-cineole (eucalyptol), and citronellal are frequently used as natural isolates to synthesize nature-identical chemicals (Surburg and Panten 2006), which would pass isotopic testing.

In many cases the naturally derived precursors are actually less expensive than the same compounds if they were to be made synthetically. For example, α-pinene is obtained from turpentine sulfate (a waste product of paper mills), limonene can be extracted from cheap orange oils (US$5 kg^{-1}), 1,8-cineole (eucalyptol) is extracted from *Eucalyptus* essential oil, and citral is extracted from lemongrass essential oils (Sell 2003). But when the natural precursors are isolated, there are typically characteristic pyrolyzed markers that arise from the isolation process. There are potentially more markers found in natural precursor-based synthetic compounds than in petrochemical-based synthetic compounds; therefore achieving an acceptable level of purification of the natural precursors can be challenging unless the precursor can be crystallized. Trace impurities in the natural precursors are also affected by the reaction carried out in synthesizing essential oil molecules.

References

Aprotosoaie AC, Hăncianu M, Costache I, Miron A (2014) Linalool: a review on a key odorant molecule with valuable biological properties. Flavour Fragr J 29:193–219. https://doi.org/10.1002/ffj.3197

Biedermann W, Koller H, Wedemeyer K (1978) Process for preparing thymol. United States Patent 4,086,283

Bordenca C, Allison RK (1951) l-Carvone from d-limonene. Ind Eng Chem 43(5):1196–1198

Brumer M (2014) The mystery of drugs and perfumed olive oil: "Samim" and "besamim" in incense and holy anointment oil. J Intercult Interdiscip Archaeol 2014(1):89–102

Carpenter MS, Easter WM (1955) The isopropyl cresols. J Org Chem 20(4):401–411

Chan WK, Tan LTH, Chan KG, Lee LH, Goh BH (2016) Nerolidol: a sesquiterpene alcohol with multi-faceted pharmacological and biological activities. Molecules 21:529. https://doi.org/10.3390/molecules21050529

Culp RA, Noakes JE (1990) Identification of isotopically manipulated cinnamic aldehyde and benzaldehyde. J Agric Food Chem 38:1249–1255

Diaz-Chavez ML, Moniodis J, Madilao LL, Jancsik S, Keeling CI, Barbour EL, Ghisalberti EL, Plummer JA, Jones CG, Bohlmann J, Cirino PC (2013) Biosynthesis of sandalwood oil: *Santalum album* CYP76F cytochromes P450 produce santalols and bergamotol. PLoS One 8:e75053

Do TKT, Hadji-Minaglou F, Antoniotti S, Fernandez X (2015) Authenticity of essential oils. Trends Anal Chem 66:146–157. https://doi.org/10.1016/j.trac.2014.10.007

Ernst E (2002) Adulteration of Chinese herbal medicines with synthetic drugs: a systematic review. J Intern Med 252:107–113

Everstine K, Spink J, Kennedy S (2013) Economically motivated adulteration (EMA) of food: common characteristics of EMA incidents. J Food Prot 76(4):723–735. https://doi.org/10.4315/0362-028X.JFP-12-399

Firenzuoli F, Jaitak V, Horvath G, Bassolé IHN, Setzer WN, Gori L (2014) Essential oils: new perspectives in human health and wellness. Evid Based Complement Alternat Med 2014:467363

Frey C (1988) Detection of synthetic flavorant addition to some essential oils by selected ion monitoring GC/MS. In: Lawrence BM, Mookherjee BD, Willis BJ (eds) Flavors and fragrance: a world perspective. Elsevier BV, Amsterdam, pp 517–524

Guenther E (1992) The essential oils, volumes I–VI. Krieger Publishing Company, Malabar

Harrewijn P, van Oosten AM, Piron PGM (2000) Natural terpenoids as messengers. Springer-Science+Business Media, Dordrecht

Heuberger E, Hongratanaworakit T, Böhm C, Weber R (2001) Effects of chiral fragrances on human autonomic nervous system parameters and self-evaluation. Chem Senses 26(3):281–292

Höld KM, Sirisoma NS, Ikeda T, Narahashi T, Casida JE (2000) α-Thujone (the active component of absinthe): γ-aminobutyric acid type A receptor modulation and metabolic detoxification. Proc Natl Acad Sci U S A 97(8):3826–3831

Huang WF, Wen KC, Hsiao ML (1997) Adulteration by synthetic therapeutic substances of traditional Chinese medicines in Taiwan. J Clin Pharmacol 37(4):344–350

International Organization for Standardization, ISO (2013) ISO 9235:2013: aromatic natural raw materials—vocabulary. https://www.iso.org/standard/51017.html. Accessed 28 Sep 2017

Krock KA, Ragunathan N, Wilkins CL (1994) Multidimensional gas chromatography coupled with infrared and mass spectrometry for analysis of *Eucalyptus* essential oils. Anal Chem 66(4):425–430

Kubeczka KH (2010) History and sources of essential oil research. In: Başer KHC, Buchbauer G (eds) Handbook of essential oils. CRC Press, Boca Raton, pp 3–38

Lawrence BM (2002) Commercial essential oils: truths and consequences. In: Swift KAD (ed) Advances in flavours and fragrances. Royal Society of Chemistry, Cambridge, pp 57–83

Leiner J, Stolle A, Ondruschka B, Netscher T, Bonrath W (2013) Thermal behavior of pinan-2-ol and linalool. Molecules 18:8358–8375. https://doi.org/10.3390/molecules18078358

Marriott PJ, Shellie R, Cornwell C (2001) Gas chromatographic technologies for the analysis of essential oils. J Chromatogr A 936:1–22
Merriam-Webster.com (n.d.) Adulterate, Merriam-Webster Dictionary. https://www.merriam-webster.com/dictionary/adulterate. Accessed 25 Sep 2017
NIIR Board (2004) Modern technology of perfumes, flavours and essential oils, 2nd edn. National Institute of Industrial Research, New Delhi
Patočka J, Dvořák A (2004) Biomedical aspects of chiral molecules. J Appl Biomed 2:95–100
Radulović NS, Blagojević PD, Miltojević AB (2013) α-Linalool – a marker compound of forged/synthetic sweet basil (*Ocimum basilicum* L.) essential oils. J Sci Food Agric 93(13):3292–3303. https://doi.org/10.1002/jsfa.6175
Rao NR, Kiran SS, Prasanthi NL (2010) Pharmaceutical impurities: an overview. Indian J Pharm Educ Res 44(3):301–310
Remaud GS, Martin YL, Martin GG, Martin GJ (1997) Detection of sophisticated adulterations of natural vanilla flavors and extracts: application of the SNIF-NMR method to vanillin and *p*-hydroxybenzaldehyde. J Agric Food Chem 45:859–866
Richmond HH (1950) Preparation of cinnamaldehyde. United States Patent 2,529,186
Roy J (2002) Pharmaceutical impurities—A mini-review. AAPS PharmSciTech 3(2):1–6
Royals EE, Horne SE (1951) Conversion of d-limonene to l-carvone. J Am Chem Soc 73(12):5856–5857
Sadgrove N, Jones G (2015) A contemporary introduction to essential oils: chemistry, bioactivity and prospects for Australian agriculture. Agriculture 5:48–102. https://doi.org/10.3390/agriculture5010048
Schipilliti L, Dugo P, Bonaccorsi I, Mondello L (2012) Authenticity control on lemon essential oils employing gas chromatography – combustion-isotope ratio mass spectrometry (GC – C-IRMS). Food Chem 131:1523–1530. https://doi.org/10.1016/j.foodchem.2011.09.119
Schmidt BM (2016) Regulatory and quality issues with the essential oils supply chain. In: Jeliazkov VD, Cantrell CL (eds) Medicinal and aromatic crops: production, phytochemistry, and utilization. American Chemical Society, Washington, DC, pp 27–48. https://doi.org/10.1021/bk-2016-1218.ch003
Schnaubelt K (1999) Medical aromatherapy: healing with essential oils. Frog Books, Berkeley
Sell CS (2003) A fragrant introduction to terpenoid chemistry. Royal Society of Chemistry, Cambridge
Shu CK, Lawrence BM (1997) Reasons for the variation in composition of some commercial essential oils. In: Risch SJ, Ho CT (eds) Spices. American Chemical Society, Washington, DC, pp 138–159. https://doi.org/10.1021/bk-1997-0660.ch012
Singh AG, Hamal JP (2013) Traditional phytotherapy of some medicinal plants used by Tharu and Magar communities of western Nepal against dermatological disorders. Sci World 11(11):81–89
Stipanovic RD, Puckhaber LS, Bell AA, Percival AE, Jacobs J (2005) Occurrence of (+)- and (−)-gossypol in wild species of cotton and in *Gossypium hirsutum var. marie-galante* (Watt). J Agric Food Chem 53:6266–6271
Stolle A, Ondruschka B, Hopf H (2009) Thermal rearrangements of monoterpenes and monoterpenoids. Helv Chim Acta 92:1673–1719
Suga T, Lee YS, Hirata T (1983) The hydroxylation of β-terpineol and its acetate with the cultured cells of *Nicotiana tabacum*. Bull Chem Soc Jpn 56(3):784–787
Sugawara Y, Hara C, Aoki T, Sugimoto N, Masujima T (2000) Odour distinctiveness between enantiomers of linalol: difference in perception and responses elicited by sensory test and forehead surface potential wave measurement. Chem Senses 25:77–84
Surburg H, Panten J (2006) Common fragrance and flavor materials, 5th edn. Wiley-VCH, Weinheim
Tang F, Zhang Q, Nie Z, Chen B, Yao S (2009) Sample preparation for analyzing traditional Chinese medicines. Trends Anal Chem 28(11):1253–1262. https://doi.org/10.1016/j.trac.2009.09.004
Tisserand R, Young R (2014) Essential oil safety, 2nd edn. Churchill Livingstone Elsevier, Edinburgh

Tranchida PQ, Bonaccorsi I, Dugo P, Mondello L, Dugo G (2012) Analysis of *Citrus* essential oils: state of the art and future perspectives. A review. Flavour Fragr J 27:98–123. https://doi.org/10.1002/ffj.2089

Traynor S (2001) The musk dilemma. Perfumer Flavorist 26(5):28–39

Turek C, Stintzing FC (2013) Stability of essential oils: a review. Compr Rev Food Sci Food Saf 12:40–53. https://doi.org/10.1111/1541-4337.12006

Wimmer P, Buysch HJ, Puppe L (1991) Process for the preparation of thymol. United States Patent 5,030,770

World Health Organization, WHO (n.d.) Traditional and complementary medicine. http://who.int/medicines/areas/traditional/en/. Accessed 25 Sep 2017

Chapter 10
Essential Oils from Pines: Chemistry and Applications

Gaurav Kumar Silori, Naveen Kushwaha, and Vimal Kumar

10.1 Introduction

Essential oils (EOs), also referred as ethereal or volatile oils, have been used for extensive applications in pharmaceutical, medical, and perfume industries. They are aromatic oily liquids and obtained from different parts of plant, i.e., leaves, seeds, fruits, buds, flowers, wood, herbs, barks, and roots (Burt 2004). Till now, around 3000 EOs have been reported by scientific community, of which about 300 are of commercial importance (van de Braak and Leijten 1994). EOs are aromatic oily liquids and therefore have low solubility in water, however are soluble in alcohols, organic solvents, fats, and other hydrophobic substances. They are generally liquid at room temperature (Thormar 2011). Essential oils contribute only a small proportion of the wet weight of plant material, which is usually 1% or less (Pengelly 2004). Essential oils' content can vary in quality, quantity, and composition according to geographical conditions, soil composition, specific parts of plant, and plant age (Masotti et al. 2003; Angioni et al. 2006). Further, the chemical compositions of essential oils not only vary in terms of the number of molecules but also in the stereochemical types of molecules extracted, or oil extraction method. Hence, the essential oil extraction method is chosen accordingly for different applications. Steam distillation is among the most conventional methods used to extract EOs for commercial production.

Essential oils are generally described as secondary plant metabolites, which are synthesized by the plant however not necessarily be essential for plant growth and development (Croteau et al. 2000). Further, all plants do not universally synthesize essential oils, though primary metabolites are synthesized by all plants and take part

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in the essential metabolic processes of respiration and photosynthesis (Theis and Lerdau 2003).

Essential oils may contain 20–60 compounds in different composition, which eventually forms a very complex mixture. Generally two or three components have higher contribution (20–70%) in terms of concentration in a specific species when compared to other (Bakkali et al. 2008).

EOs are extensively used for bactericidal, fungicidal, and medicinal applications due to their antiseptic properties. They have been also utilized as a food preservative (Bakkali et al. 2008). Some key ingredients of EOs are used for scent and cosmetics purposes. Agriculture and medical sector are among other major beneficiaries of EOs. For instance, aroma products, soaps, food additives, creams, and industrial solvents consist D-limonene and geranyl acetate, which are major contributor in EOs. Essential oils have been found useful in massages, baths, and particularly aromatherapy. Few studies also claimed to cure organ dysfunction or systemic disorder through essential oils as EOs persist valuable medicinal properties (Silva et al. 2003; Hajhashemi et al. 2003; Perry et al. 2003). Despite the extensive use of essential oils, it is important to research their additional roles which could be exploited for the improvement of human health, agriculture, industries, etc. (Bakkali et al. 2008).

Pinus (Pinaceae), among more than 110 recognized species, is the largest extant genus of conifers and one of the most important sources of essential oils across the globe. From various studies available in literature, it has been reported that pine's essential oil contains more than four dozen constituents, out of which eight to ten have key importance. Table 10.1 shows chemical structure of few important constituents of pine EOs. Pines are largely distributed in northern hemisphere across many forest types in Europe, Asia, North Africa, North America, and Central America. *Pinus* is among diverse genus of sclerophyllous-leaved tree, which shares the same characteristics with *Quercus* and *Eucalyptus* in the northern and southern hemisphere, respectively (Keeley 2012). The genus *Pinus* contains H20% of gymnosperms, as known species, and more than 50% species in Pinaceae family, including Douglas and true firs, and spruces (Richardson and Ryan 1998).

Pines are coniferous evergreen trees. Conifers belong to gymnosperm family and generally have needle shape leaves. Pine trees are generally 3–80 m tall, with the majority of species ranging from 15 to 45 m tall (Richardson and Ryan 1998; Farjon 2005). They grow well in acidic soils, however can be grown in calcareous soils. Most species require efficient soil drainage; however a few will tolerate poorly drained wet soils (Richardson and Ryan 1998). Pine wood is traditionally used for fuelwood and timber purpose. Different type of pine species has shown medicinal properties against cough, tuberculosis, bronchitis, etc. (Silori et al. 2013). New drugs have been developed through pine species which may become fine alternate of nonsteroidal anti-inflammatory drugs (Kaushik et al. 2012). Rosin, which is obtained from the distillation of resin, has wide commercial use in adhesives, printing ink, and varnish industries (Silori et al. 2013). In this chapter a brief background of essential oils from pines and their various applications have been presented. Importance of pine as a major contributor in essential oils has also been demonstrated.

Table 10.1 Variation in yield of selected pine constituents as per species

Component	Molecular formula	*P. ponderosa* (Krauze-Baranowska et al. 2002; Kelkar et al. 2006)	*P. koraiensis* (Hong et al. 2004)	*P. pinaster* (Macchioni et al. 2002)	*P. nigra* (Macchioni et al. 2002)	*P. strobus* (Krauze-Baranowska et al. 2002)	*P. resinosa* (Krauze-Baranowska et al. 2002)	*P. brutia* (Tumen et al. 2010)	*P. roxburghii* (Zafar et al. 2010; Hassan and Amjid 2009)	*P. sylvestris* (Tumen et al. 2010)	*P. elliottii* (Zhang et al. 2016)
β-Pinene	$C_{10}H_{16}$	45.7	2.81	23.5	2.2	7.9	42.4	39.56	–	1.78	2.65
Camphene	$C_{10}H_{16}$	0.5	5.23	0.7	1.3	3.2	1.6	0.64	0.9	0.60	0.08
α-Pinene	$C_{10}H_{16}$	10.2	10.49	61.6	70.0	17.7	23.3	30.91	29.3	14.76	5.09
Sabinene	$C_{10}H_{16}$	–	0.18	–	–	–	–	–	–	–	–
3-Carene	$C_{10}H_{16}$	8.4	–	–	–	–	0.5	7.80	14.2	–	–
Myrcene	$C_{10}H_{16}$	1.4	7.22	5.2	0.6	3.6	14.5	1.13(β)	1.1(β)	0.17(β)	0.08(β)
α-Terpineol	$C_{10}H_{18}O$	1.4	0.61	0.2	–	–	0.8	0.93	4.5	0.41	–
Terpinolene	$C_{10}H_{16}$	0.81	5.64(α)	–	–	0.2	0.2	0.48(α)	–	0.04(α)	–
Limonene	$C_{10}H_{16}$	–	6.55	1.4	1.8	–	–	–	1.7	–	–
Bornyl acetate	–	–	7.13	–	–	–	–	0.22	–	0.02	–
Caryophyllene	$C_{15}H_{24}$	0.2(β)	6.51	1.0(β)	0.2(β)	3.8(β)	2.2(β)	5.01(β)	21.9	2.87(β)	–
Terpinene-4-ol	$C_{10}H_{18}O$	0.3	0.22	–	–	–	0.1	0.26	0.2	0.01	–
γ-Muurolene	–	0.2	2.62	–	–	1.4	0.1	–	–	–	–
Phellandrene	$C_{10}H_{16}$	–	0.25	–	–	0.4(α)	–	–	0.7(β)	–	0.46(β)
α-Terpinene	$C_{10}H_{16}$	0.1	0.59	–	–	–	–	–	–	–	–
Thujene	$C_{10}H_{16}$	–	1.07(β)	–	–	–	–	–	–	–	–
γ -Terpinene	$C_{10}H_{16}$	0.2	0.30	–	1.8	–	–	–	0.2	–	–
p-Cymene	$C_{10}H_{14}$	0.1	0.42	–	0.6	0.2	0.1	0.37	1.9	0.47	–
Germacrene D	$C_{15}H_{24}$	0.3	–	0.9	1.1	12.2	4.9	0.38	–	0.01	–
Spathulenol	–	–	–	–	–	–	–	–	–	–	–

(continued)

Table 10.1 (continued)

Component	Molecular formula	*P. densiflora* (Hong et al. 2004)	*P. pinea* (Macchioni et al. 2002)	*P. halepensis* (Macchioni et al. 2002)	*P. tabulaeformis* (Xie et al. 2015)	*P. henryi* (Xie et al. 2015)	*P. monticola* (Dambolena et al. 2016)	*P. massoniana* (Xie et al. 2015)	*P. wallichiana* (Dambolena et al. 2016)	*P. peuce* (Hajdari et al. 2016)
β-Pinene	$C_{10}H_{16}$	9.82	1.1	0.7	2.29	0.35	22.8	2.99	34.0	10.9
Camphene	$C_{10}H_{16}$	3.86	0.1	0.6	0.38	0.34	3.5	0.63	1.0	5.8
α-Pinene	$C_{10}H_{16}$	14.44	3.9	61.8	11.08	9.68	21	8.16	14.8	29.0
Sabinene	$C_{10}H_{16}$	0.35	–	–	0.15	0.18	–	–	–	0.06
3-Carene	$C_{10}H_{16}$	–	–	–	0.31	0.13	4.2	0.13	–	0.4
Myrcene	$C_{10}H_{16}$	12.19	2.5	20.1	–	1.04	4.2	–	1.3	0.9
α-Terpineol	$C_{10}H_{18}O$	0.50	0.8	0.2	3.43	1.32	1.3	3.34	0.3	3.4
Terpinolene	$C_{10}H_{16}$	2.87	–	–	0.24 (α)	0.59 (α)	5.1	0.17(α)	–	0.13
Limonene	$C_{10}H_{16}$	4.34	75.3	0.8	0.41	0.09	14.0	–	17.8	–
Bornyl acetate	–	5.67	–	–	4.13	2.96	–	3.83	–	7.4
Caryophyllene	$C_{15}H_{24}$	3.26	3.7	8.5	22.36 (β)	18.26(β)	0.5(β)	18.48(β)	1.8(β)	0.1(β)
Terpinene-4-ol	$C_{10}H_{18}O$	0.30	–	–		0.15	–	–	0.2	0.1
γ-Muurolene	–	0.64	–	–	1.06	–	–	–	–	–
Phellandrene	$C_{10}H_{16}$	0.45	–	–	0.12(β)	0.37	1.8(α)	0.66(β)	0.3(α)	45.1(α)
α-Terpinene	$C_{10}H_{16}$	0.33	–	–	–	0.12	0.3	0.29	0.6	0.06
Thujene	$C_{10}H_{16}$	19.33	–	–	0.13 (α)	1.72(α)	0.4(α)	2.02(α)	0.1(α)	–
γ-Terpinene	$C_{10}H_{16}$	0.33	0.1	0.1	0.48	0.13	0.6	0.52	–	0.3
p-Cymene	$C_{10}H_{14}$	0.49	0.1	0.1	–	–	–	–	0.1	–
Germacrene D	$C_{15}H_{24}$	–	0.2	0.6	7.43	2.71	–	9.78	–	16.6
Spathulenol	–	–	–	–	0.23	0.65	–	–	–	0.1

All the concentrations are shown on % basis. In literature, variation in constituent's concentrations may exist due to change in geographical and climate conditions

10.2 Pine Essential Oils: Major Constituent and Corresponding Yields

Pine essential oils contain varieties of organic constituents. Though the number of constituents is more than 50 (Tumen et al. 2010), 20 major constituents have been classified by different sources to investigate their respective yields in different pine species across the world. The major components in pine EOs are β-pinene, camphene, α-pinene, sabinene, 3-carene, myrcene, α-terpineol, terpinolene, limonene, bornyl acetate, caryophyllene, terpinene-4-ol, γ-muurolene, phellandrene, α-terpinene, thujene, γ-terpinene, p-cymene, germacrene D, and spathulenol. Figure 10.1 shows chemical structures of 20 major contributors in pines' essential oils. Variations in the yield of selected constituent as per their origin and species can be seen from Table 10.1. It can be stated that five constituents, namely, α-pinene, β-pinene, camphene, 3-carene, and myrcene, are the major contributors in essential oil formation in pines.

10.3 Distribution of Pine Species Across the Globe

According to Royal Botanic Gardens and Kew and Missouri Botanical Garden, there are around 126 recognized species, with 35 unresolved species and many more synonyms (http://www.missouribotanicalgarden.org/ 2018; https://www.kew.org/ 2018). As mentioned earlier, pine has a very large family, and they are widely spread in most parts of the globe. Forty major pine species and their availability across the globe has been mentioned in Table 10.2, of which 25 species belongs to *Pinus* subgenus while 15 are from *Strobus*. Figure 10.2 shows availability percentage of investigated species in different continents. It is evident from Fig. 10.2 that Asian, European, and North American continents together comprise around 40% of major pine species found across the globe.

10.4 Pine Oil Extraction Methods

From conventional steam distillation, Soxhlet extraction followed by solvent extraction (SE), rectification and fractionation of solvent extracts, maceration, and expression (cold pressing of citrus peels) to techniques like supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), accelerated solvent extraction (ASE), solvent-free microwave extraction (SFME), and microwave hydro-diffusion and gravity (MHG), the essential oil extraction methods have received enormous advancement. Extraction yield can be quantified by state-of-the-art techniques like GC-MS and HPLC (Sahraoui et al. 2008). A brief discussion of the abovementioned methods is provided in the following section. Figure 10.3 shows the progress in essential oil's extraction techniques in last four decades.

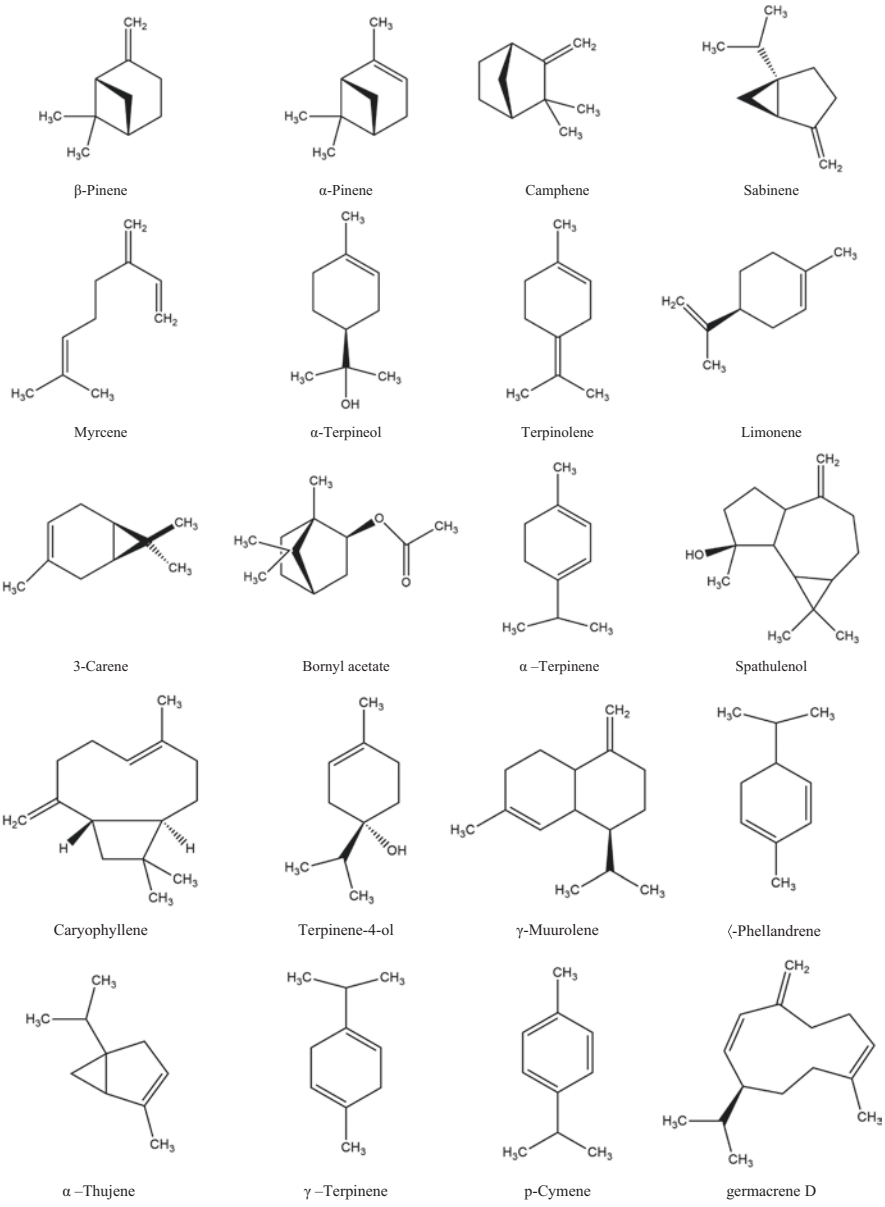

Fig. 10.1 Major constituents and their chemical structure in pine's essential oil

Table 10.2 Pine species and their availability across the globe

Section	Species	Common name	Country/region
Pinus	*P. brutia*	Eastern Mediterranean or Calabrian pine	Turkey, Crimea, Iran
	P. halepensis	Aleppo pine	Morocco, Algeria, Spain
	P. heldreichii	Bosnian pine	Southern Italy, Croatia
	P. pinaster	Maritime or cluster pine	Portugal, Northern Spain
	P. pinea	Mediterranean stone or umbrella pine	Israel, South Africa, New South Wales
	P. roxburghii	Chir pine	India, Nepal, Tibet, Pakistan
	P. densiflora	Japanese red pine	Japan, Northeastern China, Southeast Russia
	P. massoniana	Masson pine	Taiwan, Hong Kong, Northern Vietnam
	P. mugo	Dwarf mountain pine	Southwest Europe, Austria, Switzerland
	P. mugo var. *prostrata*	Dwarf mountain pine	North and Central Italy
	P. nigra subsp. *laricio*	Corsican pine	France and Italy
	P. sylvestris subsp. *scotica*	Scot pine	Western Europe to Eastern Siberia
	P. thunbergii	Japanese black pine	Japan, South Korea
	P. attenuata	Knobcone pine	Southern Oregon
	P. patula	Mexican weeping pine	Mexico, Bolivia, Kenya
	P. coulteri	Coulter or bigcone pine	Southern California, Mexico
	P. jeffreyi	Jeffrey pine	California, Oregon
	P. torreyana	Torrey pine	San Diego, Santa Rosa Island
	P. teocote	Mexican small-cone or Aztec pine	Mexico
	P. sylvestris	Scots pine	Eurasia, Siberia
	P. tabuliformis	Chinese red pine	China, North Korea
	P. elliottii	Slash pine	Southeastern United States
	P. radiata	Monterey pine	Central Coast of California
	P. ponderosa	Ponderosa or western yellow pine	Western United States and Canada
	P. banksiana	Jack pine	Canada, Minnesota

(continued)

Table 10.2 (continued)

Section	Species	Common name	Country/region
Strobus	*P. aristata*	Colorado bristlecone pine	United States
	P. cembroides	Mexican pinyon	Texas, Mexico
	P. monophylla	Single leaf pinyon	Arizona, Northwest Mexico
	P. bungeana	Lacebark pine	Northeastern and Central China
	P. gerardiana	Chilgoza or Gerard's pine	Afghanistan, Pakistan, and northwest India
	P. armandii	Chinese white or Armand's pine	China, Taiwan
	P. cembra	Swiss stone or Arolla pine	Poland, Austria, Germany
	P. flexilis	Limber or Rocky Mountain white pine	Western United States, Canada
	P. koraiensis	Korean stone pine	Korea, Northeastern China, Mongolia
	P. monticola	Western white pine	United States, Canada
	P. parviflora	Japanese white pine	Korea and Japan
	P. pumila	Dwarf stone pine	Eastern Siberia, Northern Japan, and Korea
	P. peuce	Macedonian or Balkan (white) pine	Bulgaria, Albania
	P. strobus	Eastern white pine	Eastern North America
	P. wallichiana	Himalayan blue pine	Himalaya, Bhutan

Farjon 2005; Ioannou et al. 2014; Gernandt et al. 2005; Richardson and Rundel 1998

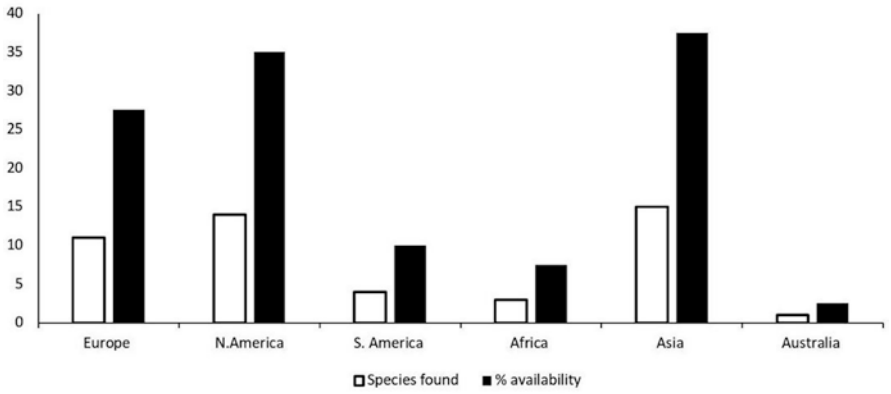

Fig. 10.2 Percentage availability of considered pine species across the globe

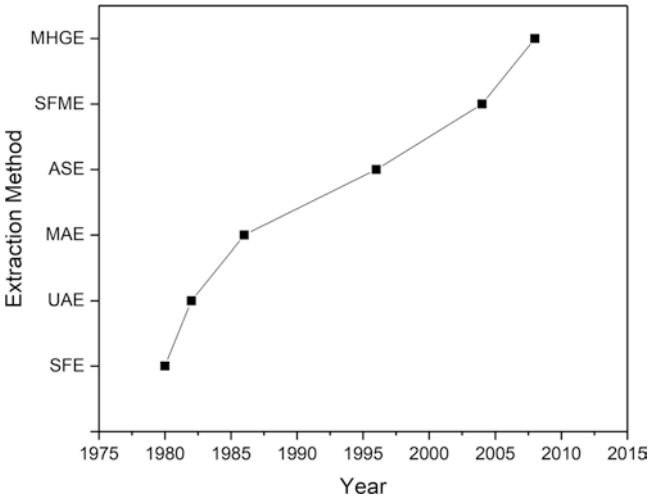

Fig. 10.3 Progress in essential oil's extraction techniques in last four decades

10.4.1 Steam Distillation (SD)

The vapor produced by the steam generator passes through the ground plant material, stored in apparatus. The vapors generated are then condensed using a condenser, and the condensate is collected in a flask. The collected oil is dried with anhydrous Na_2SO_4 and stored at particular temperature until used. Extraction needs to be performed at least three times (Sahraoui et al. 2008). Figure 10.4 shows a typical setup of steam distillation extraction process.

10.4.2 Maceration

The technique involves soaking of raw plant material, which is generally grinded/powdered form, in a close container with a solvent for a period of over 72 h. The container is frequent agitated, the process is held at room temperature. Conduction and convections are the two mediums of heat transfer in this method. The solvent is chosen according to desired component, for example, methanol is best solvent for extraction of secondary metabolites (Handa et al. 2008).

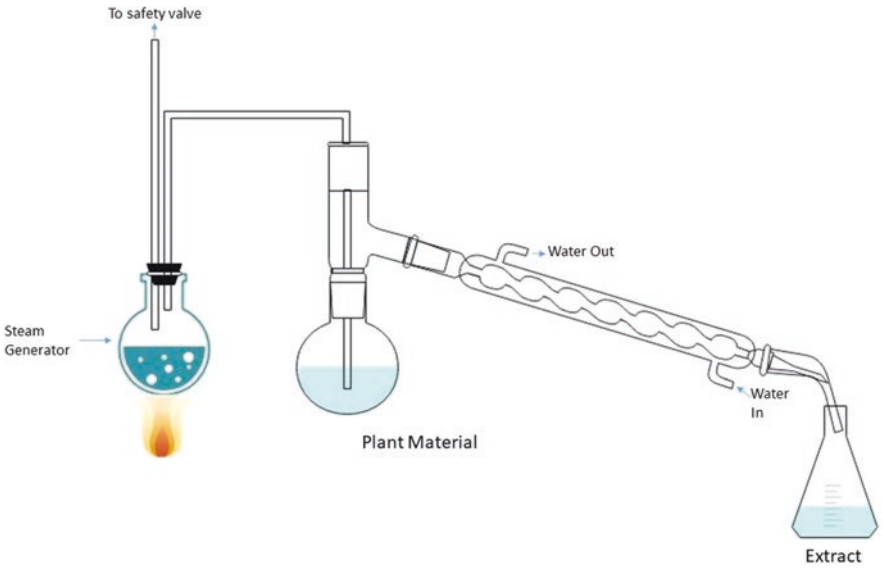

Fig. 10.4 Schematic of steam distillation extraction process

10.4.3 Soxhlet Extraction (SE)

This method is among continuous solid/liquid extraction methods. Finely ground sample of needles/plant is "thimble" made from a strong filter paper or cellulose. In thimble chamber of the Soxhlet apparatus, extraction solvent, such as methanol, hexane, or acetonitrile, is vaporized into the sample thimble after heating and then condensed back. The liquid is dropped back into the bottom flask after reaching at siphon arm and the process is continued. This process takes place again and again until all the material in the finely ground sample is extracted into an organic solvent which generally takes 18–20 h time (Jensen 2007).

10.4.4 Supercritical Fluid Extraction (SFE)

Supercritical fluid (SF), which is also known as dense-gas, is a special class of solvents containing the solvating property of liquids but behaves like a gas, simultaneously (Orav et al. 1998). The plant material is extracted with supercritical fluid which is generally carbon dioxide (Schaneberg and Khan 2002). For supercritical CO_2, extraction temperature and pressure are maintained above 304 K and 74 bar, respectively. The properties of supercritical fluid can be altered through change in temperature and pressure. However, high pressure requirement in this process makes it costly when compared to conventional extraction processes.

10.4.5 Microwave-Assisted Extraction (MAE)

The process contains a microwave unit, equipped with a circulatory Dean-Stark apparatus. The grounded plant material is soaked in a specified solvent for a specified time in a round bottom flask. After required pre-treatments, the mixture is placed into microwave oven where disruptions of plant cells happen at high temperature; the volatile essential oils come out from the oil glands and rises toward the condenser where they are condensed. Subsequently, EOs are separated from other impurities in collector (Thakker et al. 2016). Figure 10.5 demonstrates a typical MAE process.

10.4.6 Ultrasound-Assisted Extraction (UAE)

It is also referred as sonication extraction and involves the use of ultrasound irradiation, which generally ranges from 20 kHz to 2000 kHz. The extraction is performed in an ultrasound cleaning bath with the help of indirect sonication. The ground material is charged with the batch of organic solvent (polar or nonpolar as per sample requirement). The sonication is held for a particular time, which is generally

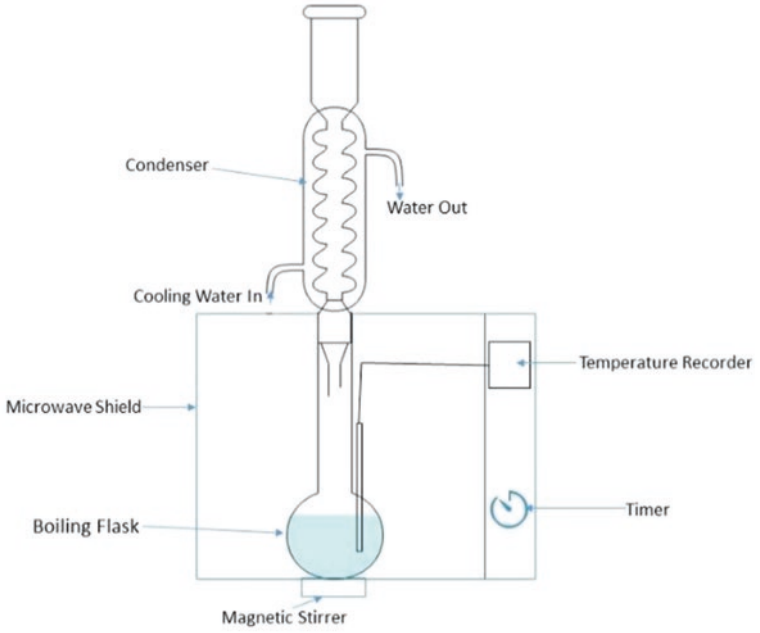

Fig. 10.5 Schematic of microwave-assisted extraction process

30 min. The organic layer obtained after sonication is transferred to a separation funnel where saturated NaCl solutions is mixed with it. The separated organic layer is obtained followed by the required washing process. Physical and chemical properties of the materials subjected to ultrasound gets altered and disrupt the plant cell wall, facilitating release of compounds and enhancing mass transport of the solvents into the plant cells. The procedure is simple and relatively low-cost technology that can be used in both small and larger scale of phytochemical extraction (Kimbaris et al. 2006).

10.4.7 Accelerated Solvent Extraction (ASE)

It is an enhanced form of liquid solvent extraction compared to maceration and Soxhlet extraction as the method uses a minimal amount of solvent. The process requires accelerated solvent extractor and the solvent. Solvent (ml) to sample (g) ratio is kept around 2.5–3. The extraction time is generally 10–12 min. Sample is stored with inert materials in a stainless steel cell to avoid the aggregation in the sample. Stored cells contain layers of sand sample mixture. This state-of-the-art extraction technology is capable to regulate temperature and pressure for each individual sample and generally requires less than an hour for extraction (Schaneberg and Khan 2002).

10.4.8 Solvent-Free Microwave Extraction (SFME)

SFME comprises microwave-assisted dry distillation at atmospheric pressure. SFME is based on a relatively simple principle and does not require any solvent or water (Filly et al. 2014). Instead, the inherent water content of plant material is heated selectively, which results in tissue bursting, and finally release of essential oil. In a study, it was claimed that the essential oils extracted by SFME method took nine times less time when compared to conventional hydro-distillation method, subjected to same yield (Lucchesi et al. 2004). SFME is among green technologies and has several benefits over conventional method used in extraction of aromatic plants.

10.4.9 Microwave Hydro-Diffusion and Gravity (MHG) Extraction

MHG is among newly developed green techniques for extraction of EOs. It is based on two phenomena, i.e., hydro-diffusion and gravity. It is alike SFME till release of extract (water and EOs). Further, hydro-diffusion phenomenon allows the extract to diffuse outside the plant material. The extract is collected and separated in a vessel

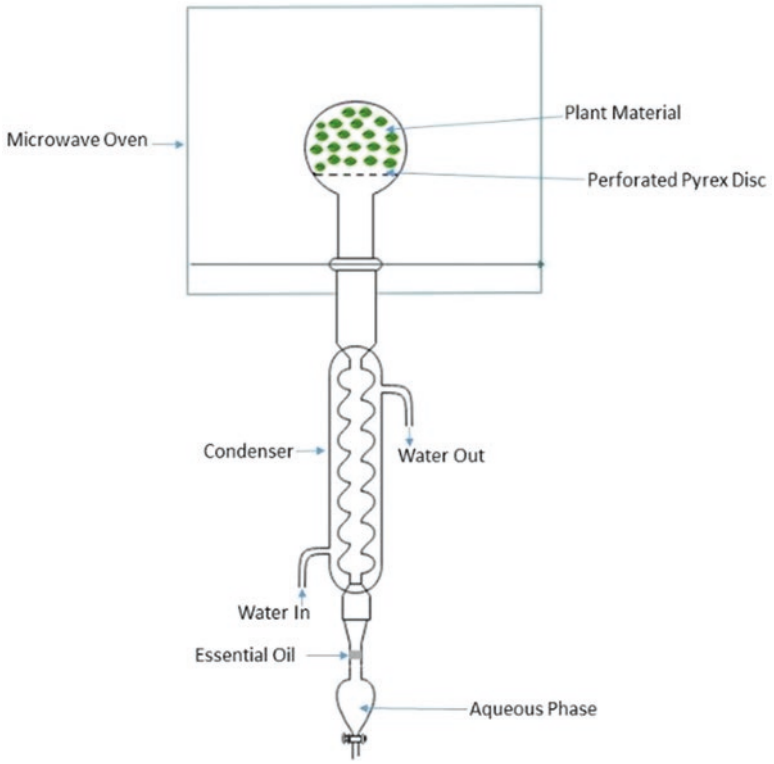

Fig. 10.6 Schematic of microwave hydro-diffusion and gravity extraction process

based on their specific gravity. Because of its least energy requirements, MHG has established its superiority over existing MAE, SFME, or modified hydro-distillation techniques. In a study, MHG was compared with a conventional technique, hydro-distillation (HD), for the extraction of essential oil from two aromatic herbs. The essential oils extracted through MHG were quantitatively (yield) and qualitatively (aromatic profile) similar to those obtained by conventional hydro-distillation while reducing extraction time by six times (Vian et al. 2008). When it comes to industrial implications, MHG technique is highly recommended having least amount of greenhouse gases. A schematic of MHG extraction process is shown in Fig. 10.6.

10.5 Chemistry of Pine Oils

Primary metabolites are universally available in plant families and constitute an essential life cycle. They are categorized into four subgroups, namely, proteins, carbohydrates, nucleic acids, and lipids (Bu'Lock 1965; Mann et al. 1994; Başer and

Buchbauer 2010). Secondary metabolites do not occur universally in each species and are usually classified into terpenoids, shikimates, polyketides, and alkaloids. Pine species contains a number of pathways pertaining to chemical structure of constituents; however in this section, the two key pathways, shikimate and terpenoids, are discussed which are readily found in pine species. Both pathways have very complex mechanism, and only brief descriptions have been presented in the following sections.

10.5.1 Shikimate Pathway

Being a key precursor for flavonoids and lignin, shikimic acid holds position as a key synthetic intermediate for plants (Bu'Lock 1965; Mann et al. 1994). While flavonoids act as antioxidants and UV protector, lignin is responsible for woody tissue formation in plants. Phosphoenolpyruvate and erythrose-4 phosphate are the two major synthesizers of shikimic acid in plants as shown in Fig. 10.7. Benzoic acid and its derivate, widespread for their use as ester, are formed by the aromatization of shikimic acid provided no further addition of three carbon atoms from phosphoenolpyruvate (Mann et al. 1994).

10.5.2 Terpenoid Pathway

As far as EOs are concerned, terpenoid is among the most important pathways existing in pine species (Singh and Sharma 2015; Eggersdorfer 2012). When first formed, terpene structure contains a multiple of five carbon atoms. Terpenoid with 10 carbon atoms per molecule are referred as monoterpenoids. Further, terpenoids with 5, 15,

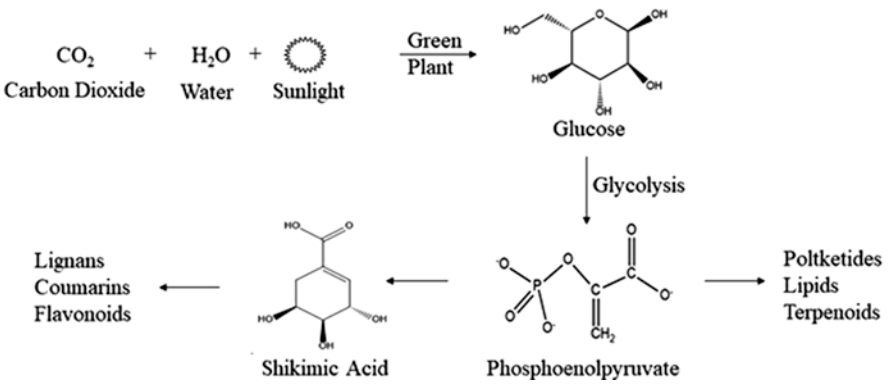

Fig. 10.7 A schematic of secondary metabolites biosynthesis

and 20 carbon atoms are known as hemiterpenoids, sesquiterpenoids, and diterpenoids, respectively (Eggersdorfer 2012; Simonsen 1931). Hemiterpenoids, sesquiterpenoids, and monoterpenoids are sufficiently volatile to be component of EOs and can be further elaborated as follows (Eggersdorfer 2012):

(i) Hemiterpenoids: Most R-OH, R-CHO, and R-CO-OR´ components with a CH_3CH_2CH $(CH3)_2$ skeleton.
(ii) Monoterpenoids: Myrcene, ß-pinene, 3-carene, α -phellandrene, and ß-phellandrene.
(iii) Sesquiterpenoids: α-Bisabolol, farnesol, santalols, ionones, and nerolidol.

A brief of some key ingredients of pine essential oils are as follows:

Alpha-Pinene ($C_{10}H_{16}$) α-Pinene is one of two isomers of pinene, an organic compound belonging to terpene family, which contains reactive four-membered ring. It is found in the oils of different species of coniferous trees, notably the pine (MacTavish 1934).

3-Carene ($C_{10}H_{16}$) Carene, or delta-3-carene, belongs to a class of monoterpenes which consists fused ring alkanes specifically cyclohexane and cyclopropane. It is a colorless liquid having pungent odor. Carene is insoluble in water but soluble in fats and oil (Eggersdorfer 2012).

Myrcene ($C_{10}H_{16}$) Myrcene belongs to monoterpene family and is an olefinic natural organic hydrocarbon. At 400°C, it is obtained through pyrolysis of β-pinene. It is rarely obtained directly from plants (Behr and Johnen 2009).

Terpineol ($C_{10}H_{18}O$) A naturally occurring alcohol from monoterpene family consisting four types of isomers, viz., alpha-, beta-, and gamma-terpineol and terpinen-4-ol. Generally, terpineol is mixture of the abovementioned isomers with alpha-terpineol as a major constituent (Eggersdorfer 2012; Behr and Johnen 2009).

Camphene ($C_{10}H_{16}$) Camphene is a volatile monoterpene, which is soluble in organic solvents and almost insoluble in water. For commercial purposes it is produced by catalytic isomerization of alpha-pinene. It is used as food additive and fragrances (Bigley et al. 1981).

Limonene ($C_{10}H_{16}$) Limonene belongs to class of colorless cyclic terpene. It is commercially used to synthesize carvone, which is used in food and flavor industry. Steam distillation and centrifugal separation are two primary techniques, which are used to obtain limonene for commercial purposes from citrus fruits (Sun 2007).

10.6 Application of Pines' Essential Oils

10.6.1 Medicinal Use

From various literature resources, it has been found that pines contain several medicinal properties, which can be utilized for the interest of mankind. Various efforts have been made over last few decades to identify antimicrobial and antiviral properties of pines. Moreira et al. (2005) examined the antimicrobial activities of essential oils against four strains of *E. coli*. On the basis of findings, it was suggested that the EO can be used as food antimicrobial preservatives (Moreira et al. 2005). Bhalla et al. (2013) successfully reviewed the possibility of essential oils' constituents for the treatment of cancerous disease. It was reported that EOs have potential to enhance activity of white blood cells, which are responsible for removing foreign materials and microbes from the body (Bhalla et al. 2013).

It has been reported that the pine needles comprise about 1.5–2.5% shikimic acid, although a little change with season and age has also been observed. The studies carried out in available literature confirm that few pine species contain a good enough amount of a key precursor which is used in the production of Tamiflu®, an antiviral drug used in the treatment of H5N1 flu (Xie et al. 2012; Sui 2008). Chen et al. (2014) achieved about a 6% yield of shikimic acid from Masson pine needles, which is possibly the highest extracted yield from any pine species till now (Chen et al. 2014). Since pine needles are inexpensive and readily available in North Asia, North America, and Europe, there is a strong possibility to utilize them as a drug manufacturer against less available star anise species.

Another major ingredient in pine EOs are terpenoids, which have antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, anti-inflammatory, and immunomodulatory properties and have been found useful in the prevention and therapy of several diseases, including cancer (Theis and Lerdau 2003; Medeiros Leite et al. 2007). Terpenoids have also been found effective as natural insecticides and can be used in storing agriculture products. Various studies have suggested to pay substantial attention to introduce terpenes into modern therapies (Paduch et al. 2007). Wound-healing mechanism can be enhanced by incorporating EOs in polymer matrix.

Resin is a viscous liquid generally obtained from the pine tree bark. Rosin, which is obtained from the distillation of resin, has many applications. Three esters of rosin, viz., glycerol, sorbitol, and mannitol, are proposed to be used in chewing gums and sweeteners as they contain antidiabetic properties (Fulzele et al. 2004). Rosins have been utilized for manufacturing microcapsules and nanoparticles, and their properties have been examined in vitro and ex vivo studies (Lee et al. 2005).

10.6.2 Industrial Uses

While pines contain several medicinal properties, they have industrial significance too. Terpenes, one of the major constituents of pine's essential oil, have use in the preparation of perfumes and insect repellants. Turpentine oil is used in the preparation of cosmetics, further the characteristic fragrances of terpenes make them useful in aromatherapy too. Terpenes like limonene and pinene are used as air fresheners (Kandi et al. 2015).

Rosin is useful in the manufacturing of adhesives, printing ink, soldering fluxes, varnishes, and sealing waxes. It has also been used as a glazing agent in many consumables including medicines and chewing gums. Other uses of rosin include plasters and ointments where it forms a key ingredient. Rosin has a good electric isolation and thus has suitability to be used in electric cables. Rosin has been mixed with pitch to be used in optical component making (Silori et al. 2013).

Besides essential oils from pines, pine needles have also been used to produce electricity at large scale through gasification process. A 10 KV plant setup has been installed to produce electricity from dry pine needles in Uttarakhand state of India (Mishra and Vlosky 2015; http://avani-kumaon.org/ n.d.). As pine species are sufficiently available across the globe, the work has fair chance to meet huge electricity demand of beneficiaries. Table 10.3 shows applications of the major EOs' constituents.

10.7 Summary

As evident from the present study and available literature, it can be clearly stated that pines have contributed extensively in essential oils. Essential oils from pines and pine needles have been used for diverse range of applications varying from pharmaceuticals, medical, etc. to energy production for domestic (in rural areas) as well as industrial applications. Essential oils from pine contain more than 50 components in which five constituents, namely, α-pinene, β-pinene, camphene, 3-carene, and myrcene, are major. Further, with the advent in process intensification in process industry new technologies have been developed for the extraction of EOs from pine. A few of them, such as ultra-sonication irradiation and microwave-assisted techniques, are six to ten times faster than the conventional techniques. However, these techniques are still mainly used in the laboratories and not much attention has been made at the industrial level. Further, two major metabolite pathways for shikimate and terpenoids were discussed in brief as they have high medicinal values.

Table 10.3 Major constituents of pines' essential oil and their applications

Pines' EO constituents	Uses	Ref.
β-Pinene	Cleaning solvent, antimicrobial properties against bacterial cells	Kelkar et al. (2006); Rivas da Silva et al. (2012)
Camphene	Food additives, plasticizer for resins and lacquers, feedstock for preparation of fragrance components	Verschueren (2001); Sell (2006)
α-Pinene	Cosmetics and odor agents, insecticides, camphene, esters, lubricating oil solvents, and plasticizers	O'Neil (2006); Lewis Sr. (2007)
Sabinene	Exhibits anti-inflammatory, antimicrobial, and antifungal properties	Valente et al. (2013)
3-Carene	Feedstock for perfumes, cosmetics, flavors, and terpene resins, serves as an antihistamine by reducing excess menstrual flow or perspiration, delta-3-carene is used as a central nervous system depressant	Ocete et al. (1989); Cavaleiro et al. (2006)
Myrcene	Production of 7-hydroxygeranyl-neryl dialkylamine Feedstock for geraniol, nerol, linalool, and isophytol. Preparation of perfume chemicals and flavoring	Eggersdorfer (2012); Lewis Sr. (2007)
α-Terpineol	Solvent for resins and cellulose esters and ethers Perfumes, soaps, disinfectant, antioxidant, flavoring agent	Lewis Sr. (2007); Tobin et al. (1976)
Terpinolene	Solvent for resins, manufacturing of synthetic resins and synthetic flavors, improvement the odor of industrial and household products	Lewis Sr. (2007); Eggersdorfer (2012)
Limonene	Starting material for the synthesis of (R)-(−)-carvone, gallstone solubilizer, used in many food, soap, and perfume products for its lemon-like flavor. Has antimicrobial, antiviral, antifungal, antilarval, insect attractant, and repellent properties	Sell (2006); Lewis Sr. (2007); Shepard (1986); Bingham et al. (2001)
Bornyl acetate	Used in perfumes and for flavoring. Bornyl acetate exhibit anti-inflammatory property and it is used as an analgesic	de Cássia da Silveira e Sá et al. (2013)
Caryophyllene	Anti-inflammatory and analgesic, antianxiety, and antidepressant	Bahi et al. (2014); Klauke et al. (2014)
Terpinene-4-ol	Pepper oils and in perfumery, medication, and therapeutics	Fahlbusch et al. (2003)
Phellandrene	Flavoring and perfumery	Hawley (1977)

(continued)

Table 10.3 (continued)

Pines' EO constituents	Uses	Ref.
α -Terpinene	Maintain the oxidative stability of food, cosmetics, and medicaments, for odor purposes in industrial fluids	Rudback et al. (2012)
Thujene	Antimicrobial activity	Sadhasivam et al. (2016)
γ -Terpinene	In the production of pharmaceutical drugs and perfumes. Cytotoxic, antimicrobial, and anti-inflammatory properties	Soukoulis and Hirsch (2004); Sharifi-Rad et al. (2015)
p-Cymene	Manufacturing of p-cresol and carvacrol, used as a solvent and heat transfer fluid, feedstock for the polycyclic musk (fixolide or tonalide), perfumery, solvent for dyes and varnishes	Sell (2006); Lewis Sr. (2007); Eggersdorfer (2012); Fahlbusch et al. (2003)
Germacrene D	Antibacterial property, cytotoxicity	Salvador et al. (2011); Olajuyigbe and Ashafa (2014)
Spathulenol	Anesthetic in nature and used to manufacture a drug which is used to cause dilation of the blood vessels	https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:132824 (2018)

References

Angioni A, Barra A, Coroneo V, Dessi S, Cabras P (2006) Chemical composition, seasonal variability, and antifungal activity of *Lavandula stoechas* L. ssp. stoechas essential oils from stem/leaves and Flowers. J Agric Food Chem 54:4364–4370

Bahi A, Al Mansouri S, Al Memari E, Al Ameri M, Nurulain SM, Ojha S (2014) beta-Caryophyllene, a CB2 receptor agonist produces multiple behavioral changes relevant to anxiety and depression in mice. Physiol Behav 135:119–124

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils – a review. Food Chem Toxicol 46:446–475

K. Başer, G. Buchbauer, Handbook of essential oils: science, technology, and applications, 2010, CRC Press (Taylor & Francis), Boca Ration

Behr A, Johnen L (2009) Myrcene as a natural base chemical in sustainable chemistry: a critical review. ChemSusChem 2:1072–1095

Bhalla Y, Gupta VK, Jaitak V (2013) Anticancer activity of essential oils: a review. J Sci Food Agric 93:3643–3653

Bigley WS, Plapp FW, Hanna RL, Harding JA (1981) Effect of toxaphene, camphene, and cedar oil on methyl parathion residues on cotton. Bull Environ Contam Toxicol 27:90–94

Bingham E, Cohrssen B, Powell CH (2001) Patty's Toxicology, 5th edn. Wiley, New York

Bu'Lock JD (1965) The biosynthesis of natural products; an introduction to secondary metabolism. McGraw-Hill, London/New York

Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol 94:223–253

de Cássia da Silveira e Sá R, Andrade LN, de Sousa DP (2013) A review on anti-inflammatory activity of monoterpenes. Molecules 18:1227–1254

Cavaleiro C, Pinto E, Goncalves MJ, Salgueiro L (2006) Antifungal activity of Juniperus essential oils against dermatophyte, Aspergillus and Candida strains. J Appl Microbiol 100:1333–1338
Chen X, Li Yuan LW, Wang M, Lei Y (2014) Content analysis of Shikimic acid in the Masson pine needles and antiplatelet-aggregating activity. Int J Agric Sci Technol 2:110–114
Croteau R, Kutchan TM, Lewis NG (2000) Natural products (secondary metabolites). In: Biochemistry and molecular biology of plants. American Society of Plant Physiologists, Rock Ville, pp 1250–1318
Dambolena JS, Gallucci MN, Luna A, Gonzalez SB, Guerra PE, Zunino MP (2016) Composition, antifungal and antifumonisin activity of *Pinus wallichiana*, Pinus monticola and *Pinus strobus* essential oils from Patagonia Argentina. J Essent Oil-Bearing Plants 19:1769–1775
van de Braak SAAJ, Leijten GCJJ (1994) A.I. ('s-H.T.O. for Education, Research, Essential oils and oleoresins: a survey in the Netherlands and other major markets in the European Union, CBI, Centre for the Promotion of Imports from Developing Countries
Eggersdorfer M (2012) Terpenes. In: Ullmann's encyclopedia of industrial chemistry, 7th edn. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. https://doi.org/10.1002/14356007.a26_205
Fahlbusch K-G, Hammerschmidt F-J, Panten J, Pickenhagen W, Schatkowski D, Bauer K, Garbe D, Surburg H (2003) Flavors and fragrances. Wiley Online Library, https://doi.org/10.1002/14356007.a11_141
Farjon A (2005) Pines: drawings and descriptions of the genus Pinus. Brill Publishers, Leiden
Filly A, Fernandez X, Minuti M, Visinoni F, Cravotto G, Chemat F (2014) Solvent-free microwave extraction of essential oil from aromatic herbs: From laboratory to pilot and industrial scale. Food Chem 150:193–198
Fulzele SV, Satturwar PM, Kasliwal RH, Dorle AK (2004) Preparation and evaluation of microcapsules using polymerized rosin as a novel wall forming material. J Microencapsul 21:83–89
Gernandt DS, Geada López G, Ortiz García S, Liston A (2005) Phylogeny and classification of Pinus. Taxon 54:29–42
Hajdari A, Mustafa B, Nebija D, Selimi H, Veselaj Z, Breznica P, Quave CL, Novak J (2016) Essential oil composition of *Pinus peuce* Griseb. needles and twigs from two national parks of Kosovo. Sci World J 2016
Hajhashemi V, Ghannadi A, Sharif B (2003) Anti-inflammatory and analgesic properties of the leaf extracts and essential oil of *Lavandula angustifolia* mill. J Ethnopharmacol 89:67–71
Handa SS, Khanuja SPS, Longo G, Rakesh DD (2008) Extraction technologies for medicinal and aromatic plants. ICS UNIDO, Trieste, Italy
Hassan A, Amjid I (2009) Gas chromatography-mass spectrometric studies of essential oil of Pinus roxburghaii stems and their antibacterial and antifungal activities. J Med Plants Res 3:670–673
Hawley GG (1977) Condensed chemical dictionary, 9th edn. Van Nostrand Reinhold Inc., New York
Hong E-J, Na K-J, Choi I-G, Choi K-C, Jeung E-B (2004) Antibacterial and antifungal effects of essential oils from coniferous trees. Biol Pharm Bull 27:863–866
http://avani-kumaon.org/, (n.d.). Accessed on 06-08-2018
http://www.missouribotanicalgarden.org/. Accessed on 06-08-2018
https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:132824. Accessed on 06-08-2018
https://www.kew.org/. Accessed on 06-08-2018
Ioannou E, Koutsaviti A, Tzakou O, Roussis V (2014) The genus Pinus: a comparative study on the needle essential oil composition of 46 pine species. Phytochem Rev 13:741–768
Jensen WB (2007) The origin of the Soxhlet extractor. J Chem Educ 84:1913
Kandi S, Godishala V, Rao P, Ramana KV (2015) Biomedical significance of terpenes: an insight. Biomed Biotechnol 3:8–10
Kaushik D, Kumar A, Kaushik P, Rana AC (2012) Analgesic and anti-inflammatory activity of pinus roxburghii sarg. Adv Pharmacol Sci 2012
Keeley JE (2012) Ecology and evolution of pine life histories. Ann For Sci 69:445–453
Kelkar VM, Geils BW, Becker DR, Overby ST, Neary DG (2006) How to recover more value from small pine trees: essential oils and resins. Biomass Bioenergy 30:316–320

Kimbaris AC, Siatis NG, Daferera DJ, Tarantilis PA, Pappas CS, Polissiou MG (2006) Comparison of distillation and ultrasound-assisted extraction methods for the isolation of sensitive aroma compounds from garlic (*Allium sativum*). Ultrason Sonochem 13:54–60

Klauke A-L, Racz I, Pradier B, Markert A, Zimmer AM, Gertsch J, Zimmer A (2014) The cannabinoid CB(2) receptor-selective phytocannabinoid beta-caryophyllene exerts analgesic effects in mouse models of inflammatory and neuropathic pain. Eur Neuropsychopharmacol 24:608–620

Krauze-Baranowska M, Mardarowicz M, Wiwart M, Pobłocka L, Dynowska M (2002) Antifungal activity of the essential oils from some species of the genus Pinus. Zeitschrift Für Naturforsch C 57:478–482

Lee CM, Lim S, Kim GY, Kim DW, Joon HR, Lee KY (2005) Rosin nanoparticles as a drug delivery carrier for the controlled release of hydrocortisone. Biotechnol Lett 27:1487–1490

Lewis RJ Sr (2007) Hawley's condensed chemical dictionary, 15th edn. Wiley Online Library, https://doi.org/10.1002/9780470114735

Lucchesi ME, Chemat F, Smadja J (2004) Solvent-free microwave extraction of essential oil from aromatic herbs: Comparison with conventional hydro-distillation. J Chromatogr A 1043:323–327

Macchioni F, Cioni PL, Flamini G, Morelli I, Perrucci S, Franceschi A, Macchioni G, Ceccarini L (2002) Acaricidal activity of pine essential oils and their main components against Tyrophagus putrescentiae, a stored food mite. J Agric Food Chem 50:4586–4588

MacTavish WC (1934) A textbook of organic chemistry. Third edition revised (Chamberlain, Joseph Scudder). J Chem Educ 11:633

Mann J, Davidson RS, Hobbs JB, Banthorpe DV, Harborne JB (1994) Natural product-their chemistry and biological significance. Longman, Harlow

Masotti V, Juteau F, Bessière JM, Viano J (2003) Seasonal and phenological variations of the essential oil from the narrow endemic species Artemisia molinieri and its biological activities. J Agric Food Chem 51:7115–7121

Medeiros Leite A, De Oliveira Lima E, Leite De Souza E, De M, Diniz MF, Trajano VN, Almeida De Medeiros I (2007) Inhibitory effect of β-pinene, α-pinene and eugenol on the growth of potential infectious endocarditis causing Gram-positive bacteria. Rev Bras Ciências Farm Brazilian J Pharm Sci 43:121–126

A. Mishra, R. Vlosky, A Case Study of Electricity Generation from Pine Needles in Rural Uttarakhand, India, Louisiana State University Agriculture Center, Baton Rouge (LA) (2015) http://www.woodema.org/conferences/2015_Dubrovnik_presentations/F06_Mishra-Vlosky.pdf

Moreira MR, Ponce AG, Del Valle CE, Roura SI (2005) Inhibitory parameters of essential oils to reduce a foodborne pathogen. LWT – Food Sci Technol 38:565–570

O'Neil MJ (2006) The Merck Index – an encyclopedia of chemicals, drugs, and biologicals. Royal Society of Chemistry (UK)

Ocete MA, Risco S, Zarzuelo A, Jimenez J (1989) Pharmacological activity of the essential oil of Bupleurum gibraltaricum: anti-inflammatory activity and effects on isolated rat uteri. J Ethnopharmacol 25:305–313

Olajuyigbe O, Ashafa A (2014) Chemical Composition and Antibacterial Activity of Essential Oil of *Cosmos bipinnatus* Cav. Leaves from South Africa. Iran J Pharm Res 13:1417–1423

Orav A, Kailas T, Koel M (1998) Simultaneous distillation, extraction and supercritical fluid extraction for isolating volatiles and other materials from conifer needles. J Essent Oil Res 10:387–393

Paduch R, Kandefer-Szerszeń M, Trytek M, Fiedurek J (2007) Terpenes: substances useful in human healthcare. Arch Immunol Ther Exp 55:315–327

Pengelly A (2004) The constituents of medicinal plants: an introduction to the chemistry and therapeutics of herbal medicines, 2nd edn. Allen & Unwin Academic, Crows Nest, New South Wales

Perry NSL, Bollen C, Perry EK, Ballard C (2003) Salvia for dementia therapy: review of pharmacological activity and pilot tolerability clinical trial. Pharmacol Biochem Behav 75:651–659

Richardson DM, Rundel PW (1998), Ecology and biogeography of Pinus: an introduction. In: Richardson, D.M. (Ed.), Ecology and Biogeography of Pinus. Cambridge University Press, Cambridge

Richardson DM, Ryan MG (1998) The complete pine. In: Ecology and biogeography of Pinus. Cambridge University Press, Cambridge
Rivas da Silva AC, Lopes PM, Barros de Azevedo MM, Costa DC, Alviano CS, Alviano DS (2012) Biological activities of α-pinene and β-pinene enantiomers. Molecules 17:6305–6316
Rudback J, Bergstrom MA, Borje A, Nilsson U, Karlberg A-T (2012) alpha-Terpinene, an antioxidant in tea tree oil, autoxidizes rapidly to skin allergens on air exposure. Chem Res Toxicol 25:713–721
Sadhasivam S, Palanivel S, Ghosh S (2016) Synergistic antimicrobial activity of *Boswellia serrata* Roxb. ex Colebr. (Burseraceae) essential oil with various azoles against pathogens associated with skin, scalp and nail infections. Lett Appl Microbiol 63:495–501
Sahraoui N, Vian MA, Bornard I, Boutekedjiret C, Chemat F (2008) Improved microwave steam distillation apparatus for isolation of essential oils. Comparison with conventional steam distillation. J Chromatogr A 1210:229–233
Salvador MJ, de Carvalho JE, Wisniewski-Jr A, Kassuya CAL, Santos ÉP, Riva D, Stefanello MÉA (2011) Chemical composition and cytotoxic activity of the essential oil from the leaves of Casearia lasiophylla. Rev Bras Farmacogn 21:864–868
Schaneberg BT, Khan IA (2002) Comparison of extraction methods for marker compounds in the essential oil of lemon grass by GC. J Agric Food Chem 50:1345–1349
Sell CS (2006) Terpenoids. In: Kirk-Othmer encyclopedia of chemical technology. Wiley Online Library. https://doi.org/10.1002/0471238961.2005181602120504.a01.pub2
Sharifi-Rad J, Sharifi-Rad M, Hoseini-Alfatemi SM, Iriti M, Sharifi-Rad M, Sharifi-Rad M (2015) Composition, cytotoxic and antimicrobial activities of Satureja intermedia C.A.Mey essential oil. Int J Mol Sci 16:17812–17825
Shepard TH (1986) Catalog of teratogenic agents, 5th edn. The Johns Hopkins University Press, Maryland
Silori GK, Pant G, Singh JK, Kumar P (2013) Hay and Tendril classifiable deforestation of pine and its impacts – a survey of Himalayan Region in India. IOSR J Environ Sci Toxicol Food Technol 6:06–14
Silva J, Abebe W, Sousa SM, Duarte VG, Machado MIL, Matos FJA (2003) Analgesic and anti-inflammatory effects of essential oils of Eucalyptus. J Ethnopharmacol 89:277–283
Simonsen JL (1931) The terpenes. Cambridge University Press, Cambridge
Singh B, Sharma RA (2015) Plant terpenes: defense responses, phylogenetic analysis, regulation and clinical applications. 3 Biotech 5:129–151
Soukoulis S, Hirsch R (2004) The effects of a tea tree oil-containing gel on plaque and chronic gingivitis. Aust Dent J 49:78–83
Sui R (2008) Separation of Shikimic acid from pine needles. Chem Eng Technol 31:469–473
Sun J (2007) D-limonene: safety and clinical applications. Altern Med Rev 12:259–264
Thakker MR, Parikh JK, Desai MA (2016) Microwave assisted extraction of essential oil from the leaves of Palmarosa: multi-response optimization and predictive modelling. Ind Crop Prod 86:311–319
Theis N, Lerdau M (2003) The evolution of function in plant secondary metabolites. Int J Plant Sci 164:S93–S102
Thormar H (ed) (2011) Lipids and essential oils as antimicrobial agents, 1st edn. Wiley, Chichester
Tobin T, Swerczek TW, Blake JW (1976) Pine oil toxicity in the horse: drug detection, residues and pathological changes. Res Commun Chem Pathol Pharmacol 15:291–301
Tumen I, Hafizoglu H, Kilic A, Dönmez IE, Sivrikaya H, Reunanen M (2010) Yields and constituents of essential oil from cones of Pinaceae spp. Natively grown in Turkey. Molecules 15:5797–5806
Valente J, Zuzarte M, Goncalves MJ, Lopes MC, Cavaleiro C, Salgueiro L, Cruz MT (2013) Antifungal, antioxidant and anti-inflammatory activities of Oenanthe crocata L. essential oil. Food Chem Toxicol 62:349–354
Verschueren K (2001) Handbook of environmental data on organic chemicals, 4th edn. Wiley, New York

Vian MA, Fernandez X, Visinoni F, Chemat F (2008) Microwave hydrodiffusion and gravity, a new technique for extraction of essential oils. J Chromatogr A 1190:14–17

Xie JY, Lan JF, Yu L, Wu CM (2012) Study on extraction of Shikimic acid from pine needles of *Pinus Elliottii* Engelm by means of microwave pretreatment. Adv Mater Res 455–456:752–759

Xie Q, Liu Z, Li Z (2015) Chemical composition and antioxidant activity of essential oil of six Pinus taxa native to China. Molecules 20:9380–9392

Zafar I, Fatima A, Khan SJ, Rehman Z, Mehmud S (2010) GC-MS studies of needles essential oil of Pinus roxburghaii and their antimicrobial activity from Pakistan. Electron J Environ Agric Food Chem 9:468–473

Zhang S, Jiang J, Luan Q (2016) Genetic and correlation analysis of oleoresin chemical components in slash pine. Genet Mol Res 15

Part IV
Strategies and Technologies for Essential Oil Production

Chapter 11
Biotechnological Approaches to Increase Essential Oil Yield and Quality in Aromatic Plants: The *Lavandula latifolia* (Spike Lavender) Example. Past and Recommendations for the Future

Juan Segura, Jesús Muñoz-Bertomeu, Isabel Mendoza-Poudereux, and Isabel Arrillaga

11.1 Introduction

Aromatic plants are typically defined by their production of so-called essential oils (EOs), which are stored in glandular trichomes, located on the surface of both flowers and leaves (Hallahan 2000; Tissier et al. 2017). These EOs comprise a combination of hydrophobic and volatile compounds of variable chemical nature, and the name "essential" derives from the word "essence," due to the aromatic nature of the oils (Lubbe and Verpoorte 2011). EOs have ecological and physiological functions in plant interactions with biotic and abiotic factors (e.g., pollinator attraction, defense, plant-to-plant communication, thermotolerance, and environmental stress adaptation) and in growth and development (Abbas et al. 2017; Dudareva et al. 2013; Jones et al. 2016; Pichersky and Gershenzon 2002; Vivaldo et al. 2017). On the other hand, EO constituents are of great economic interest for pharmaceutical, food, and flavor industries, cosmetics, perfumery, and aromatherapy (Lobstein and Couic-Marinier 2017; Upson and Andrews 2004; Woronuk et al. 2011).

The *Lavandula* genus of the Lamiaceae family includes approximately 400 registered cultivars, 39 plant species, and an important number of hybrids (Upson 2002; Upson and Andrews 2004). This category of plants is naturally found in the Mediterranean area along with Asia and the Middle East, being typical constituents

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of the degraded Mediterranean shrublands (Rivas-Goday and Rivas-Martínez 1967; Rivas-Martínez 1979) with scarce summer rain and distinct xerophytic characteristics. Plants belonging to the *Lavandula* genus are perennial shrubs, between 100 and 200 cm in height, with characteristic square-shaped stems bearing tomentose linear to oblong-lanceolate leaves; the upper branches have terminal spikes consisting of six- to ten-flowered verticillasters with purplish/white corollas (Tutin et al. 1972). Only 3 out of the 39 *Lavandula* species have a high economic value in the commercial production of EOs; these are *Lavandula angustifolia* Mill. (true lavender), *Lavandula latifolia* Medik. (Spike lavender, Fig. 11.1), and *Lavandula* × *intermedia* Emeric ex Loisel. (lavandin), a sterile hybrid of *L. angustifolia* and *L. latifolia*. Several species within the genus are used as ornamental and melliferous plants, including the three previously mentioned species in addition to *L. dentata* L., *L. stoechas* L., and *L. pedunculata* (Mill.) Cav. (Upson and Andrews 2004; Urwin and Mailer 2008).

Usually, the EOs from *Lavandula* species are obtained similarly to other aromatic plants by steam distillation from either flowering spikes or leaves (fresh or dry); during this process, pressurized water vapor disrupts the glandular trichomes releasing the oil and the mixture condenses thanks to a cooling system. The mixture then enters the *essencier*, a gravity settler, and once decantation is finished, two phases are retrieved (Lesage-Meessen et al. 2015): a supernatant oil, designated EO,

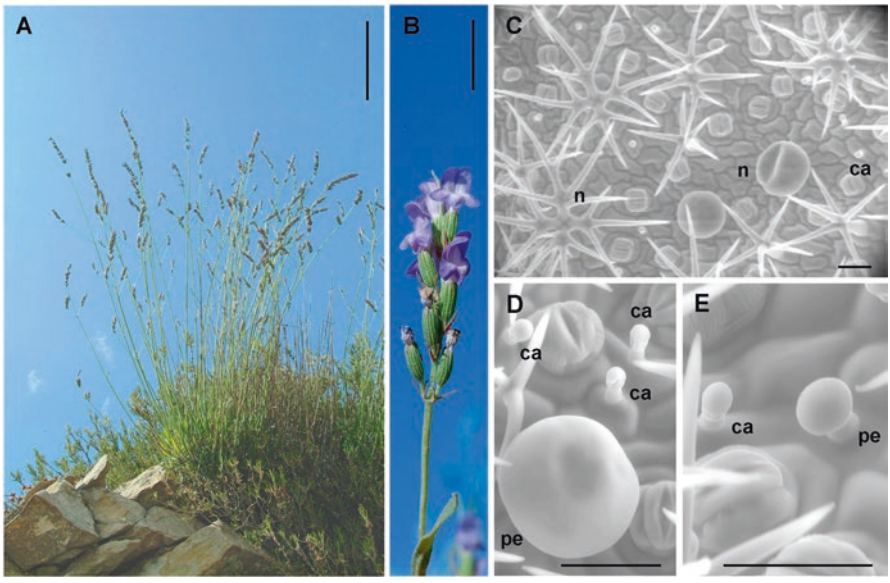

Fig. 11.1 (**a**) Spike lavender in its natural environment; (**b**) detail on spike lavender flowers; (**c**–**e**) spike lavender leaves observed under a scanning electron microscope showing glandular capitate (ca) or peltate (pe) trichomes and non-glandular trichomes (n). (Photo from JM Muñoz-Bertomeu). Bars (**a**) 20 cm; (**b**) 1 cm; (**c**) 50 μm

and the aqueous part, rich in biologically active compounds with applications in cosmetology (Smigielski et al. 2013).

The constituents of an EO may be classified into two principal groups: (1) hydrocarbons (monoterpenes and sesquiterpenes) and (2) oxygenated compounds derived from these hydrocarbons, including alcohols, aldehydes, esters, ketones, phenols, oxides, etc. (Arimura et al. 2017; Ashour et al. 2010).

The amount, chemical composition, and quality of the EOs depend on many genotypic and phenotypic factors, such as variety, the botanical organ they are isolated from, the plant's developmental stage, soil and climatic conditions, season of harvest, drying methods in the case of dry material, or even extraction techniques (Aprotosoaie et al. 2017; Dušková et al. 2016; Kara and Baydar 2013).

Lavender EOs consist of a complex mixture of tens to hundreds of mono- and sesquiterpene alcohols, esters, oxides, and ketones (Daviet and Schalk 2010; Harborne and Williams 2002; Aprotosoaie et al. 2017). The main constituents of these EOs are monoterpenes, such as camphor, 1,8-cineole, linalyl acetate, linalool, β-ocimene, or terpinen-4-ol. Sesquiterpenes (as caryophyllene and nerolidol) and other terpenoids such as perillyl alcohol are also present in traces. A review of the composition of the essential oil from 17 *Lavandula* species has been recently published (Aprotosoaie et al. 2017). These authors provide a systematic view of the chemistry of lavender essential oils mainly considering investigations within the last 15 years. The review reports the characteristic constituents and chemotypes of each studied *Lavandula* species; also, the intra- and interspecific chemical variability of the oils is discussed in relation to the geographic area, onto- and morphogenetic factors, and extraction methods.

The quality of lavender EOs depends first on the amount of desirable major flavor compounds, especially the monoterpenes linalool and linalyl acetate (syn. linalool acetate) that are characteristic terpenes of lavender scent, but also on the specific aromatic bouquet given by several minor compounds (Dušková et al. 2016). Only a few *L. angustifolia* cultivars produce EOs of high quality, which are predominantly used in the perfume industry (Despinasse et al. 2017). Other monoterpenes such as camphor and borneol produce an undesirable odor, diminishing the quality of the oil (Dušková et al. 2016). Nevertheless, those EOs richer in camphor are of interest in aromatherapy and phytotherapy (Herraiz-Peñalver et al. 2013).

Traditionally, spike lavender is used as a raw material in perfumery and cosmetics due to the olfactory properties of its essential oil that contains the monoterpenes camphor, cineol, and linalool as major constituents (Aprotosoaie et al. 2017 and references therein). In addition, this oil exerts major effects on the central nervous system (anxiolytic, sedative, anticonvulsant, analgesic, local anesthetic activity), and it also shows antioxidant, antimicrobial, anti-inflammatory, spasmolytic, and carminative properties, which render it highly appreciated in phytotherapy and aromatherapy (Cavanagh and Wilkinson 2002; El Alaoui et al. 2017; Manion and Widder 2017; Woronuk et al. 2011). Several investigations also report on the biocide action of plant essential oils, including those from lavender, which could potentiate its use as eco-friendly pesticides (Haig et al. 2009; Gómez-Mateo et al. 2016; Varona et al. 2010).

Despite the multipurpose applications, high economic importance, and good adaptation to environmental conditions of spike lavender, its cultivation has been displaced in Spain in the last years by the more productive hybrid lavandin, which is characterized by a higher yield of essential oil per hectare (Renaud et al. 2001) but a lower market price. This situation prompted several investigations in which the phytochemical structure of the natural populations of the species was studied (Herraiz-Peñalver et al. 2013; Muñoz-Bertomeu et al. 2007a; Salido et al. 2004). These publications revealed a great intraspecific variability in the chemical composition of the spike lavender oils that have many implications for the genetic improvement of the species. Knowledge on the partitioning of phytochemical variation within and among populations may assist in the definition of adequate units that may allow selecting genotypes with homogeneous productions in EO quantity and quality. Thus, several plants from populations with linalool-rich EOs identified by Muñoz-Bertomeu et al. (2007a) and Herraiz-Peñalver et al. (2013) may be of interest for selection of parental strains in new crosses and preservation of germplasm. The selected genotypes could be maintained ex situ by applying the available in vitro cloning protocols for the species (for review see Gonçalves and Romano 2013; Segura and Calvo 1991). In addition to conventional methods to select high-yielding essential oil genotypes, the metabolic engineering of the terpene biosynthetic pathways offers an alternative biotechnological way to improve the production of EO in spike lavender, as we shall describe below.

The newest advances in synthetic biology can be used to optimize the design and synthesis of pathways that produce natural products. This includes novel DNA construction technologies and the use of genetic parts for the precise control of expression and for synthetic regulatory circuits, including clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR interference (CRISPRi) (Smanski et al. 2016; Kim et al. 2016). The results reviewed here will help in guiding these new strategies into EO metabolic engineering of this economically important aromatic crop.

11.2 Metabolic Pathways for the Biosynthesis of Essential Oil Constituents in the *Lavandula* Genus

As previously stated, mono- and sesquiterpenes (the C10 and C15 isoprenoids, respectively) are the major fractions of lavender genus EOs. The biosynthesis of these compounds is conceptually divided into four stages (Lange and Ahkami 2013).

Stage 1 encompasses the synthesis of the universal C5 terpene precursor isopentenyl diphosphate (IPP) and its allylic isomer dimethylallyl diphosphate (DMAPP), which in plants are produced via two compartmentalized pathways (Fig. 11.2; Rodríguez-Concepción and Boronat 2002; Vranová et al. 2013; Zebec et al. 2016). One is the methyl-D-erythritol 4-phosphate pathway that starts with the biosynthesis

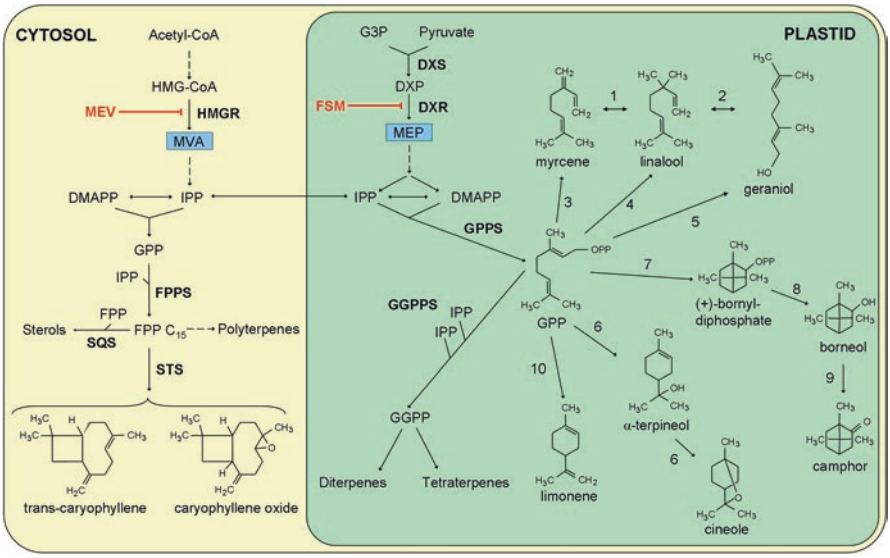

Fig. 11.2 Overview of the biosynthesis of isoprenoids in plants. The following enzymes are indicated in boldface: DXR, 1-deoxy-D-xylulose-5-phosphate reductoisomerase; DXS, 1-deoxy-D-xylulose-5-phosphate synthase; FPPS, farnesyl diphosphate synthase; GGPPS, geranylgeranyl diphosphate synthase; GPPS, geranyl diphosphate synthase; HMGR, 3-hydroxy-3-methylglutaryl CoA reductase; SQS, squalene synthase; STS, sesquiterpene synthase. Enzymes catalyzing monoterpene synthesis are indicated by numbers: (1) linalool dehydratase-hydrolase; (2) geraniol hydroxy-mutase; (3) myrcene synthase; (4) S-linalool synthase; (5) geraniol synthase; (6) cineole synthase; (7) bornyl diphosphate synthase; (8) bornyl diphosphate diphosphatase; (9) borneol dehydrogenase; (10) limonene synthase. The first intermediate specific to each pathway is boxed: DMAPP, dimethylallyl diphosphate; DXP, 1-deoxy-D-xylulose-5-phosphate; FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate; GPP, geranyl diphosphate; G3P, D-glyceraldehyde-3-phosphate; HMG-CoA, 3-hydroxy-3-methylglutaril-CoA; IPP, isopentenyl diphosphate; MEP, methyl-D-erythritol-4-phosphate; MVA, mevalonic acid. Pathways inhibitors: MEV, mevinoline; FSM, fosmidomycin

of 1-deoxy-D-xylulose-5-phosphate (DXP) from pyruvate and glyceraldehyde-3-phosphate (G3P), comprising seven enzymatic steps all located to plastids (Lu et al. 2016). The other is the classical mevalonic acid pathway, comprised of six enzymatic steps located in the cytosol, endoplasmic reticulum, and peroxisomes (Lange and Ahkami 2013). The MEP pathway starts by the transketolase-type condensation, catalyzed by DXP synthase (DXS), of two carbons from pyruvate with G3P to produce DXP (Rohmer 2003). The posterior biosynthesis of MEP, catalyzed by DXP reductoisomerase (DXR), is the preceding step for the C5 units' formation, following the reactions in which the last enzyme of the MEP pathway, hydroxymethylbutenyl diphosphate reductase (HDR), simultaneously synthesizes IPP and DMAPP. HMG-CoA (3-hydroxy-3-methylglutaryl-coenzymeA) reductase (HMGR) converts HMG-CoA to MVA using NADPH as a cofactor, the first committed step of the MVA pathway (Campos and Boronat 1995). Finally, MVA is transformed into IPP by the sequential action of three enzymes: MVA kinase,

phosphomevalonate kinase, and pyrophosphomevalonate decarboxylase (McGarvey and Croteau 1995).

Stage 2 of mono- and sesquiterpene biosynthesis involves condensation reactions of IPP and DMAPP catalyzed by chain-length specific prenyltransferases. The condensation of one molecule each of IPP and DMAPP, catalyzed by geranyl diphosphate synthase, renders geranyl diphosphate (GPP), the C10 precursor of monoterpenes. A condensation of a DMAPP unit with two molecules of IPP generates farnesyl diphosphate (FPP), the C15 direct sesquiterpenes precursor, which is catalyzed by FPP synthase (FPS).

At Stage 3, monoterpenes are produced from GPP by the action of monoterpene synthases, while sesquiterpene synthases transform FPP to various sesquiterpenes. In general, monoterpene synthases localize to plastids, whereas sesquiterpene synthases are cytosolic enzymes (Chen et al. 2011).

Finally, at stage 4, mono- and sesquiterpenes may be further modified through the actions of cytochrome P450 hydroxylases, reductases, dehydrogenases, and transferases to yield a wide range of end products (Woronuk et al. 2011). Figure 11.2 also summarizes the possible biogenetic routes for the most common plastidial-produced acyclic (myrcene and linalool) and cyclic (limonene, α-pinene, cineole, camphor, α-terpineol, and borneol) monoterpenes and the cytosolic sesquiterpenes trans-caryophyllene and its oxygenated derivative, found in spike lavender oils.

11.3 Metabolic Engineering of Monoterpene Biosynthesis in Spike Lavender

Although knowledge about the regulation of the biosynthesis of terpenes is incomplete, several studies (Banerjee and Sharkey 2014; Daviet and Schalk 2010; Dudareva et al. 2004; Rodríguez-Concepción 2006; Vranová et al. 2013) show that the yield of end products depends on both the supply of GPP units and the level of expression of each of the terpene synthases. This allows two possible strategies to undertake the metabolic engineering of monoterpene biosynthesis:

(a) Manipulation of the initial steps, that is, those implicated in the synthesis of IPP and DMAPP that will cause an increased amount of monoterpenes.
(b) The manipulation of the final steps of the pathway, that is, the monoterpene synthases catalyzing the synthesis of monoterpenes; this second approach would cause changes mainly in the qualitative profile of produced monoterpenes.

Both strategies have been employed for the genetic engineering of aromatic plants such as mint (Lange et al. 2011; Mahmoud and Croteau 2002), basil (Wang et al. 2016), true lavender (Lane et al. 2010), and spike lavender. Here we summarize work done to improve the EO yield and quality in spike lavender.

11.3.1 Increasing C5 Precursors

The ways in which the two terpene synthesis pathways can be well regulated are not yet unraveled (Banerjee and Sharkey 2014; Daviet and Schalk 2010; Rodríguez-Concepción 2006; Vranová et al. 2013). It is known that at least three enzymes (DXS, DXR, and HDR) are involved in plastidial pathway (MEP) regulation (Córdoba et al. 2009; Rodríguez-Concepción 2006). The results obtained for both plants and bacteria indicate that the HDR and DXS enzymes mainly control the flux through the said pathway, both at transcriptional and post-transcriptional level in response to out-cellular signals of different nature: metabolic, developmental, or environmental (for review see Córdoba et al. 2009; Rodríguez-Concepción 2006). The role of DXR, responsible for the conversion of DXP into MEP, remains unclear in regard to the regulation of the MEP pathway. Hence, although DXR activity in bacteria did not limit isoprenoid biosynthesis, in plants their regulatory role appears to be species dependent (Rodríguez-Concepción 2006). Furthermore, the MVA pathway is mainly controlled at the HMGR level (Enfissi et al. 2005); it is acknowledged that HMGR activity regulates both the flux through the MVA pathway and the eventual production of isoprenoid end products (Leivar et al. 2011; Rodríguez-Concepción 2006). A recent investigation supports the role of mevalonate kinase (PMK) as an unsuspected regulatory hub in the plant MVA pathway and hint at a role of isopentenyl phosphate (IP) in regulating the formation of both MVA and MEP pathway-derived terpenoids (Henry et al. 2018).

To elucidate the role of DXS, DXR, and HMGR enzymes in the biosynthesis of monoterpenes in spike lavender, all three enzymes have been overexpressed separately. The *DXS* (Muñoz-Bertomeu et al. 2006), *DXR* (Mendoza-Poudereux et al. 2014a), and *HMGR* (Muñoz-Bertomeu et al. 2007b) in *Arabidopsis thaliana* (L.) Heynh genes, under the control of the constitutive CaMV 35S promoter (Fig. 11.3; constructions kindly provided by Professor A. Boronat, University of Barcelona,

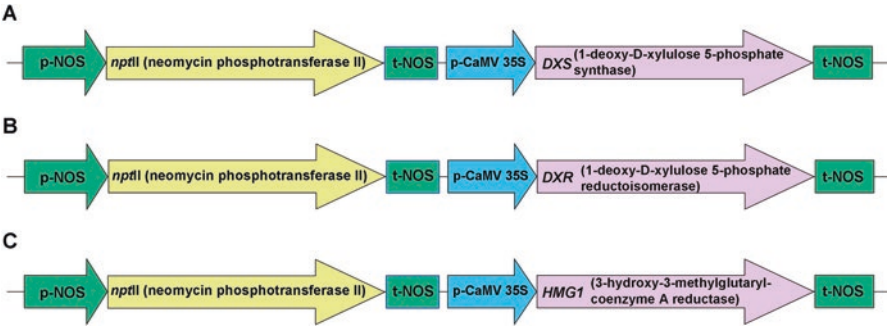

Fig. 11.3 Schematic diagram of the T-DNA constructions employed for genetic transformation of *Lavandula latifolia* using the protocol previously developed by our group (Nebauer et al. 2000). (**a**) pLBI1DXSBS1, (**b**) pLBI1DXSR10, and (**c**) pBICD1 containing a neomycin phosphotransferase II (*npt*II) marker gene and a truncated form of the Arabidopsis (*A. thaliana*) *HMG1* cDNA. All the constructions were kindly provided by Professor A Boronat (University of Barcelona, Spain)

Spain), were transferred into the spike lavender genome. For that, the spike lavender leaf transformation protocol previously described (Nebauer et al. 2000) was followed. T0 and their respective self-derived T1 progenies were carefully characterized, by monitoring for each transgene the copy number and the expression level of the genes, the essential oil profile, and even the photosynthetic pigment content.

11.3.1.1 Upregulation of DXS Increases Essential Oil Production in Spike Lavender Without Apparent Detrimental Effects on Plant Development and Fitness

Transgenic T0 plants overexpressing the *DXS* gene increased their essential oil production from 1.2- to 1.7-fold in flowers and from 2.0- to 4.6-fold in leaves, as compared to controls (Fig. 11.4). Analysis performed on self-derived T1 progenies revealed Mendelian segregation ratios of the *DXS* transgene (data not shown). Besides, T1 plants inheriting the *DXS* transgene had significantly increased essential oil yield. The correlation was especially apparent when comparing in developing leaves *DXS* transgene expression levels and monoterpene content (Fig. 11.5). In addition, overexpression of *DXS* transgene in spike lavender leads to increased

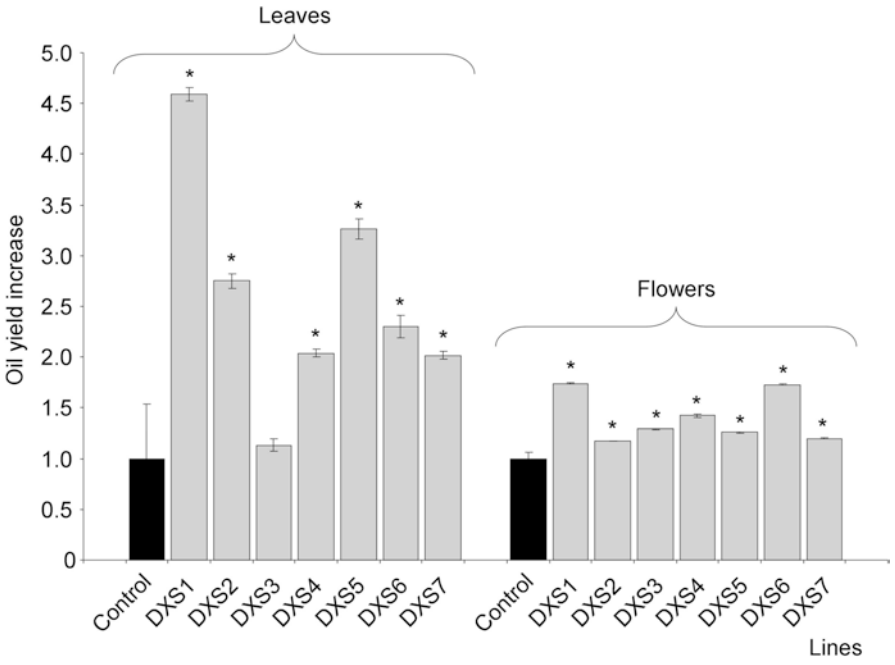

Fig. 11.4 Essential oil yield increase (fold-change) in leaves and flowers of control and transgenic T0 spike lavender plants transformed with *Arabidopsis DXS* gene. Data represent means ± SD of four measurements. (Adapted from Muñoz-Bertomeu et al. 2006)

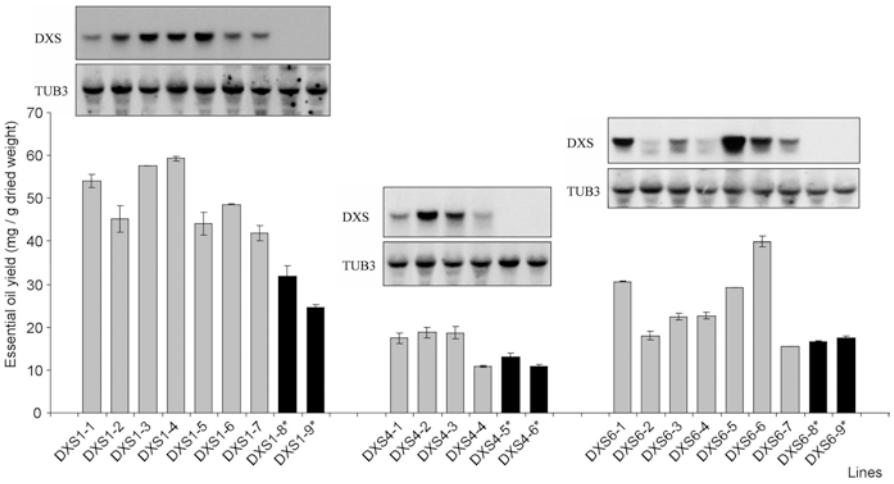

Fig. 11.5 Essential oil yield (mg/g dried weight) from leaves of representative transgenic T1 spike lavender plants obtained from controlled self-pollination of T0 transgenic DXS1, DXS4, and DXS6 lines. Reported values for each T1 plant represent the mean ± SD of four measurements. DXS1-8 and DXS1-9, DXS4-5 and DXS4-6, and DXS6-8 and DXS6-9 are T1 plants that did not inherit the DXS transgene. Northern blotting tests of *DXS* gene for each progeny are also shown. The expression of the *TUB3* gene is shown to verify equal loading. (Adapted from Muñoz-Bertomeu et al. 2006)

linalool content in essential oil from flowers but not from leaves (Muñoz-Bertomeu et al. 2006).

The results available show that metabolic flux control through the MEP pathway differs between spike lavender and peppermint, another economically important Lamiaceae plant species. Thus, upregulation of DXS in *L. latifolia* led to the highest improved essential oil production reported thus far in this species (up to 74% in flowers and up to 359% in leaves (Muñoz-Bertomeu et al. 2006). In contrast, upregulation of the same enzyme in peppermint did not result in significant increase in essential oil production in any of the 28 transgenic lines produced (Lange et al. 2011).

Volatile terpenoid metabolic engineering can inflict an expense on plant fitness and growth, caused by the reduced resource of primary metabolism precursors (Aharoni et al. 2005 and references therein). This is apparently not the case, however, for spike lavender since the photosynthetic pigment content did not differ significantly in *DXS* overexpressing transgenic and control plants. This means *DXS* overexpression did not increase the MEP-derived photosynthetic pigments. These findings imply that constitutive *DXS* overexpression does not translate into equally high DXS activity in all the tissues, organs, and even cellular types in the plant (Muñoz-Bertomeu et al. 2006). As reported by Guevara-García et al. (2005), the MEP pathway is regulated at different levels, both transcriptional and post-transcriptional. This provides an explanation for which *DXS* overexpression in *L. latifolia* raises EO yield in flower and leaf glandular trichomes without increasing

the yield of other terpene-derived compounds in other cell tissues, such as chlorophylls and carotenoids in photosynthetic cells. This makes the *DXS* gene an interesting target for biotechnological interventions for increasing the total yield in this plant species.

11.3.1.2 DXR is Not a Rate-Determining Enzyme for Essential Oil Production in Spike Lavender

DXR enzyme catalyzes the first committed step of the MEP pathway (Carretero-Paulet et al. 2002). When it was first approached, it was expected that upregulation of this enzyme in *L. latifolia* would lead to increased amounts of essential oil, replicating previously accomplished results by overexpressing the *DXS* gene (Muñoz-Bertomeu et al. 2006; see Sect. 11.3.1.1). This was not the general effect in most of the T0 lines (Mendoza-Poudereux et al. 2014a). Only two out of the seven transgenic T0 spike lavender plants that were analyzed produced more essential oils than controls; nevertheless, these increased essential oil phenotypes are hardly imputable to the *DXR* transgene effect since correlation between transcript accumulation and monoterpene production could not be established in T0 (Fig. 11.6; Mendoza-Poudereux et al. 2014a). Specifically, the DXR2 line showed an increase in the essential oil content in leaves (2.0-fold for young leaves and 1.3-fold for mature leaves) and flowers (1.3-fold) and line DXR5 also showed a higher essential oil production than controls but only in the youngest leaves (1.6-fold). Similar results

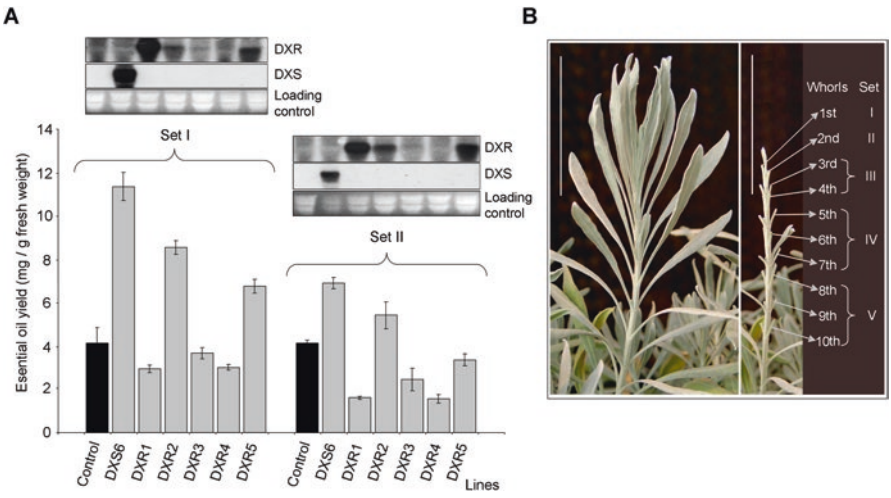

Fig. 11.6 (**a**) Essential oil yield (mg/g fresh weight) in leaves from Set I (first and second whorls) and Set II (third whorl) of control and transgenic T0 DXR and DXS spike lavender plants. Reported values represent the means ± SD of three measurements. Northern blotting tests of *DRS* gene for each set and gel-loading control are also shown. (**b**) Detail of the first to tenth whorl leaves grouped in five developmental stages (Set I–V) used in the experiments (Mendoza-Poudereux et al. 2014a)

were obtained when total chlorophyll and carotenoid content in both T0 transgenic plants and their progenies were analyzed (Mendoza-Poudereux et al. 2014a). These results imply that DXR enzyme does not play a crucial role in the synthesis of plastidial monoterpene precursors and may not be used efficiently for biotechnological purposes. This leaves the DXS enzyme as the main controls point of the flux through the MEP pathway in *L. latifolia*. This is in sharp contrast to what was actually reported for peppermint, where constitutive expression of the *DXR* gene lead to a generalized increase in essential oil yield (Mahmoud and Croteau 2001), which corroborates the differences mentioned earlier in the control of metabolic flux trough the MEP pathway in both Lamiaceae species.

11.3.1.3 Upregulation of HMGR Increases Essential Oil Yields as Well as End Product Phytosterols

As stated in Sect. 11.3.1.1, the upregulation of DXS in spike lavender plants was followed by boosted monoterpene levels. While these findings provide confirmation to the contribution of the MEP pathway in the synthesis of EOs in this species, the possibility that the MVA pathway could provide EOs precursors is not excluded. Likewise, metabolic cross-talk between both the terpene synthesis pathways is well documented (Henry et al. 2018; Vranová et al. 2013 and references therein). To investigate the potential role of the MVA pathway in the synthesis of EOs in *L. latifolia*, a truncated form of the *A. thaliana HMG1* cDNA was introduced and overexpressed in this species (Fig. 11.3) encoding the catalytic domain of the HMGR1S isoform (Muñoz-Bertomeu et al. 2007b). As shown in Fig. 11.7, upregulation of HMGR1S improved EO production. Monoterpenes constituted the largest percentage of the oil production in transgenic *L. latifolia* plants, but sesquiterpenes presented the top increases when compared to controls, with average increases of 1.4- versus 1.8-fold (flowers) and 1.5- versus 3.0-fold (leaves). It is known that sesquiterpenes are formed in the cytoplasm, which could explain this differential behavior (Dudareva et al. 2006). Besides, offspring that inherited the *HMG1* transgene had significantly increased EO production (data not shown). All these findings apparently support the contribution of the MVA pathway in some form to the biosynthesis of EO in *L. latifolia*.

As expected, the *HMGR1S* overexpression also enhanced the sterol amount, specifically stigmasterol and β-sitosterol, in transgenic T0 spike lavender plants (increased averages of almost twofold). This increased sterol phenotype of transgenic T0 *L. latifolia* plants was inherited by their progenies, which confirms that flux leading to stigmasterol and β-sitosterol was under HMGR control (Fig. 11.8). Furthermore, *HMGR1S* overexpression had no effect on the photosynthetic pigments' (chlorophylls and carotenoids) content in those plants (data not shown), confirming previous findings on terpene pathways compartmentalization in photosynthetic tissues (Lichtenthaler 1999).

Taking all these findings, we hypothesized that in *L. latifolia* there is tissue/organ-dependent metabolic cross-talk between MEP and MVA pathways, possibly

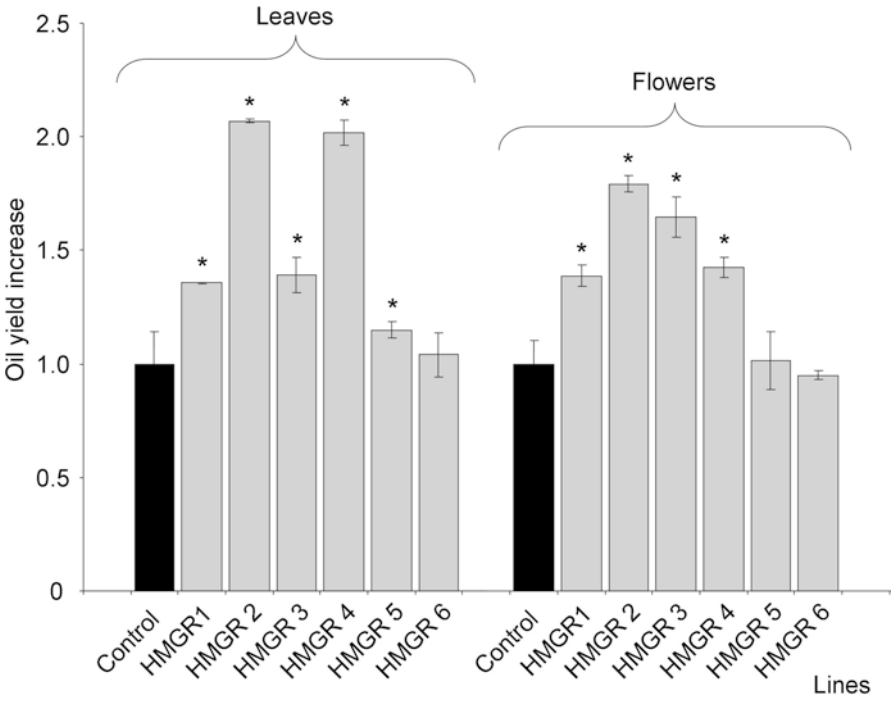

Fig. 11.7 Essential oil yield increase (fold-change) in leaves and flowers of control and transgenic T0 spike lavender plants transformed with the *Arabidopsis HMG1* gene. Reported values represent the mean ± SD of four measurements. (Adapted from Muñoz-Bertomeu et al. 2007b)

through the recently discovered mevalonate kinase-catalyzed pathway (Henry et al. 2018). Apparently, precise compartmentalization in photosynthetic tissues takes place, where the *HMG1* transgene overexpression leads to an increased sterol yield without affecting photosynthetic pigments. Nevertheless, in non-photosynthetic cells of glandular trichomes, the *HMGR1S* overexpression might offer MVA-derived compounds for both plastidial monoterpene and cytosolic sesquiterpene synthesis, heading for a rise in EO production. How exactly the mevalonate kinase-catalyzed pathway (Henry et al. 2018) may have a role on this is still something to be unraveled.

Inhibitors and ^{13}C-Labeling Experiments Demonstrated Cross-Talk Between MVA and MEP Pathways in Spike Lavender

To investigate whether the MVA pathway might be a source of C5 units to the plastids for monoterpene synthesis, both inhibitor-based and ^{13}C labeling experiments were performed using *L. latifolia* seedlings (Mendoza-Poudereux et al. 2015). Particularly, metabolic fluxes of both terpene synthesis pathways were disturbed

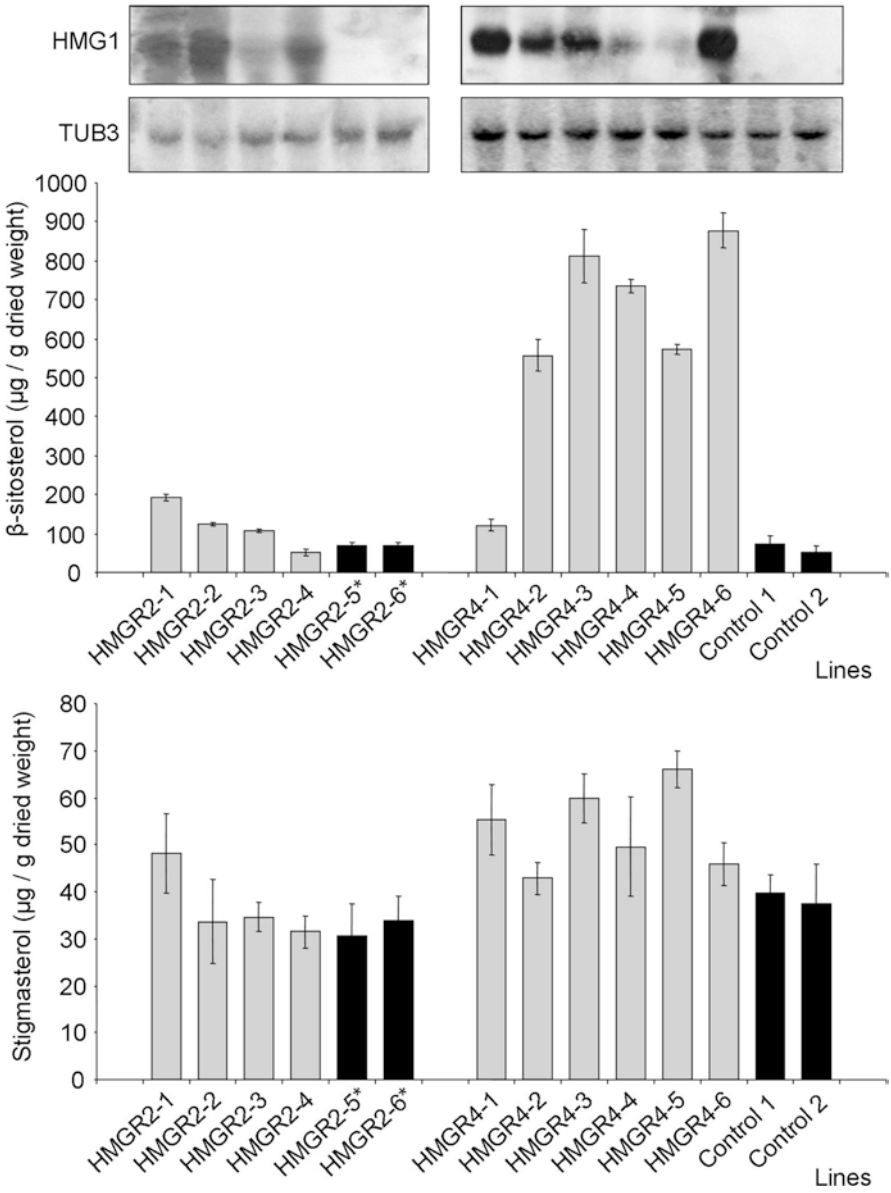

Fig. 11.8 Sterol content (microgram per gram-dried weight) from leaves of representative transgenic T1 spike lavender plants obtained from controlled self-pollination of T0 transgenic HMGR2 and HMGR4 lines. HMGR2-5 and HMGR2-6 are T1 plants that did not inherit the *HMG1* transgene. Reported values for each T1 plant represent the mean ± SD of four measurements. Northern blotting tests of *HMG1* gene for each progeny are also shown. The expression of the *TUB3* gene is shown to verify equal loading. (Adapted from Muñoz-Bertomeu et al. 2007b)

with fosmidomycin (FSM) or mevinolin (MEV), specific inhibitors of the MEP and MVA pathways, respectively (Fig. 11.2). MEV competitively inhibits the HMGR enzyme, while FSM is an inhibitor of the DXR enzyme; both inhibitors have been used in terpene metabolism research before (Bach and Lichtenthaler 1983; Re et al. 1995; Rodríguez-Concepción 2006). Moreover, ^{13}C-labeling experiments using [U-$^{13}C_6$] glucose tracer were performed, attempting to measure the relative contributions of MEP and MVA pathways in monoterpene synthesis (camphor and cineol) of wild-type and *HMGR* overexpressing transgenic plants.

The results showed that MEV concentrations higher than 0.5 mM significantly reduced plant development, but left the photosynthetic pigment or EO synthesis untouched. Instead, FSM concentrations higher than 20 mM blocked the synthesis of photosynthetic pigments or EO and consequently reduced stem development (Mendoza-Poudereux et al. 2015). Mevalonate (MVA) recovered the normal phenotype of plants treated with MEV. Furthermore, in FSM-treated sprouts, MVA partially restored the biosynthesis of photosynthetic pigments (carotenoids and chlorophylls) and, less so, of EOs (Fig. 11.9). These findings suggest that some of

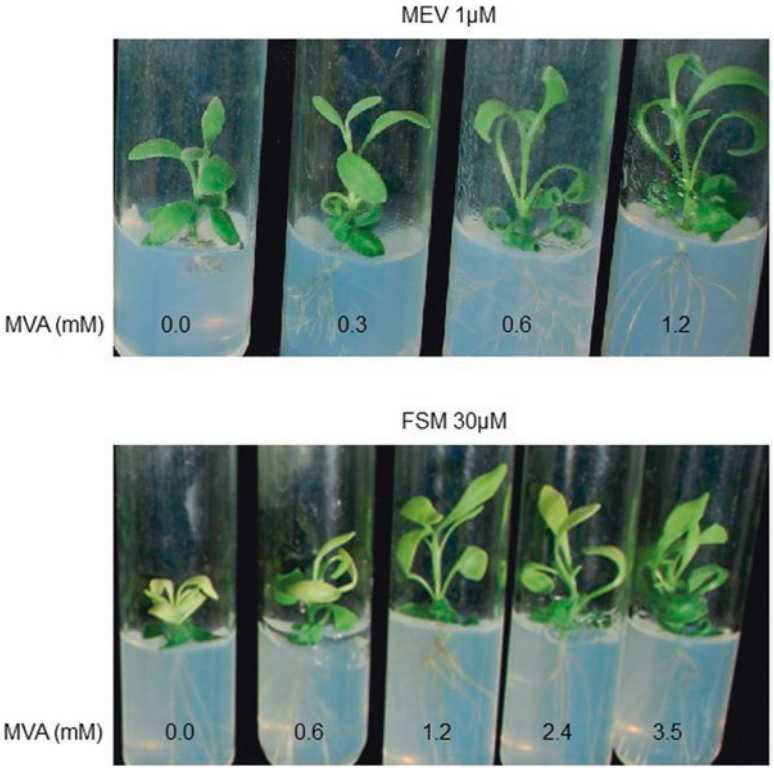

Fig. 11.9 Phenotype recovery with increasing concentrations of MVA (0.0, 0.3, 0.6, 1.2, 2.4, or 3.5 mM) in WT spike lavender shoot apices grown in the presence of 1 mM MEV (top) or 30 μM FSM (bottom). (Adapted from Mendoza-Poudereux et al. 2015)

the IPP flux produced by the cytosolic pathway may be transferred to the chloroplast and be used for the biosynthesis of carotenoids and chlorophylls in *L. latifolia* as previously reported in *Arabidopsis* and tobacco (Hemmerlin et al. 2003; Nagata et al. 2002).

To give a more accurate view of the possible cross-talk between MEP and MVA pathways, labeling experiments in which spike lavender transgenic HMGR5 and control plants were grown on medium containing 2 g/L [U-$^{13}C_6$] glucose and 30 g/L of unlabeled sucrose for 7, 14, 21, or 28 days were performed. After the feeding periods, leaf EO was extracted with chloroform and analyzed by GC/MS. Camphor and 1,8-cineole, the most abundant monoterpenes in the spike lavender leaf EO (Muñoz-Bertomeu et al. 2007b), were chosen as the reference monoterpenes to estimate the relative carbon flux through both terpenes pathway. GC/MS analysis of these monoterpenes indicated that their C5-precursors (IPP and DMAPP) are predominantly biosynthesized via the MEP pathway. However, based on the isotopologue profiles, a contribution of the MVA pathway, although minor, was patent. This contribution was bigger in transgenic spike lavender plants overexpressing the HMGR enzyme than in the controls. Both the inhibitor treatments and labeling experiments provide evidence for transport of MVA-derived precursors from the cytosol to the plastids in leaves of spike lavender (Mendoza-Poudereux et al. 2015).

11.3.2 Overexpression of Terpene Synthases Modifies the Leaf Essential Oil Profile in Spike Lavender

To test if the manipulation of the final steps of the monoterpene biosynthetic pathway can be used to modify essential oil profile, the monoterpene limonene (LS) and linalool (LIS) synthases were upregulated in spike lavender (Mendoza-Poudereux et al. 2014b; Muñoz-Bertomeu et al. 2008). Limonene and linalool, the respective products of the catalytic activity of these enzymes (Fig. 11.2), are implicated distinctively in the biological properties and quality of the spike lavender essential oil.

11.3.2.1 Expression of Spearmint Limonene Synthase in Transgenic Spike Lavender Alters Monoterpene Composition in Developing Leaves

Limonene is used as an insecticide to control ectoparasites of pet animals and might be employed for pest and weed control in organic agriculture, once their phytotoxicity on non-target animals has been investigated (Ibrahim et al. 2001). Limonene, however, is a minor constituent of the *L. latifolia* EO (Muñoz-Bertomeu et al. 2007a), and so, hypothetically, it should be possible to raise its yield easily by metabolic engineering.

Spearmint (*Mentha spicata* L.) limonene synthase (*MsLS*) gene, provided by Prof. Croteau (Washington State University, Pullman, USA), was constitutively expressed in spike lavender (Muñoz-Bertomeu et al. 2008). LS enzyme catalyzes the conversion of geranyl diphosphate into limonene (Fig. 11.2). This *LS* transgene overexpression did not consistently affect EO profile from flowers or mixed leaves from fourth to tenth whorls (Muñoz-Bertomeu et al. 2008). It is known that leaf age influences production of monoterpenes in some aromatic species (Dudai et al. 2001; Gershenzon et al. 2000; Turner et al. 2000). Therefore, an experiment using leaves sampled at different developmental stages was designed and completed. EO increase was higher in developing than in mature leaves in all plants (data not shown). The contents of the most common monoterpenes (borneol, camphor, cineole, limonene, linalool, myrcene, α-pinene, and α-terpineol) found in *L. latifolia* oils were increased in developing leaves from the first whorl and decreased over the leaves' lifespan. The content of the sesquiterpene trans-caryophyllene followed the same pattern, but that of its oxygenated derivative increased with leaf age. This robust developmental regulation of mono- and sesquiterpene biosynthesis was reproducibly observed in all transgenic progenies, irrespective of *LS* transgene inheritance (Muñoz-Bertomeu et al. 2008).

Figure 11.10 shows the limonene content in T0 leaves at different developmental stages; it is worth noting that the limonene increase in the youngest transgenic leaves reached up to a 450% compared to control plants, which also matched with the highest transcript accumulation of the *LS* gene. Also, and as expected, *LS* overexpression did not change the chlorophyll and carotenoid content in *L. latifolia* leaves (Muñoz-Bertomeu et al. 2008).

In brief, these results show that spearmint *LS* gene overexpression can modify monoterpene profile in *L. latifolia* oil. The detected alterations in EO composition of the *LS* transgenic plants are age regulated, being especially evident in young leaves.

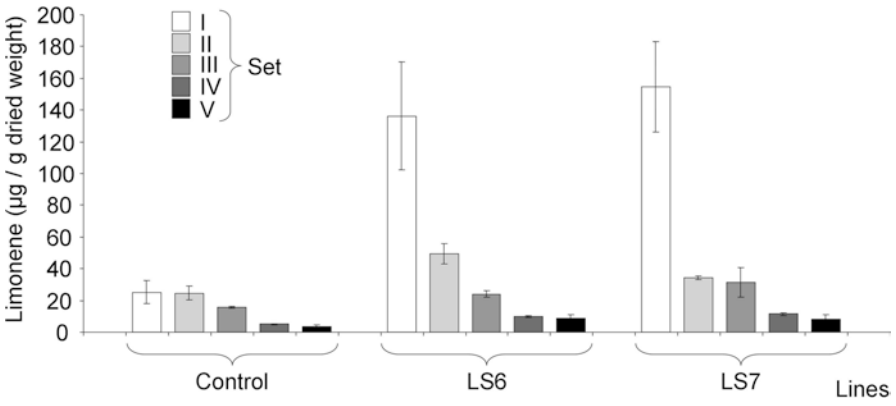

Fig. 11.10 Effect of leaf developmental stage (see Fig. 11.6b) on the production (microgram per gram fresh weight) of limonene in essential oil of controls and T0 transgenic LS6 and LS7 spike lavender lines. Reported values represent the mean ± SD of four measurements (Muñoz-Bertomeu et al. 2008)

11.3.2.2 Expression of Linalool Synthase in Transgenic Spike Lavender Alters Monoterpene Composition in Developing Leaves

Essential oils with high linalool content and low camphor content are preferred for the perfume and cosmetic industries (Dušková et al. 2016). In addition, linalool is a critical precursor for vitamin E, vitamin A, farnesol, citronellol, and ionones and also has antifungal, antimicrobial, and insecticidal properties (Aprotosoaie et al. 2014; Beier et al. 2014; Herman et al. 2016). However, the monoterpene profile of spike lavender essential oil has high relative amounts of camphor and cineole at the expense of linalool and linalyl acetate, which results in a more pungent scent (Lis-Balchin 2002). Consequently, from an economical point of view, the biotechnological breeding of spike lavender is required to improve the quality of its EO by increasing the ratio of linalool to camphor.

The linalool synthase (*LIS*) gene from *Clarkia breweri* (A. Gray) Greene, provided by Professor Pichersky (University of Michigan, USA), was constitutively expressed in spike lavender, and the T0 transgenic plants obtained showed a significant increase in linalool content as compared to control (Mendoza-Poudereux et al. 2014b). The positive effect of *LIS* transgene was particularly striking in the youngest leaves of two transgenic lines, where linalool increased up to a 1000% (Fig. 11.11). This high linalool-producing phenotype was maintained in leaf EO of the progenies that inherited the transgene (Fig. 11.12).

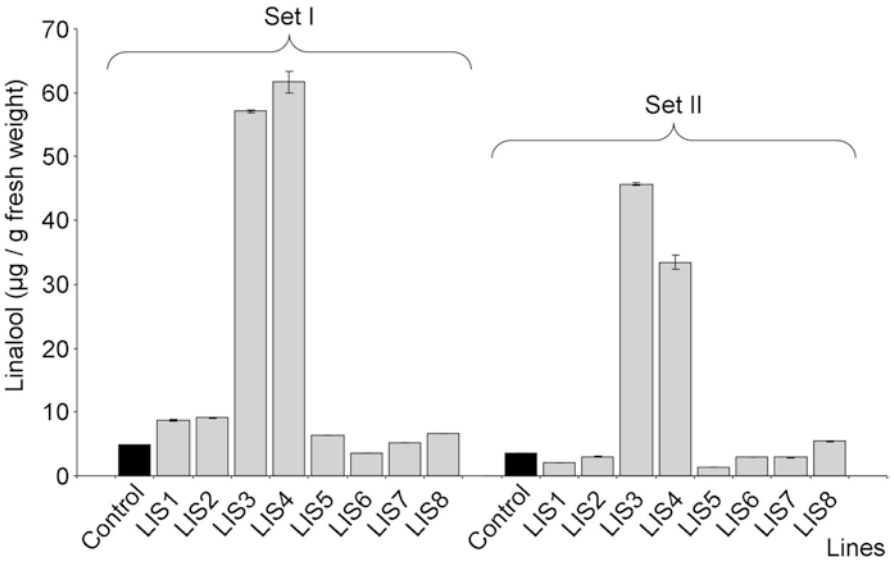

Fig. 11.11 Effect of leaf developmental stage (Set I and II, see Fig. 11.6B) on the production (microgram per gram fresh weight) of linalool in essential oil of controls and T0 transgenic LIS spike lavender lines. Reported values represent the mean ± SD of at least three measurements. (Adapted from Mendoza-Poudereux et al. 2014b)

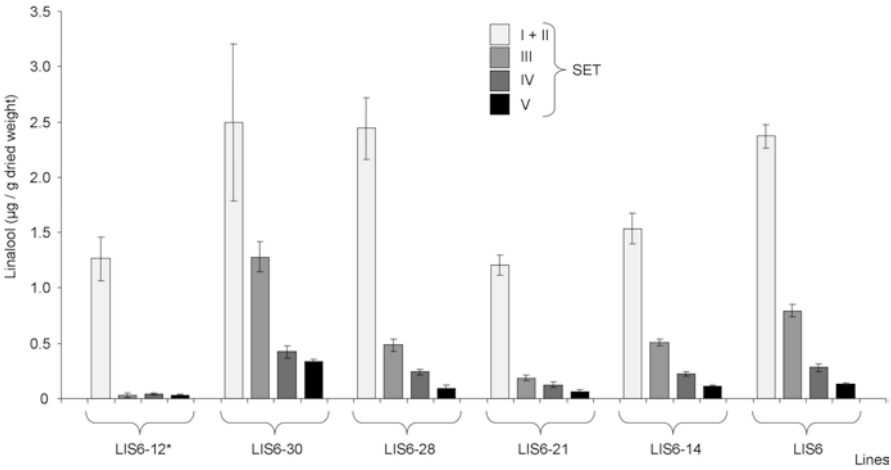

Fig. 11.12 Effect of leaf developmental stage (Sets I–V, see Fig. 11.6b) on the linalool content (microgram per gram of fresh weight) in spike lavender essential oils from transgenic T0 LIS6 and some of their T1 progenies. LIS6-12 T1 progeny did not inherit the *LIS* transgene. Reported values represent the mean ± SD of at least three measurements. (Adapted from Mendoza-Poudereux et al. 2014b)

Interestingly, EO from these transgenic spike lavender flowers did not show the increased linalool phenotype found in the leaves. Linalool content is the main difference between leaf and flower spike lavender EO (traces in leaves and more than 15% of the total oil in flowers), which suggest a strong spatial regulation of the LIS enzyme as has been reported for other monoterpene synthases (Dudareva et al. 2004; Irmisch et al. 2012; Tholl 2006). Based on our results, we hypothesize that the *LIS* transgene only increases linalool content in organs with a low LIS activity (leaves). This is in accordance with previous studies in other plants, where the lack of linalool increase was due to its conversion into another compound in order to store it or as a side effect of the normal metabolism of monoterpenes in plants (Lavy et al. 2002; Lewinsohn et al. 2001; Lücker et al. 2001). A statistical limitation due to the availability of IPP might also be the cause for this phenomenon in flowers.

11.3.3 Transgenic Spike Lavender Plants Co-expressing DXS *and* LIS *Genes Negatively Affect Linalool Content in Leaf Essential Oil*

As stated in Sect. 11.3.2.2, the overexpression of the *LIS* gene changes the monoterpene profile of the spike lavender leaf EO by significantly increasing its linalool content. However, a clear correlation between the overexpression of this gene and

the leaf EO yield could not be established (Mendoza-Poudereux et al. 2014b). Since an increased EO yield in spike lavender is dependent on the C5 unit precursors supply, we undertook controlled crosses to generate double transgenic spike lavender plants that contained both the *DXS* gene, whose overexpression significantly increases EO content in spike lavender (Muñoz-Bertomeu et al. 2006) and the *LIS* gene (Mendoza-Poudereux et al. 2014b). The analysis of the leaf EO showed, however, that both linalool content and EO yield from double transgenic plants were significantly lower than that obtained in the DXS6 mother plant (Fig. 11.6). Northern blotting of double transgenic plants showed that transcript levels of both *DXS* and *LIS* genes were minimal. Because of this, the low EO yield and the reduction in the linalool content of double transgenic plants as compared to the DXS6 mother plant could be explained by assuming effects of co-suppression of the transgenes, as has been already observed in other plant species (Baulcombe 2004; Kanazawa 2008). Thus, the generation of double transgenic spike lavender plants overexpressing both *DXS* and *LIS* genes does not appear to be an appropriate strategy for the biotechnological improvement of spike lavender since neither linalool content nor EO yield could be improved Fig. 11.13.

11.4 Concluding Remarks and Future Prospects

This review demonstrates the potential of the first step of the MEP pathway, catalyzed by the DXS enzyme, as a site for metabolic engineering of the aromatic species spike lavender, where end-product monoterpenes' yield is enhanced by increasing the supply of precursors to specific branches of the isoprenoid pathway. In contrast, DXR enzyme does not play a crucial role in the synthesis of plastidial monoterpene precursors in this species. The upregulation of HMGR enzyme also leads to an increased yield of essential oil in spike lavender, suggesting that MVA pathway also provides C5 precursors for the biosynthesis of monoterpenes. Specific inhibitors and ^{13}C-labeling experiments using [U-$^{13}C_6$] glucose partially support this cross-talk between both pathways.

The constitutive overexpression of either *LS* or *LIS* genes in spike lavender, in which limonene and linalool are minor components of the leaf essential oil, leads to significant increases in the accumulation of these monoterpenes, especially in young leaves, which also indicates a good correlation between *LS* or *LIS* transcript levels and limonene or linalool accumulation.

Given the commercial significance of *Lavandula latifolia* for pharmaceutical, cosmetic, and food industries, the results reviewed here provide useful information for improved breeding. Besides the biotechnologically relevant enhancement in the yield of spike lavender oil, plants stably overexpressing genes of MEP and MVA pathways as well as specific monoterpenes synthases provide a valuable model for studying the monoterpene biosynthesis pathways and their regulatory mechanisms.

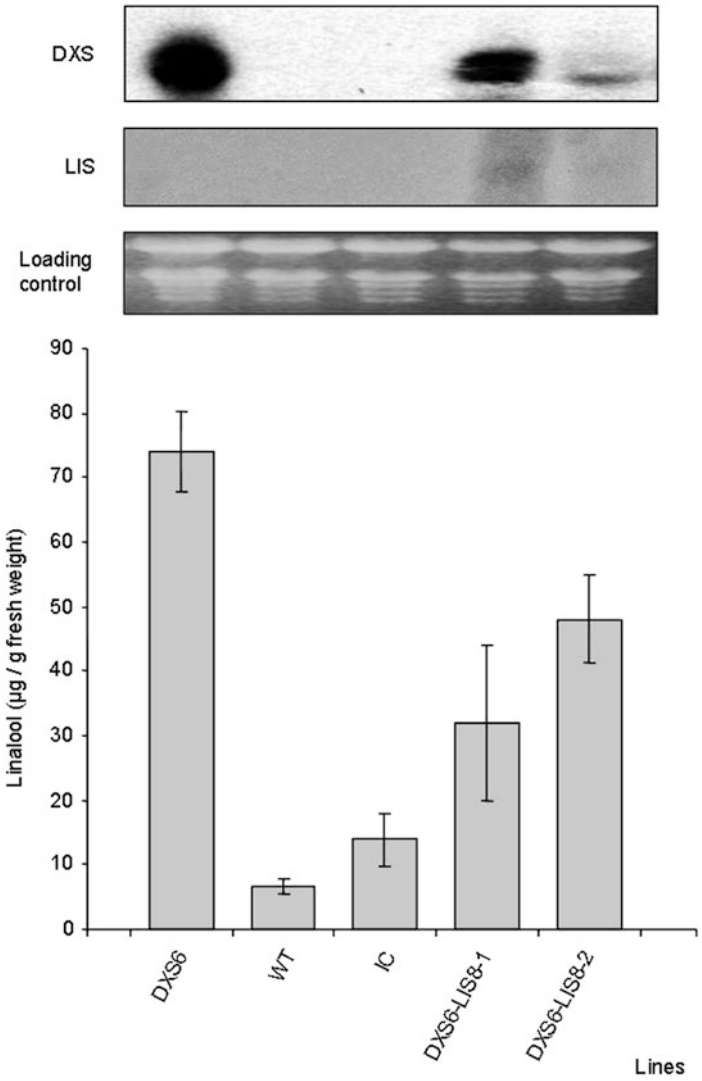

Fig. 11.13 Linalool content (microgram per gram of fresh weight) in pooled leaves from first to third whorls (see Fig. 11.6b) in DXS6 parental and double transgenic DXS6-LIS8 spike lavender plants. WT, wild type plant; IC, internal control (progeny that did not inherit any of the genes). Reported values represent the mean ± SD of three measurements. Expression analysis of the DXS and LIS transgenes in pooled leaves from first to third whorls (see Fig. 11.6b) and gel-loading control are also shown. (Adapted from Mendoza-Poudereux et al. 2014b)

Enhanced mevalonate levels in mutant strains of *Saccharomyces cerevisiae* generated using the CRISP/CAS system have been reported (Jakočiūnas et al. 2015). In addition, by exploiting engineered *Escherichia coli*, harboring a biosynthetic mevalonate (MVA) pathway and plant-derived terpenoid synthases, the

CRISPRi system successfully modulated the expression of all the MVA pathway genes in the context of operon and blocked the transcription of the acetoacetyl-CoA thiolase enzyme that catalyzes the first step in the MVA pathway (Kim et al. 2016). The authors conclude that CRISPRi is revealed as a robust tool for systematic modulation of biosynthetic and endogenous gene expression. Thus, it might be used to tune the metabolic pathway for the biosynthesis of monoterpenes in spike lavender. Here we have presented three genes (*DXS, HMGR,* and *LIS*) that could be targeted to increase the amount and quality of spike lavender EOs and two other genes (*DXR* and *LS*) that could be in principle avoided for any new interventions regarding enhancing monoterpene production, easing the new steps into the metabolic engineering of spike lavender.

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References

Abbas F, Ke Y, Yu R, Yue Y, Amanullah S, Jahangir MM, Fan Y (2017) Volatile terpenoids: multiple functions, biosynthesis, modulation and manipulation by genetic engineering. Planta 246:803–816

Aharoni A, Jongsma MA, Bouwmeester HJ (2005) Volatile science? Metabolic engineering of terpenoids in plants. Trends Plant Sci 10:594–602

Aprotosoaie AC, Hăncianu M, Costache II, Miron A (2014) Linalool: a review on a key odorant molecule with valuable biological properties. Flavour Fragr J 29:193–219

Aprotosoaie AC, Gille E, Trifan A, Luca VS, Miron A (2017) Essential oils of *Lavandula* genus: a systematic review of their chemistry. Phytochem Rev 16:761–799

Arimura G, Matsui K, Koeduka T, Holopainen JK (2017) Biosynthesis and regulation of plant volatiles and their functional roles in ecosystem interactions and global environmental changes. In: Arimura G, Maffei M (eds) Plant specialized metabolism: genomics, biochemistry, and biological functions. CRC Press, Taylor & Francis Group, Boca Raton, pp 185–238

Ashour M, Wink M, Gershenzon J (2010) Biochemistry of terpenoids: monoterpenes, sesquiterpenes and diterpenes. In: Wink M (ed) Biochemistry of plant secondary metabolism. Annu Plant Rev 40. Wiley-Blackwell, Oxford, pp 258–303

Bach TJ, Lichtenthaler HK (1983) Inhibition by mevinolin of plant growth, sterol formation and pigment accumulation. Physiol Plant 9:50–60

Banerjee A, Sharkey TD (2014) Methylerythritol 4-phosphate (MEP) pathway metabolic regulation. Nat Prod Rep 31:1043–1055

Baulcombe D (2004) RNA silencing in plants. Nature 431:356–363

Beier RC, Byrd JA, Kubena LF, Hume ME, McReynolds JL, Anderson RC, Nisbet DJ (2014) Evaluation of linalool, a natural antimicrobial and insecticidal essential oil from basil: effects on poultry. Poult Sci 93:267–272

Campos N, Boronat A (1995) Targeting and topology in the membrane of plant 3-hydroxy-3-methylglutaryl coenzyme A reductase. Plant Cell 7:2163–2174

Carretero-Paulet L, Ahumada I, Cunillera N, Rodríguez-Concepción M, Ferrer A, Boronat A, Campos N (2002) Expression and molecular analysis of the *Arabidopsis DXR* gene encoding

1-deoxy-D-xylulose 5- phosphate reductoisomerase, the first committed enzyme of the 2-C-methyl-D- erythritol-4- phosphate pathway. Plant Physiol 129:1581–1591
Cavanagh HMA, Wilkinson IM (2002) Biological activities of lavender essential oil. Phytother Res 16:301–308
Chen F, Tholl D, Bohlmann J, Pichersky E (2011) The family of terpene synthases in plants: a mid-size family of genes for specialized metabolism that is highly diversified throughout the kingdom. Plant J 66:212–229
Córdoba E, Salmi M, León P (2009) Unravelling the regulatory mechanisms that modulate the MEP pathway in higher plants. J Exp Bot 60:2933–2943
Daviet L, Schalk M (2010) Biotechnology in plant essential oil production: progress and perspective in metabolic engineering of the terpene pathway. Flavour Fragr J 25:123–127
Despinasse Y, Fiorucci S, Antonczak S, Moja S, Bony A, Nicolè F, Baudino S, Magnard JL, Jullien F (2017) Bornyl-diphosphate synthase from *Lavandula angustifolia*: a major monoterpene synthase involved in essential oil quality. Phytochemistry 137:24–33
Dudai N, Larkov O, Ravid U, Putiesky E, Lewinsohn E (2001) Developmental control of monoterpene content and composition in *Micromeria fruticosa* (L.) Druce. Ann Bot 88:349–354
Dudareva N, Pichersky E, Gershenzon J (2004) Biochemistry of plant volatiles. Plant Physiol 135:1893–1902
Dudareva N, Negre F, Nagegowda DA, Orlova I (2006) Plant volatiles: recent advances and future perspectives. Crit Rev Plant Sci 25:417–440
Dudareva N, Klempien A, Muhlemann JK, Kaplan I (2013) Biosynthesis, function and metabolic engineering of plant volatile organic compounds. New Phytol 198:16–32
Dušková E, Dušek K, Indrák P, Smékalová K (2016) Postharvest changes in essential oil content and quality of lavender flowers. Ind Crop Prod 79:225–231
El Alaoui C, Chemin J, Fechtali T, Lory P (2017) Modulation of T-type Ca^{2+} channels by Lavender and Rosemary extracts. PLoS One 12:e0186864
Enfissi EMA, Fraser PD, Lois L, Boronat A, Schuch W, Bramley PM (2005) Metabolic engineering of the mevalonate and non- mevalonate isopentenyl diphosphate-forming pathways for the production of health-promoting isoprenoids in tomato. Plant Biotechnol J 3:17–27
Gershenzon J, McConkey M, Croteau R (2000) Regulation of monoterpene accumulation in leaves of peppermint. Plant Physiol 122:205–213
Gómez-Mateo M, Navarro C, Merino G, Valero A (2016) Evaluation of different Mediterranean essential oils as prophylactic agents in anisakidosis. Pharm Biol 55:456–461
Gonçalves S, Romano A (2013) In vitro culture of lavenders (*Lavandula* spp.) and the production of secondary metabolites. Biotechnol Adv 31:166–174
Guevara-García A, San Román C, Arroyo A, Cortés ME, Gutiérrez-Nava ML, León P (2005) Characterization of the *Arabidopsis clb6* mutant illustrates the importance of posttranscriptional regulation of the methyl-D-erythritol 4-phosphate pathway. Plant Cell 17:628–643
Haig TJ, Haig TJ, Seal AN, Pratley JE, An M, Wu H (2009) Lavender as a source of novel plant compounds for the development of a natural herbicide. J Chem Ecol 35:1129–1136
Hallahan DL (2000) Monoterpenoid biosynthesis in glandular trichomes of labiate plants. Adv Bot Res 31:77–120
Harborne JB, Williams CA (2002) Phytochemistry of the genus *Lavandula*. In: Lis-Balchim M (ed) Lavender. Taylor & Francis Inc, New York, pp 86–99
Hemmerlin A, Hoeffler JF, Meyer O, Tritsch D, Kagan IA, Grosdemange-Billiard C, Rohmer M, Bach TJ (2003) Cross-talk between the cytosolic mevalonate and the plastidial methylerythritol phosphate pathways in tobacco bright yellow-2 cells. J Biol Chem 278:26666–26676
Henry LK, Thomas ST, Widhalm JR, Lynch JH, Davis TC, Kessler SA, Bohlman J, Dudareva N (2018) Contribution of isopentenyl phosphate to plant terpenoid metabolism. Nat Plants 4:721–729
Herman A, Tambor K, Herman A (2016) Linalool affects the antimicrobial efficacy of essential oils. Curr Microbiol 72:165–172
Herraiz-Peñalver D, Cases MA, Varela F, Navarrete P, Sánchez-Vioque R, Usano-Alemany J (2013) Chemical characterization of *Lavandula latifolia* Medik. essential oil from Spanish wild populations. Biochem Syst Ecol 46:59–68

Ibrahim MA, Kainulainen P, Aflatuni A, Tiilikkala K, Holopainen JK (2001) Insecticidal, repellent, antimicrobial activity and phytotoxicity of essential oils: with special reference to limonene and its suitability for control insect pests. Agric Food Sci Finl 10:243–259

Irmisch S, Krause ST, Kunert G, Gershenzon J, Degenhardt J, Köllner TB (2012) The organ-specific expression of terpene synthase genes contributes to the terpene hydrocarbon composition of chamomile essential oils. BMC Plant Biol 12:84

Jakočiūnas T, Bonde I, Herrgård M, Harrison SJ, Kristensen M, Pedersen LE, Jensen MK, Keasling JD (2015) Multiplex metabolic pathway engineering using CRISPR/Cas9 in *Saccharomyces cerevisiae*. Metab Eng 28:213–222

Jones AMP, Shukla MR, Sherif SM, Brown PB, Saxena PK (2016) Growth regulating properties of isoprene and isoprenoid-based essential oils. Plant Cell Rep 35:91–102

Kanazawa A (2008) RNA silencing manifested as visibly altered phenotypes in plants. Plant Biotechnol 25:423–435

Kara N, Baydar H (2013) Determination of lavender and lavandin cultivars (*Lavandula* sp.) containing high quality essential oil in Isparta, Turkey. Turk J Field Crops 18:58–65

Kim SK, Han GH, Seong W, Kim H, Kim SW, Lee DH, Lee SG (2016) CRISPR interference-guided balancing of a biosynthetic mevalonate pathway increases terpenoid production. Metab Eng 38:228–240

Lane A, Boecklemann A, Woronuk GN, Sarker L, Soheil S (2010) A genomics resource for investigating regulation of essential oil production in *Lavandula angustifolia*. Planta 231:835–845

Lange BM, Ahkami A (2013) Metabolic engineering of plant monoterpenes, sesquiterpenes and diterpenes-current status and future opportunities. Plant Biotech J 11:169–196

Lange BM, Mahmoud SS, Wildung MR, Turner GW, Davis EM, Lange I, Baker RC, Boydston RA, Croteau RB (2011) Improving peppermint essential oil yield and composition by metabolic engineering. Proc Natl Acad Sci U S A 108:16944–16949

Lavy M, Zuker A, Lewinsohn E, Larkov O, Ravid U, Vainstain A, Weiss D (2002) Linalool and linalool oxide production in transgenic carnation flowers expressing the *Clarkia breweri* linalool synthase gene. Mol Breed 9:103–111

Leivar P, Antolin-Llovera M, Ferrero S, Closa M, Arro M, Ferrer A, Boronat A, Campos N (2011) Multilevel control of Arabidopsis 3-hydroxy-3-methylglutaryl coenzyme A reductase by protein phosphatase 2A. Plant Cell 23:1494–1511

Lesage-Meessen L, Bou M, Sigoillot JC, Faulds C, Lomascolo A (2015) Essential oils and distilled straws of lavender and lavandin: a review of current use and potential application in white biotechnology. Appl Microbiol Biotechnol 99:3375–3385

Lewinsohn E, Schalechet F, Wilkinson J, Matsui K, Tadmor Y, Nam KH, Amar O, Lastochkin E, Larkov O, Ravid U, Hiatt W, Gepstein S, Pichersky E (2001) Enhanced levels of the aroma and flavor compound S-linalool by metabolic engineering of the terpenoid pathway in tomato fruits. Plant Physiol 27:1256–1265

Lichtenthaler HK (1999) The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. Annu Rev Plant Physiol Plant Mol Biol 50:47–65

Lis-Balchin M (2002) Chemical composition of essential oils from different species, hybrids and cultivars of *Lavandula*. In: Lis-Balchin M (ed) Lavender. Taylor & Francis Inc, New York, pp 251–262

Lobstein A, Couic-Marinier F (2017) Huile essentielle de *Lavande officinale*. Actual Pharm 565:57–60

Lu X, Tang K, Ping P (2016) Plant metabolic engineering strategies for the production of pharmaceutical terpenoids. Front Plant Sci 7:1647

Lubbe A, Verpoorte R (2011) Cultivation of medicinal and aromatic plants for specialty industrial materials. Ind Crop Prod 34:785–801

Lücker J, Bouwmeester HJ, Schwab W, Blaas J, van der Plas LHW, Verhoeven HA (2001) Expressionof *Clarkia* S-linalool synthase in transgenic petunia plants results in the accumulation of S-linalyl-β-D-glucopyranoside. Plant J 27:315–324

Mahmoud SS, Croteau RB (2001) Metabolic engineering of essential oil yield and composition in mint by altering expression of deoxyxylulose phosphate reductoisomerase and menthofuran synthase. Proc Natl Acad Sci U S A 98:8915–8920

Mahmoud SS, Croteau RB (2002) Strategies for transgenic manipulation of monoterpene biosynthesis in plants. Trends Plant Sci 7:366–373
Manion CR, Widder RM (2017) Essentials of essential oils. Am J Health Syst Pharm 74:e153–e162
McGarvey DJ, Croteau R (1995) Terpenoid metabolism. Plant Cell 7:1015–1026
Mendoza-Poudereux I, Muñoz-Bertomeu J, Arrillaga I, Segura J (2014a) Deoxyxylulose 5-phosphate reductoisomerase is not a rate-determining enzyme for essential oil production in spike lavender. J Plant Physiol 171:1564–1570
Mendoza-Poudereux I, Muñoz-Bertomeu J, Navarro A, Arrillaga I, Segura J (2014b) Enhanced levels of S-linalool by metabolic engineering of the terpenoid pathway in spike lavender leaves. Metab Eng 23:136–144
Mendoza-Poudereux I, Kutzner E, Huber C, Segura J, Eisenreich W, Arrillaga I (2015) Metabolic cross-talk between pathways of terpenoid backbone biosynthesis in spike lavender. Plant Physiol Biochem 95:113–120
Muñoz-Bertomeu J, Arrillaga I, Ros R, Segura J (2006) Up-regulation of 1-deoxy-D-xylulose-5-phosphate synthase enhances production of essential oils in transgenic spike lavender. Plant Physiol 142:890–900
Muñoz-Bertomeu J, Arrillaga I, Segura J (2007a) Essential oil variation within and among natural populations of *Lavandula latifolia* and its relation to their ecological areas. Biochem Syst Ecol 35:479–488
Muñoz-Bertomeu J, Arrillaga I, Ros R, Segura J (2007b) Up- regulation of an N-terminal truncated 3-hydroxy-3-methylglutaryl CoA reductase enhances production of essential oils and sterols in transgenic *Lavandula latifolia*. Plant Biotech J 5:746–758
Muñoz-Bertomeu J, Ros R, Arrillaga I, Segura J (2008) Expression of spearmint limonene synthase in transgenic spike lavender results in an altered mono-terpene composition in developing leaves. Metab Eng 10:166–177
Nagata N, Suzuki M, Yoshida S, Muranaka T (2002) Mevalonic acid partially restores chloroplast and etioplast development in Arabidopsis lacking the non-mevalonate pathway. Planta 216:345–350
Nebauer SG, Arrillaga I, del Castillo-Agudo L, Segura J (2000) *Agrobacterium tumefaciens*-mediated transformation of the aromatic shrub *Lavandula latifolia*. Mol Breed 6:539–552
Pichersky E, Gershenzon J (2002) The formation and function of plant volatiles: perfumes for pollinator attraction and defense. Curr Opin Plant Biol 5:237–243
Re EB, Jones D, Learned RM (1995) Co-expression of native and introduced genes reveals cryptic regulation of HMG-CoA reductase expression in *Arabidopsis*. Plant J 7:771–784
Renaud ENC, Charles DJ, Simon JE (2001) Essential oil quantity and composition from 10 cultivars of organically grown Lavender and Lavandin. J Essent Oil Res 13:269–273
Rivas-Goday S, Rivas-Martínez S (1967) Matorrales y tomillares de la Península Ibérica comprendidos en la clase *Ononido-Rosmarinetea* Rr. – Bl. 1947. Anal Inst Bot Cavanilles 25:1–183
Rivas-Martínez S (1979) Brezales y jarales de Europa occidental (Revisión Fitosociológica de las clases *Calluno-ulicetea* y *Cisto-Lavanduletea*). Lazaroa 1:5–127
Rodríguez-Concepción M (2006) Early steps in isoprenoid biosynthesis: multilevel regulation of the supply of common precursors in plant cells. Phytochem Rev 5:1–15
Rodríguez-Concepción M, Boronat A (2002) Elucidation of the methylerythritol phosphate pathway for isoprenoid biosynthesis in bacteria and plastids. A metabolic milestone achieved through genomics. Plant Physiol 130:1079–1089
Rohmer M (2003) Mevalonate-independent methylerythritol phosphate pathway for isoprenoid biosynthesis. Elucidation and distribution. Pure Appl Chem 75:375–388
Salido S, Altarejos J, Nogueras M, Sánchez A, Luque P (2004) Chemical composition and seasonal variations of spike lavender oil from Southern Spain. J Essent Oil Res 16:206–210
Segura J, Calvo MC (1991) *Lavandula* spp. (lavender): in vitro culture, regeneration of plants, and the formation of essential oils and pigments. In: Bajaj YPS (ed) Medicinal and aromatic plants III, biotechnology in agriculture and forestry, vol 15. Springer-Verlag, Berlin-Heidelberg-New York, pp 283–310

Smanski MJ, Zhou H, Claesen J, Shen B, Fischbach MA, Voigt CA (2016) Synthetic biology to access and expand nature's chemical diversity. Nat Rev Microbiol 14:135–149

Smigielski K, Prusinowska R, Krosowiak K, Sikora M (2013) Comparison of qualitative and quantitative chemical composition of hydrolate and essential oils of lavender (*Lavandula angustifolia*). J Essent Oil Res 25:291–299

Tholl D (2006) Terpene synthases and the regulation, diversity and biological roles of terpene metabolism. Curr Opin Plant Biol 9:297–304

Tissier A, Morgan JA, Dudareva N (2017) Plant volatiles: Going "In" but not "Out" of trichome cavities. Trends Plant Sci 22:930–938

Turner GW, Gershenzon J, Croteau RB (2000) Development of peltate glandular trichomes of peppermint. Plant Physiol 124:665–679

Tutin TO, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA (1972) Flora europea, vol 3. University Press, Cambridge

Upson T (2002) The taxonomy of the genus *Lavandula* L. In: Lis-Balchin M (ed) Lavender: the genus *Lavandula*. Taylor & Francis, London, pp 2–34

Upson T, Andrews S (2004) The genus *Lavandula*. Royal Botanic Gardens, Kew

Urwin NAR, Mailer RJ (2008) Oil content and fatty acid profiles of seed oil from the genus *Lavandula*. J Am Oil Chem Soc 85:491–492

Varona S, Kareth S, Martín A, Cocero MJ (2010) Formulation of lavandin essential oil with biopolymers by PGSS for application as biocide in ecological agriculture. J Supercrit Fluids 54:369–377

Vivaldo G, Masi E, Taiti C, Caldarelli G, Mancuso S (2017) The network of plants volatile organic compounds. Sci Rep 7:11050

Vranová E, Coman D, Gruissem W (2013) Network analysis of the MVA and MEP pathways for isoprenoid synthesis. Annu Rev Plant Biol 64:665–700

Wang Q, Reddy VA, Panicker D, Mao HZ, Kumar N, Rajan C, Venkatesh PN, Chua NH, Sarojam R (2016) Metabolic engineering of terpene biosynthesis in plants using a trichome-specific transcription factor *MsYABBY5* from spearmint (*Mentha spicata*). Plant Biotechnol J 14:1619–1632

Woronuk G, Demissie Rheault M, Mahmoud S (2011) Biosynthesis and therapeutic properties of *Lavandul*a essential oil constituents. Planta Med 77:7–15

Zebec Z, Wilkes J, Jervis AJ, Scrutton NS, Takano E, Breitling R (2016) Towards synthesis of monoterpenes and derivatives using synthetic biology. Curr Opin Chem Biol 34:37–43

Chapter 12
The Phytochemical Composition, Biological Effects and Biotechnological Approaches to the Production of High-Value Essential Oil from Geranium

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Abbreviations

BAP	Benzyl amino purine
DXR	1-Deoxy-d-xylulose reductoisomerase
DXS	1-Deoxy-d-xylulose-5-phosphate synthase
HMGR	3-Hydroxy-3-methylglutaryl-CoA reductase
MEP	2-C-Methyl-D-erythritol 4-phosphate
MVA	Mevalonic acid

12.1 Introduction

Geraniaceae family plants (*Pelargonium* sp.) are perennial medicinal and essential oil-yielding branched herbs, growing in subtropical and temperate climates. *Pelargonium* genus comprises more than 750 species, and most of them originated from Europe and Africa. They have remarkable commercial applications due to their characteristic essential oil. The essential oil distillate of a high-value Geraniaceae plant, rose-scented geranium (Fig. 12.1), has a very strong, pleasant and rosy fragrance with a minty top; therefore, it is used as a substitution of the expensive rose oil, and is also known as 'poor man's rose oil'. The essential oil of rose-scented geranium was extensively used as a flavouring agent in the food, cosmetic, perfumery, and pharmaceutical industries. The rose-scented geranium essential oil is also well known for its effectiveness in various health-related treatments such as aromatherapy and for its antimicrobial properties (Narnoliya et al. 2017, 2018a; Jadaun et al. 2017). In India, geranium was introduced in the

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Fig. 12.1 Rose-scented geranium plant

nineteenth century in the southern climate, and now it grows in different parts of India. They are also used as ornamental plants, for example, *Pelargonium x hortorum* and *Pelargonium graveolens*, and grown in gardens and parks to provide a pleasant fragrance (Ravindra and Kulkarni 2015).

There are four species of geranium which have commercial applications: zonal geranium (*Pelargonium x hortorum*), scented geranium (*Pelargonium* sp.), regal pelargonium (*Pelargonium x domesticum*) and regal ivy geranium (*Pelargonium peltatum*). The essential oil of geranium is synthesized in specialized cells known as trichomes. The vegetative and reproductive organs of rose-scented geranium are reported to have non-glandular and glandular trichomes. Generally, glandular trichomes are the major reservoirs for essential oil (Boukhris et al. 2013; Narnoliya et al. 2017). More than 200 species of geranium occur naturally, out of which *P. graveolens*, *P. odoratissimum*, *P. radens*, and *P. capitatum* are more commonly used for harvesting the essential oil. The hydro-distillation method is commonly used for extraction of oil from the aerial part, especially leaves and stem. Essential oil of rose-scented geranium is composed of complex volatile phytochemicals, produced as secondary metabolites, such as terpenes, esters, aldehydes, ketones, alcohols, and phenols. Generally, they play a crucial role in ecological adjustment of the plant and protect it from pathogen and herbivore attacks. Thus, essential oil components are the key substances of rose-scented geranium for its defence system (Babu and Kaul 2005; Jadaun et al. 2017; Ravindra and Kulkarni 2015).

Geranium oil comprises more than 120 phytoconstituents, which include monoterpenes, sesquiterpenes, diterpenes, and low molecular weight aroma compounds. There are three main components, linalool, citronellol and geraniol, and their esters, which constitute more than 60% of total essential oil, and they are responsible for determining its odour. Other components are menthone, nerol, isomenthone, rose

oxides, terpineol, pinene and myrcene (Jadaun et al. 2017; Ravindra and Kulkarni 2015). Thus, terpenes are the major contributors in the essential oil of rose-scented geranium, and these terpenes are biosynthesized through the terpenoid pathway. It was less explored in terms of its genomics, transcriptomics, gene expression and enzyme characterization. Recently, the transcriptomic information of rose-scented geranium leaf has been reported, which provides a foundation for the molecular study of primary and secondary metabolism (Narnoliya et al. 2017, 2018a). Recently, a gene, 1-deoxy-d-xylulose-5-phosphate synthase (DXS) was cloned from this plant and its recombinant protein was physico-kinetically characterized and heterologously overexpressed in *Withania somnifera* to evaluate the effect of DXS on withanolides (Jadaun et al. 2017).

Hence, recent genomics and functionality studies provided a platform to enhance the quantity and quality of essential oil. Using modern biotechnological and synthetic biological approaches, with the aid of available biological information, it could be possible to enhance the productivity of rose-scented geranium's essential oil. Herein, we discuss the phytochemical composition of geranium essential oil, and its biological effects. Further, different biotechnological approaches have also been explored to enhance the production of high-value essential oil.

12.2 Phytochemical Composition of Geranium

Rose-scented geranium is famous for its fragrance produced by its high-value essential oil. Aroma and fragrance of an oil depend on its composition. Essential oil of geranium contains more than 200 types of organic compounds, of which, terpenes, phenylpropanoids, and some other low molecular weight phytoconstituents occur predominantly (Table 12.1). Terpenes constitute the major part of essential oil. Terpene is composed of a five-carbon isoprene unit (CH_2-C (CH_3)-CH-CH_2). The common formula of terpene is $(C_5H_8)_n$, where 'n' is the number of isoprene units. Further, depending on the number of isoprene units, terpenes are classified into different categories such as monoterpenes (2 isoprene units, i.e., 10 carbons), sesquiterpenes (3 isoprene units, i.e., 15 carbons), diterpenes (4 isoprene units, i.e., 20 carbons), triterpenes (6 isoprene units, i.e., 30 carbons) and tetraterpenes (8 isoprene units, i.e., 40 carbons). In essential oil, these terpenes are present either in their simple form or in alcoholic, ketonic, aldehyde, and ester forms, and sometimes as chlorinated or oxygenated derivatives. On the basis of carbon arrangement, these terpenes are present in different structures like acyclic, monocyclic, and bicyclic structures.

The other main component of essential oil is phenylpropanoid, a derivative of the aromatic amino acid phenylalanine, synthesized *via* the shikimic acid pathway. Cinnamic acid and para-hydroxycinnamic acid are the precursors for generation of a variety of phenylpropanoids. Generally, they are present in nonvolatile glycosylated forms, but whenever they are catalysed by enzymatic reactions, the resultant

Table 12.1 Different types of chemicals extracted from rose-scented geranium

Chemical Category	Examples of Chemicals
Aliphatic hydrocarbons	Butane; isoprene; 1,3-pentadiene; hexane; isooctane; octadecane; nonadecane; nonadecene; eicosane; henicosane; decosane; tricosane; tetracosane; pentacosane
Aromatic hydrocarbons	Toluene; p-cymene
Terpene hydrocarbons	α-Pinene; β-pinene; α-phellandrene; β-phellandrene; camphene; myrcene; sabinene; limonene; γ-terpinene; terpinolene; cis-β-ocimene; trans-β-ocimene; dehydro-1,8-cineole; 1,4-cineole; p-menthadiene; perillene; piperitone
Sesquiterpene hydrocarbons	α-Copaene; α-cadinene; γ-cadinene; δ-cadinene; guaia-6-9-diene; β-bisabolene; α-calcorene, calamenene, β-selinene, α-muurolene; γ-muurolene; α-bourbonene; β-bourbonene; 11-orbourbonene; β-caryophyllene; γ-caryophyllene; bicyclo-germacrene; germacrene D; longifolene; β-gurjunene; β-farnesene; (E,E)-α-farnesene; α-cubebene; β-cubebene; β-elemene; β-maaline; α-humulene; viridiflorene; zonzrene; α-ylangene; allo-aromadendrene; selina- 4,11-diene; α-guaiene
Aliphatic alcohols	Methanol; ethanol; t-butanol; pentanol; 1-penten-3-ol-2-propanol; hexanol; 2-methylpropanol; 2-dimethylpropanol; 2-methylbutanol; 2-methyl-3-buten-2-ol; 3-metylbutanol; 3-methylpentan-1-ol; cis-3-hexenol; trans-2-hexenol; 3-hexen-1-ol; octanol; 1-octen-3-ol; 2-octanol
Terpene alcohols	Geraniol; isogeraniol; isopulegol; 7-hydroxy-6, 7-dihydrogeraniol; nerol; epi-photonerol A; linalool; menthol; isomenthol; neoisomenthol; α-terpineol; citronellol; 7-hydroxydyhydrocitronellol; borneol; isoborneol; terpinen-4-ol
Aromatic alcohols	2-Phenylethyl alcohol
Sesquiterpene alcohols	10-Epi-γ-eudesmol; β-eudesmol; 11-selina-4-α-ol; junenol; farnesol; guaiol; spathulenol; T-cadinol; elemol
Aliphatic esters	Methyl formate; methyl butyrate; 2-methylbutyl formate; 3-methybutyl formate; 2-methylpropyl formate; 3-methylpentyl formate; ethyl formate; butyl formate; propyl formate; 2-propyl formate; hexyl formate; benzyl tiglate; (Z)-3-hexenyl acetate
Aromatic esters	2-Phenylethyl tiglate; 2-phenylethyl propionate; 2-phenylethyl butyrate; 2-phenylethyl isobutyrate; 2-phenylethyl isovalerate; 2-phenylethyl acetate
Terpene esters	3-Hexenyl acetate; geranyl formate; geranyl butyrate; geranyl isobutyrate; geranyl 2-methyl butyrate; geranyl tiglate; geranyl acetate; geranyl propionate; geranyl valerate; geranyl 3-methylvalerate; geranyl 4-methylvalerate; geranyl hexanoate; geranyl heptanoate; geranyl nonanoate; geranyl isovalerate; methyl geranate; geranyl 3-methyl pentanoate; geranyl octanoate; citronellyl acetate; citronellyl formate; citronellyl butyrate; citronellyl tiglate; citronellyl propionate; citronellyl valerate; citronellyl 4-methylvalerate; citronellyl isovalerate; citronellyl hexanoate; citronellyl isohexanoate; citronellyl heptanoate; citronellyl octanoate; citronellyl nonanoate; furopelargonic acetate; linalyl acetate; bornyl acetate; neryl acetate; neryl formate

(continued)

Table 12.1 (continued)

Chemical Category	Examples of Chemicals
Aliphatic ketones	Acetone; 2-butanone; 2-pentanone; 3-methyl-2-butanone; 2-methyl-3-pentanone; 4-methyl-2-pentanone; 2-methylcyclopentanone; 3-methylcyclo-pentanone; 3-metylcyclohexanone; 4-methyl-3-penten-2-one; 2-hexanone; methylheptenone; 6-methyl-5-hepten-2-one; methyl-3-methylcyclo-pentenyl ketone
Terpene ketones	Menthone; isomenthone
Sesquiterpene ketones	1,7-Dihydrofurapelargone; furapelargone A; furapelargone B; 7,8 dihydrofurapelargone
Aliphatic aldehydes	Benzaldehyde; ethanol; decanal; 2-methylpropanal; 3-methyl-2-butanal; 3-methylbutanal; 2-furfuraldehyde; nonanal; (E)-2-hexenal
Terpene aldehydes	Geranial; citronellal; neral; photocitrl A; epi-photocitral A; photocitral B; p-menth-1-en-9-al
Terpene oxides	Cis-rose oxide; trans-rose oxide; cis-linalool oxide; trans-linalool oxide; anhydrolinalool oxide; bois-de-rose oxide; nerol oxide
Sesquiterpene oxides	Caryophyllene oxide
Aliphatic acids	Formic acid; propionic acid; acetic acid; caprylic acid
Terpene acids	6-oxo-6-7-dihydrocitronellic acid; geranic acid; citronellic acid
Miscellaneous	Dimethyl sulphide; eugenol; methyl eugenol; furan; α-agarofuran; juniper camphor; vetispirans theaspirans; rose furan; epoxy-rose furan

aglyconic moiety produces a characteristic aroma and flavour (Friedrich 1976; Dudareva et al. 2004).

Thus, essential oil is the key component of rose-scented geranium, and its content in geranium ranges from 0.06% to 0.16%. Approximately 20–170 kg/ha of essential oil was produced by the growers in different locations (Rao 2009). On the basis of available reports in the literature, it is confirmed that citronellol, geraniol, linalool, and citronellyl formate are the major phytoconstituents of geranium essential oil, but their ratio is variable with cultivation zone and season (Džamić et al. 2014). Geranium, grown in Tajikistan, showed that 79 compounds acquire 95.1% of the total essential oil. In this essential oil, 37.5% citronellol, 6% geraniol, 3.7% caryophyllene oxide, 3.1% menthone, 3% linalool, 2.7% β-bourbonene, 2.1% isomenthone, 2.0% geranyl formate, and 3.1% menthone were reported to be present (Sharopov et al. 2014). Citronellol (36.4%), and citronellyl formate (12.1%) were also found as main components of oil isolated from aerial parts of geranium, grown in Isfahan Province and Central Iran (Ghannadi et al. 2012). Essential oil of aerial parts of geranium grown in Dr. Josif Pančić, an institute of medicinal plants, Belgrade, was reported to contain 55 compounds, constituting 99.32% in weight of total oil in which 59.74% and 0.49% comprises oxygenated monoterpenes and monoterpene hydrocarbons, respectively. Citronellol (24.54%), geraniol (15.33%), citronellyl formate (10.66%) and linalool (9.80%) are present predominantly (Džamić et al. 2014).

There are several factors, such as environmental conditions (climate, soil, humidity, fertilizer and seasonal variation), genotypic and physiological conditions of plants and distillation method, which affect the essential oil yield and composition of phytoconstituents in oil (Rao 2009; Sangwan et al. 2001; Verma et al. 2013; Cannon et al. 2013). There is also a clear ageing effect on the composition of oil in geranium, as shown in a report by Rajeswara Rao et al. (1993) that essential oil yield (1.56%) and geraniol content (34.6%) were the highest in the youngest leaf. Essential oil yield not only varies with different cultivars, but even the same cultivar may produce essential oil of altered composition in different seasons. Different cultivars of geranium, such as Bourbon, CIM-Pawan and Kelkar, showed variations in essential oil production from 0.05% to 0.12%, depending on the season of its cultivation (Verma et al. 2014). Oil composition fluctuates with climatic conditions, as citronellol-, nerol-, geraniol- and menthone-rich oil was obtained from plants grown in temperate climates of high-altitude regions; on the other side, isomenthone-, linalool-, citronellyl formate-rich oil was found in plants grown in lower altitudes (Rajeswara Rao et al. 1990).

Apart from climatic conditions, soil conditions also have a significant impact on the essential oil yield in geranium. An experiment was performed on growing geranium plants under water-stress conditions, and it was observed that essential oil yield was indirectly proportional to the duration of interval periods of irrigation (Putievsky et al. 1990). The nearby vegetation in cultivation area also affects oil yield and composition, as reported earlier that presence and absence of weeds affect the ratio of phytoconstituents in oil. However, some experiments showed that growing another crop at a particular distance from geranium crop does not affect the oil composition up to a significant level, and hence, income can be doubled from the same land area (Rajeswara Rao and Bhattacharya 1997; Singh et al. 2013). The phytochemical composition of rose-scented geranium oil is presented in Table 12.1.

12.3 Biosynthesis of Essential Oil

There are two types of phytoconstituents present in rose-scented geranium, that is, terpene (major) and phenylpropanoids (minor). Terpenes are synthesized through the terpenoid/isoprenoid pathway and phenylpropanoids through the shikimate pathway.

12.3.1 Terpene Biosynthetic Pathway

Terpenes are synthesized by the participation of two pathways, one is the cytosolic mevalonate (MVA) or classical acetate pathway, and another is the plastidial non-mevalonate or 2-C-methyl-D-erythritol 4-phosphate (MEP) or glyceraldehyde phosphate/pyruvate or 1-deoxy-D-xylulose 5-phosphate (DXP) pathway (Fig. 12.2).

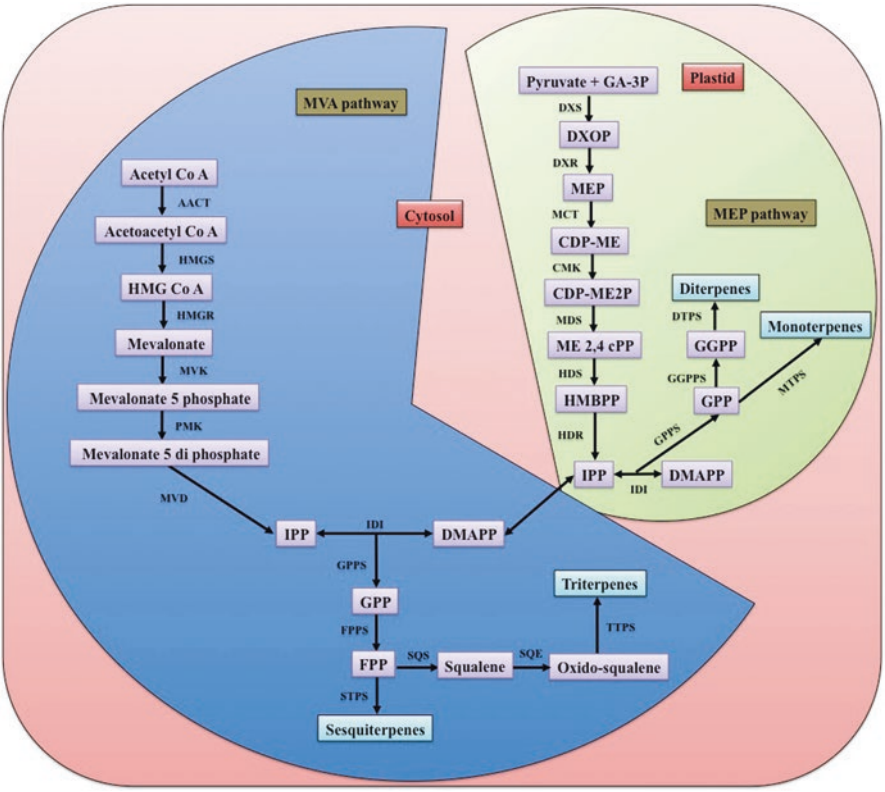

Fig. 12.2 The terpene biosynthetic pathway in rose-scented geranium. AACT acetoacetyl-CoA thiolase/acetyl-CoA acetyltransferase, HMGS hydroxymethylglutaryl-CoA synthase, HMGR hydroxymethylglutaryl-CoA reductase, MVK mevalonate kinase, PMK phosphomevalonate kinase, MVD mevalonate diphosphate decarboxylase, DXS 1-deoxy-D-xylulose 5-phosphate synthase, DXR 1-deoxy-D-xylulose 5-phosphate reductoisomerase, MCT 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase, CMK 4-(cytidine 5′-diphospho)-2-C-methyl-D-erythritol kinase, MDS 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase, HDS (E)-4-hydroxy-3-methylbut-2-enyl diphosphate synthase, HDR (E)-4-hydroxy-3-methylbut 2-enyl diphosphate reductase, IDI isopentenyl-diphosphate delta isomerase, GPPS geranyl diphosphate synthase, FPPS farnesyl pyrophosphate synthase, GGPPS geranylgeranyl diphosphate synthase, MTPS: monoterpene synthase, STPS sesquiterpene synthase, DTPS diterpene synthase, HMG CoA hydroxymethylglutaryl-CoA, IPP isopentenyl pyrophosphate, DMAPP dimethylallyl pyrophosphate GA-3P: glyceraldehyde 3-phosphate, DXOP 1-deoxy-D-xylulose-5-phosphate, MEP 2-C-methyl-d-erythritol-phosphate, CDP-ME 4-(cytidine 5′-diphospho)-2-C-methyl-d-erythritol, CDP-ME2P 2-phospho 4-(cytidine 5′-diphospho)2-c-methyl-d-erythritol, ME 2,4 cPP C-methyl-D-erythritol 2,4-cyclodiphosphate, HMBPP 1-hydroxy-2-methyl-2-butenyl 4-diphosphate, GPP geranyl pyrophosphate, FPP farnesyl pyrophosphate, GGPP geranylgeranyl pyrophosphate, MVA mevalonic acid

There are several evidences to prove that the MVA pathway is operated in cytosol, and endoplasmic reticulum plays a major role in the biosynthesis of sesquiterpenes (C15), triterpenes (C30), and polyterpenes, while the MEP pathway is dedicated for synthesis of isoprenes (C5), monoterpenes (C10), diterpenes (C20), and tetraterpenes (C40). The names of the pathways indicate the name of their first products such as mevalonic acid and 1-deoxy-D-xylulose 5-phosphate in the MVA and MEP pathways, respectively. Isopentenyl pyrophosphate (IPP), and dimethylallyl diphosphate (DMAPP), generated from both the pathways, function as precursors for manufacturing a plethora of terpenes. Terpenes are biosynthesized as a result of several enzymatic reactions catalyzed by terpene synthase enzymes. For many years, MVA was thought to be solely responsible for the synthesis of terpenes, but radioactive tracer experiments revealed the discovery of the MEP pathway for terpene biosynthesis (Lichtenthaler 1999; Eisenreich et al. 2004). Although both pathways contribute to the biosynthesis of geranium essential oil, the MEP pathway is predominantly involved, as evident by the higher concentration of monoterpenes than sesquiterpenes in geranium oil (Jadaun et al. 2017).

The MVA pathway starts with condensation reaction of two molecules of acetyl CoA, which produces acetoacetyl CoA that further undergoes condensation reaction with another acetyl CoA, and forms 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) as the product. This HMG-CoA is reduced into mevalonic acid (MVA) by the action of the enzyme HMG-CoA reductase (HMGR). Mevalonic acid kinase (MVK) and phosphomevalonate kinase (PMK) further convert MVA into mevalonate diphosphate through the phosphorylation process. Mevalonate-5-diphosphate decarboxylase (MPD) catalyses an ATP-coupled decarboxylation reaction for the production of isopentenyl diphosphate (IPP). IPP can be converted into dimethylallyl diphosphate (DMAPP) by an enzyme, IPP/DMAPP isomerase (IDI) (Fig. 12.2). Recently, a modified MVA pathway was proposed in which mevalonate-5-phosphate underwent decarboxylation forming isopentenylphosphate, which can be transformed into isopentenyl diphosphate (IPP) by the enzyme isopentenyl phosphate kinase (IPK) (Chen and Poulter 2010; Hayakawa et al. 2017).

D-Glyceraldehyde 3-phosphate and pyruvate are the precursor molecules in the MEP pathway. They condense together and form the first intermediate 1-deoxy-D-xylulose 5-phosphate (DXP). In the next step, DXP is reductively isomerized by reducto-isomerase (DXR/IspC) into MEP and subsequently couples with cytidine 5′-triphosphate (CTP), generating methyl erythritol cytidyl diphosphate (CDP-ME) by CDP-ME synthetase (IspD). Then, CDP-ME is phosphorylated to produce 4-diphosphocytidyl-2-C methyl-D-erythritol-2-phosphate (CDP-MEP). An ATP-dependent enzyme IspE catalyses this reaction. At the next level, cyclization of CDP-MEP is performed by IspF, which leads to the generation of 2-C-methyl-D erythritol-2,4- cyclodiphosphate (MEcPP). Further, IspG catalysed transformation of MEcPP into 4-hydroxy-3-methylbutenyl 1-diphosphate (HMBPP). The final step of this pathway is performed by the IspH protein, which generates IPP and DMAPP.

For the synthesis of diverse terpenes, isoprene units are joined together in a head-to-tail pattern. Geranyl pyrophosphate (GPP), a monoterpene precursor molecule, is synthesized by the action of the geranyl pyrophosphate synthase (GPPS) enzyme.

Further, addition of one more isoprene unit with GPP by farnesyl pyrophosphate synthase (FPPS) leads to generation of a sesquiterpene precursor, farnesyl pyrophosphate (FPP). Head-to-tail condensation of FPP with IPP produces geranylgeranyl pyrophosphate (GGPP), a diterpene. In the next stage, addition of one more IPP with GGPP forms C25 compounds known as sesterterpene. Interestingly, condensation of farnesyl pyrophosphate (FPP) is used for the synthesis of the C30 triterpene compound (squalene), and in a similar fashion, tail-to-tail condensation of geranylgeranyl pyrophosphate (GGPP) results in the synthesis of C40 molecules, tetraterpenes (Narnoliya et al. 2017, 2018b).

The arrangement of carbon molecules in the chain takes place according to the types of terpenes produced. The most common pattern is cyclization of terpenes, which takes place through generation of an intermediate carbenium ion. For example, heterolysis of the carbon oxygen bond of geranyl pyrophosphate produces geranyl carbocation, and when this carbocation reacts with water, it produces geraniol and subsequently its oxidation leads to synthesis of citral. Granyl carbocation undergoes intramolecular electrophilic addition reaction to generate monocyclic carbocation, which produces limonene after a proton elimination reaction. Further, many other different kinds of intra- and intermolecular interactions are required for the production of diversified terpene molecules (Narnoliya et al. 2017, 2018b).

12.3.2 Phenylpropanoid Biosynthesis

Phenylpropanoids are the aromatic compounds which are synthesized by the shikimate acid pathway, and aromatic amino acids phenylalanine and tyrosine are the precursors of this pathway. The shikimic acid pathway starts with the joining of D-erythrose 4-phosphate with phosphoenol pyruvic acid, and shikimic acid is produced as an intermediate, followed by the generation of chorismate. This chorismate is further utilized in the generation of phenylalanine, which is converted into cinnamic acid, and further, it leads to the generation of multiple types of phenylpropanoids.

12.4 Pharmacological Properties of Geranium

Pelargonium contains a number of pharmacological properties. However, there are very limited reports available on the pharmacological properties of rose-scented geranium. Herein, we discuss the pharmacological properties related to *Pelargonium* sp. including rose-scented geranium.

12.4.1 Antibacterial Properties

Geranium essential oil possesses significant antibacterial properties. Bigos et al. (2012) stated that geranium oil possesses a compelling antibacterial property against clinical isolates of *Staphylococcus aureus* strain ATCC 433000, which contains multidrug-resistant capacity. Due to its antibacterial effect and almost zero toxicity, geranium oil can be used in food processing. During quiche filling, addition of geranium essential oil at different concentrations (250 ppm, 500 ppm and 1000 ppm) showed significant antibacterial property (Lis-Balchin et al. 1998). Essential oil can be applied in combination with other oils or with standard drugs, and results of such combinatorial experiments exhibit unexpectedly higher inhibitory rates. For example, when citricidal™ and geranium oil were applied together, this combination showed a highly inhibitory influence against MRSA (methicillin-resistant *S. aureus*) and geranium oil methicillin-sensitive *S. aureus* (Edwards-Jones et al. 2004). Besides, *S. aureus* is also effective against other gram-positive strains like *Bacillus cereus*, and *Bacillus subtilis* (Silva and Fernandes 2010). Carmen and Hancu (2014) also found that geranium essential oil is able to inhibit the growth of gram-negative bacteria (*E. coli*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*) as well as gram-positive bacteria (*S. aureus*, and *Enterococcus faecalis*). Surprisingly, geranium oil, either in free form or in capsulated form, acts as an inhibitory factor against *Mycobacterium* sp., the most targeted pathogen of current drug professionals. Geranium oil is effective against *M. abscessus*, *M. massiliense*, *M. smegmatis*, and M. *a*vium, even at minimal inhibitory concentrations (MICs) [17.9–35.9 μg/ml] (Giongo et al. 2015). Along with *Mycobacterium* sp., geranium oil has potent antimicrobial activities against other screened pathogenic bacteria such as *S. aureus*, *Streptococcus*, *Staphylococcus*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, and *Salmonella enteritidis* (Giongo et al. 2015).

12.4.2 Antifungal Properties

In addition to the antimicrobial properties against many gram-positive and gram-negative bacteria, geranium oil is also found to be effective against *Candida albicans* and *Cryptococcus neoformans* fungi, causing severe diseases in humans. Further, the effect of the individual component of geranium oil was estimated, and results suggested that citronellol exhibits the most effective fungicidal property, followed by geraniol, isomenthone, geranyl formate and citronellyl formate (Rath et al. 2005). In another study, geranium oil also exhibited significant antifungal property against *C. albicans* (Carmen and Hancu 2014). When essential oil was supplemented in nanocapsule, it exhibited significant inhibitory effects on the growth of more than one *Candida* species, *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, *C. geochares*, *C. magnoliae*, *C. kefyr*, *C. guilliermondii*, *C. catenulata*, *C. membranefaciens*, *C. lusitaniae*, and *C. dubliniensis* (Giongo et al. 2015).

Antifungal properties of *Geranium herbarium* was tested against *Saprolegnia parasitica*, an oomycete pathogen which causes diseases in freshwater fishes, affecting the fish market. In a study, rainbow trout (*Oncorhynchus mykiss*) eggs were infected with *S. parasitica* and then treated with geranium oil at different concentrations (1 ppm, 5 ppm, 10 ppm, 25 ppm, 50 ppm, and 100 ppm). The geranium oil concentration of 100 ppm was noted as minimum inhibitory concentration (MIC) that makes a significant difference from the control (untreated) sample (Khosravi et al. 2012). The essential oil harvested from *Pelargonium graveolens* exhibited remarkable antifungal properties against *Rhizoctonia solani*, a plant pathogenic fungus, and *Malassezia*, a fungus causing skin diseases in animals (Bouzenna and Krichen 2013; Naeini et al. 2011).

12.4.3 Anti-inflammatory and Antioxidant Properties

Essential oil of geranium displayed significant anti-inflammatory properties against mice in which ear oedema was induced by croton oil. Almost 73–88% reduction was obtained at doses of 5 and 10 ml of oil/ear, respectively. Inhibition of inflammation was also confirmed by histological analysis (Nadjib Boukhatem et al. 2013). According to Džamić et al., geranium oil exhibited potent antioxidant properties, and it successfully reduced 2,2-diphenyl-1-picrylhydrazyl (DDPH) radicals in a dose-dependent manner (Džamić et al. 2014).

12.4.4 Insecticidal Properties

The insecticidal/antifeedant property of geranium oil was well known for the past many years against various types of insects (Lis 1996). L-Quisqualic acid (C5H7N3O5), an excitatory amino acid, isolated from the petals of *Pelargonium x hortorum*, displayed a paralytic effect on Japanese beetles (Range et al. 2011). Essential oil of geranium also exhibits insecticidal properties against the insect *Rhyzopertha dominica* at a dose of 50 µl/petri dish, 8.5 cm in diameter (Bouzenna and Krichen 2013).

12.4.5 Anti-neuroinflammatory Properties

Neurodegenerative disorders like Alzheimer's disease are the consequences of neuroinflammation and neural cell death, which are caused by the activation of microglial cells, leading to the production of pro-inflammatory factors like nitric oxide (NO). Application of geranium oil showed an inhibitory effect on NO production with a reduced expression of cyclooxygenase-2 (COX-2) and nitric oxide synthase

(iNOS) enzymes. Interestingly, when individual components were used in the experiment, they were unable to show significant reduction in inflammation; therefore, the inhibitory effect of essential oil is a kind of synergistic effect (Elmann et al. 2010).

12.4.6 Immunomodulatory and Cytoprotective Properties

A drug, EPs® 7630, was formulated using the root extract of *Pelargonium sidoides*, and in Germany, this drug is approved for the treatment of bronchitis. This drug has immunomodulatory and cytoprotective effects, showing an inhibitory effect on the interaction of bacteria with its host cell. Simultaneously, it stimulates the respiratory cells by increasing the ciliary beat frequency of these cells (Moyo and Van Staden 2014). This drug was also tested against various viruses related to respiratory infections, and interestingly, this drug showed inhibitory effects against many tested viruses such as influenza A virus strains (H1N1, H3N2), parainfluenza virus, coxsackie virus and human coronavirus. However, it was found to be ineffective against adenovirus, pathogenic avian influenza A virus (H5N1) or rhinovirus (Michaelis et al. 2011). The cumulative effect of antibacterial and antiviral properties of this drug makes it an efficient drug against respiratory infections. There are a limited number of anti-HIV 1 therapies; therefore, the development of drugs against human immunodeficiency virus (HIV) is a task of global concern. In this direction, the use of aqueous extract of *Pelargonium sidoides* root was also examined against HIV 1, and this extract showed significant anti-HIV 1 activity. The mode of action of this extract is different from previously cited mechanisms as this extract poses an inhibitory impact on the attachment of HIV 1 to host cells, and this happens due to action of its phenolic components (Helfer et al. 2014).

12.5 Genomic Analysis of Geranium

Taxonomic position of geranium placed *Pelargonium* sp. in the Geraniaceae family, and almost all the cultivars grown in different parts of the world are the interspecific hybrids of *P. capitatum* (L.) L'Herit and *P. graveolens* L'Herit or *P. capitatum* (L.) L'Herit and *P. radens* H.E. Moore. Generally these cultivars are diploids with x = 11 and 2n = 77. These cultivars are sterile so only vegetative cutting is the only way of its propagation (Demarne and Van der Walt 1993). In India, the popular cultivars of geranium are Bourbon, Algerian, and Kelkar.

Nowadays, transcriptome and genomics approaches are largely used to map metabolite pathways in organisms of interest. Transcriptomic data from several medicinal and aromatic plants were available, which revealed genomic information about various valuable metabolic pathway enzymes in *Withania somnifera*, *Centella asiatica*, *Azadirachta indica*, *Ocimum* sp., etc. (Gupta et al. 2013; Sangwan et al.

2013; Krishnan et al. 2012; Narnoliya et al. 2014; Rastogi et al. 2014). Although transcriptomic data are available from the Geraniaceae family, such as *Geranium maderense*, *Pelargonium x hortorum* (Zhang et al. 2013), etc., the essential oil pathway (terpenoid pathway) was deeply explored in a transcriptomic study on rose-scented geranium (Narnoliya et al. 2017). In this analysis, a total of 78,943 unique transcripts were reported, out of which 51,802 contigs showed homology-based functional annotation. Further, putative gene(s) representing terpene, ascorbic acid, tartaric acid and anacardic acid (2-hydroxy-6-alkylbenzoic acid), biosynthetic pathways, hormone metabolism and transcription factors were identified. Transcriptomic study also helped in investigating 6040 simple sequence repeats (SSRs) in rose-scented geranium. The genes encoding DXS, 1-deoxy-d-xylulose reductoisomerase (DXR) and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) were successfully cloned, and their expression analysis was conducted in rose-scented geranium. Further, DXS gene was overexpressed homologously (rose-scented geranium) as well as heterologously (*Withania somnifera*), resulting in enhanced production of the essential oil (Jadaun et al. 2017).

Tartaric acid, a high-value food ingredient, is biosynthesized through the catabolism of ascorbic acid *via* two routes, C2/C3 (*via* threonic acid) and C4/C5 (*via* iodic acid) (Debolt et al. 2007; Loews 1999). Geranium is also able to produce a significant quantity of tartaric acid, but the plant species specific preference for the alternative cleavage pathway was unclear. Some reports suggest that geranium follows the C2/C3 cleavage route, but there are insufficient evidences to prove the absence of C4/C5 route. In our recent transcriptome analysis, a putative gene encoding the key regulator of the C4/C5 cleavage reaction, L-idonate-5-dehydrogenase, was annotated with a notable expression level, stipulating the possibilities of occurrence in both the C2/C3 and C4/C5 route for tartaric acid biosynthesis (Narnoliya et al. 2017).

12.6 Economic Status of Geranium Essential Oil

Rose-scented geranium is one of the most important commercial plants of the perfumery industry. Geranium is cultivated throughout the world for its good-quality essential oil with roselike fragrance. The demand for essential oil is flourishing rapidly due to the increased awareness of its beneficial effects. It is not only useful to the perfumery and cosmetic related activities, but also used in the food and beverage industries because of its application as food additive without any known side effects. In the global market, currently, Europe accounts as the major producer of essential oil, but Asia-Pacific is also emerging as a promising continental leader in the near future (Dhananjay et al. 2010). The estimated demand of geranium oil is around 250 tons per year. The United States, France, Germany, United Kingdom, Japan and other European countries represent a good market for geranium oil. The main producers and exporters of geranium oil are China, Egypt, Algeria, and Morocco. On the differences of the origin of geranium cultivars, they

are majorly divided into three main categories—Reunion Island, Egyptian or North African and Chinese. In Reunion Island-type cultivar, the ratio of citronellol and geraniol is almost 1:1, and the other main components are isomenthone, citronellyl formate, and guaia-6,9-diene. Although Egyptian-type oil also contains a 1:1 ratio of citronellol and geraniol, their prominent components are citronellyl formate, isomenthone, and 10-epi-eudesmol. Chinese-type oil is dominated by citronellol and citronellyl formate with low amounts of geraniol. In grading of quality, Reunion type is followed by Egyptian, and then Chinese cultivars. The price of geranium oil ranges from \$55 kg^{-1} to \$110 kg^{-1}, although it depends on oil quality, origin country, and market demand, but still, the price is quite high.

12.7 Tissue Culture Study of Geranium

The primary mode of propagation in geranium is cutting, and it rarely occurs through seeds. To maintain the cutting of geranium, a proper cultivation area is required, and due to seasonal variations, in some countries like India, specific growth chambers for protecting the plants against unfavourable conditions are also required. The number of seeds produced from some of the genotypes of geranium is low, and their viability is also very less; therefore, plant regeneration through seeds is not advisable. The cost of geranium seeds is approximately US\$120/1000 seeds; therefore, propagation through seeds is very expensive. Another drawback of seed propagation in geranium is that for some time, seed-regenerated plants lack specific horticultural attributes like semi-double florets, and non-shattering features (Harney 1982).

Another reason for low cultivation rates in geranium is susceptibility to many diseases, such as bacterial blight (*Xanthomonas campestris* pv. pelargonii, and *Ralstonia solanacearum*), Verticillium wilt, Botrytis blight (*Botrytis cinerea*), root rot (*Pythium* spp.), bacterial fasciation (*Rhodococcus fascians*), rust (*Puccinia pelargonii-zonalis*), *Pelargonium* flower break virus (PFBV), etc. (Bi et al. 1999; Nameth et al. 1999; Swanson et al. 2005). So there is a need to develop suitable technologies for achieving a variety of geranium with good agricultural traits like large leaves with high essential oil yield and disease resistance. The tissue culture technology can be applied for maintenance, and improvement of higher-yielding varieties of geranium. The tissue culture techniques can facilitate maintenance and genetic engineering of the desired character in plants, as per the growing demand of geranium in perfumery, pharmaceutical, and food industries. So, here we present a detailed study of tissue culture techniques used in the refinement of phenotypic, and genotypic constitutions of geranium.

12.7.1 Meristem Tip Culture

In 1952, Morel and Martin for the first time introduced the meristem tip culture technique in plants during the practice of production of virus-free *Dahlia* plants. Afterwards, this technology was used for many ornamental and vegetative plants for production of disease-free plants, whether they were viral, bacterial or fungal diseases (Van Zaayen et al. 1992; Smith 2013; Mohapatra and Batra 2017). Geranium is prone to several pathogens, and the most prominent are *Xanthomonas campestris* pv. pelargonii, *Verticillium dahliae,* and *Botrytis cinerea.* It is necessary to maintain disease-free stocks of cultivars for achieving good market prices (Dunbar 1990). Even chemical treatment is not so fruitful to keep plant infection free because mostly these infections are systemic in nature. Therefore, meristem culture is suggested as a useful technology for production of disease-free plants.

For meristem culture, shoot tips (0.1–0.5 mm) are used as explants for regeneration of plantlets. The plants grown from that meristem explant are further screened for obtaining pathogen-free plants, and presence of pathogen is tested at different stages of growth, and it can be done by keeping the regenerating plantlet under conditions favourable for growth of that pathogen. Modern techniques are more sensitive for testing pathogen contamination in plants. These techniques are enzyme-linked immunosorbent assay (ELISA), colorimetric assays, and polymerase chain reactions (PCR). These techniques are able to detect very low levels of infection. Several protocols have been standardized for production of disease-free geranium plants (Debergh and Maene 1977; Reuther 1982; Mithila et al. 2001).

Virus-infected leaves of *Pelargonium zonale* showed chlorotic rings, and flecks as disease symptoms. Meristem tip culture is a promising method for the production of virus-free plants. Meristem tips are grown on basal media with various combinations of plant growth hormones, and the meristem responds differently in different media. For example, meristem growth in basal media supplemented with α-naphthalene acetic acid (NAA) and coconut milk results only in callus formation, while supplementation of basal medium with low quantities of auxin, indole acetic acid and kinetin results in plantlet formation. Plants regenerated from virus-free meristem as explants are established as stocks of virus-free mother plants for future usage (Hakkaart and Hartel 1979). *Xanthomonas pelargonii*-free *P. x domesticum* is produced by this method (Cassells et al. 1987). Due to some drawbacks, such as low survival rate of explants in initial stages and low numbers of shoot production, this technique has limited applications at the industrial level (Desilets et al. 1993).

12.7.2 In Vitro Organogenesis

Mass propagation of geranium can be achieved by direct or indirect organogenesis. There are several procedures reported to produce plantlets from a variety of explants. Details of these methods, explants, and media composition are described here.

12.7.2.1 Shoot Tip as Explants

Hybrid geranium is largely a male sterile, so cutting or micropropagation is the only way for its propagation. Earlier, Hamdorf (1976) mentioned the experimental details of the regeneration protocol using shoot tips as explants, which are applicable for many varieties of *Pelargonium* hybrids (*P. zonale*, *P. peltatum*, *P. zonalex*, *P. peltatum*, *P. grandiflorum*). By using shoot tip as an explant, shoots were induced by growing the explants on the Murashige and Skoog (MS) medium containing 2.0 mg l^{-1} of zeatin and 1.9 mg l^{-1} of indoleacetic acid (IAA) (Dunbar and Stephens 1989). Desilets et al. (1993) also reported rapid multiplication of the shoot of geranium (*Pelargonium x hortorum*), where axillary meristem was grown on optimized MS media supplemented with 0.11 pM of l-naphthaleneacetic acid (NAA) and 0.89 pM of 6-benzyladenine (BA). Within one month, 40% of explants responded, giving rise to full shoots, and almost a 90% survival rate was observed during the acclimatization process.

12.7.2.2 Leaves and Petioles as Explants

Different hormonal combinations and acclimatization conditions were optimized when leaves or foliar segments were used as explants. The combination of 1.3 mg l^{-1} of benzyl amino purine (BAP) and 0.5 mg l^{-1} of NAA was found as the most efficient combination for direct regeneration of plantlets, and during acclimatization, the substrate containing coconut powder, Biosafra® (12 g l^{-1}), limestone (1 g l^{-1}) and vermiculite (1:1) was supplemented with MS salts (Arrigoni-Blank et al. 2011). Multiple shoots were obtained from mature leaf explants of *Pelargonium rapaceum* (L.) L' Hérit. The best response of explants was observed in the media having a hormonal combination of 0.1 mg l^{-1} of NAA and 0.1 mg l^{-1} of BAP (Sukhumpinij et al. 2010). Similarly, using the tissue culture technique, micropropagation of *Pelargonium sidoides* DC was accomplished successfully, and regenerated plants were hardened in a glasshouse (Theisen and Muller 2012; Moyo et al. 2013). Haploid and diploid cultivars of *P. zonale var* ver 'Kleiner Liebling' plants showed differential patterns in morphological and histological characters during regeneration from stem, leaf and petiole explants. Variations were also seen in response to growth hormones, callusing period and regeneration efficiency (Tuleja et al. 2014).

12.7.2.3 Cotyledons, Hypocotyls and Root as explants

Different parts of a seedling of *Pelargonium x hortorum* Bailey were tested as explants to obtain the best material having significant responding efficiency (Chang et al. 1996). The effects of different factors such as seedling age, growth hormone and excision orientation were studied for regeneration efficiency. Among the tested combination, IAA + zeatin-treated cotyledon explants showed the highest rate of regeneration. Substantial shoots were regenerated when the explant was isolated

from the basal regions of cotyledons of young (2–4 day old) seedlings. The maximum shoot regeneration was observed when hypocotyls were regenerated on IAA + zeatin or thidiazuron-supplemented media, while root explant regeneration was suggested on zeatin-supplemented media. Croke and Cassells (1997) achieved adventitious shoot regeneration from hypocotyl explants of *P. x hortorum*. The caulogenic potential of the root of *Pelargonium x hortorum* FI hybrids was determined by growing root explants on MS media supplemented with tri-iodobenzoic acid (TIBA) and thidiazuron (TDZ), and significant shoot regeneration was obtained (Doyle et al. 1999).

12.7.2.4 Regeneration Using Mature Seeds

Qureshi and Saxena (1992) reported the production of adventitious shoots and somatic embryos directly by culturing the mature seeds of hybrid geranium (*Pelargonium x hortorum* Bailey) on MS media supplemented with different plant growth regulators (BAP, BAP + IAA, and thidiazuron).

12.7.3 Somaclonal Variations: A Novel Source for Crop Improvement

Generally, plants regenerated from tissue culture practices are identical to source plants in morphology and genetic constitution, and these plants are termed somaclones because they are generated from the same group of somatic cells. Sometimes, during the tissue culture process, genetic variations are generated in somaclones which distinguish them from the original plant in morphology as well as in other features also; such kinds of variations in somaclones are termed somaclonal variations. These variations are the sources of new traits which may be helpful for crop improvement, and if these variations are genetically stable for many generations, then these lines can be incorporated in plant breeding programmes (Krishna et al. 2016).

In geranium, many authors reported the occurrence of somaclonal variations, which are different in morphology as well as in phytochemical compositions (Dunbar and Stephens 1989; Gupta et al. 2001; Gupta et al. 2002; Kulkarni et al. 2012; Ravindra et al. 2004; Ravindra and Kulkarni 2015). During the screening of calliclones of geranium, Saxena et al. (2008a) observed two morphotypes that differed in the dentation patterns of leaves: one morphotype was with high dentated leaves (HDLs), and another was with low dentated round leaves (LDLs). HDL and LDL were different in many agronomically important traits, such as flowering time, plant height, canopy size and the number of branches and, the most important one, essential oil composition. After greenhouse trials these morphotypes were grown and observed for genetic stability (Saxena et al. 2008a, b). Two isomenthone-rich

(64.4% and 67.7%) somaclones were obtained during the propagation practice of *Pelargonium* sp. (Kulkarni et al. 1998). Although, these variations in tissue culture may be spontaneous, sometimes, they can be incorporated through some agents like mutagens. In geranium, explants of 'Bourbon' and 'Narmada' cultivars were treated with N-nitroso-N-methylurea (NMU) to obtain somaclonal variations showing better oil yields (Kulkarni et al. 2014). By using somaclonal variation strategies, disease-resistance callus culture of *Pelargonium graveolens* cv. Hemanti was obtained, which further led to the generation of plants resistant against *Alternaria alternata* (Saxena et al. 2008a, b).

12.7.4 Somatic Embryogenesis

Somatic embryogenesis is an alternative process of generation of embryo without fusion of gametes. In this process, the somatic cell or group of somatic cell is developed into an embryo or plants after passing through specific embryological stages without undergoing callus formation (Finer 1995). Somatic embryogenesis helps in raising identical plants as parental with fewer chances of variations. Slimmon et al. (1991) optimized somatic embryogenesis in geranium (*Pelargonium x hortorum Bailey* cv. Scarlet Orbit Improved) and they reported that along with indole acetic acid, other non-indolic compounds like phenylacetic acid (PAA) were capable of inducing somatic embryogenesis (Slimmon et al. 1991). For production of somatic embryo of zonal geranium, petioles and hypocotyls and, for regal geranium, petioles were used as explants (Marsolais et al. 1991). TDZ plays an important role in somatic embryogenesis of geranium, and it was further proved by its application on different explants such as intact seedlings, etiolated hypocotyls and cotyledons (Qureshi and Saxena 1992; Visser et al. 1992; Hutchinson et al. 1997; Haensch 2004).

Croke and Cassells (1997) reported the formation of putative somatic embryos from *Pelargonium x hortorum* Baily using petioles as explants in TDZ-supplemented MS media. Different factors affecting somatic embryogenesis were also studied such as hormonal doses, pH, basal media composition and genotype. In the case of *Pelargonium* × *hederifolium* 'Bonete', when petioles were used as explants, adventitious shoot formation occurred through a mixed role of organogenesis and somatic embryogenesis mode of regeneration. Presence of TDZ along with IBA during the induction phase enhances the rate of formation of adventitious structures, and subsequently, improvement was observed in development of shoots (Wojtania et al. 2004). Somatic embryogenesis was also induced in *Pelargonium sidoides* DC, using somatic cells of inflorescence, shoots and petioles in the presence of TDZ (Duchow et al. 2015). Presence of a symbiotic bacterium positively influenced the somatic embryogenesis phenomenon in *P. x hortorum* (cv. Ringo Rose) (Visser-Tenyenhuis et al. 1994). Later, identification of this bacterium revealed that it has homology with *Bacillus circulans*, and inoculation of this bacterium with explants enhances the regeneration process in *Pelargonium x hortorum* (Murthy et al. 1999).

12.7.5 Genetic Transformation Studies

Genetic transformation technology is a successful tool for improvement of a crop, and till date there are many successful stories in front of us. In geranium, several efforts were put in to achieve model cultivars with desired attributes. The first report of geranium transformation was presented by Pellegrineschi et al. (1994); they infected the stem and leaves with *Agrobacterium rhizogenes*, and the plants that regenerated from these transformed roots exhibited some major changes that differed from the original plant, such as higher leaf and branch number, altered root morphology, reduced height and increased concentration of geraniol and other aromatic components. In 1995, Robichon and his colleagues first showed the *Agrobacterium tumefaciens* (strain EHA101, binary plasmid pKHG3)-mediated transformation of *Pelargonium x hortorum* using cotyledons and hypocotyls as explants (Robichon et al. 1995). All the regenerated plants contained normal morphology and all were fertile. In another study, different strains of *Agrobacterium tumefaciens* (strain LBA4404 and strain LBG66) were used for raising stable transgenic lines of geranium (KrishnaRaj et al. 1997).

Further, an improved method of *Agrobacterium tumefaciens* (strain LBA4404 and binary plasmid pLN54)-mediated genetic transformation of regal pelargonium (*Pelargonium x domesticum* Dubonnet) was established (Boase et al. 1998). In this study, they optimized different factors responsible for enhancement of transformation efficiency such as nature and source of explants, type and concentration of phytohormone and, most importantly, addition of a phenolic substance acetosyringone (100 μM) in the pre-culture and co-cultivation period. Transgenic geranium (*Pelargonium* sp. 'Frensham') plants were raised through *Agrobacterium*-mediated genetic transformation, and the binary vector had a gene encoding an antimicrobial protein, Ace-AMP1. The transgenic plants showed resistance against *Botrytis cinerea*, a pathogen causing leaf infection (Bi et al. 1999). Transgenic plants of *Pelargonium x hortorum* and *P. capitatum* were generated using a modified transformation protocol with two strains of *Agrobacterium tumefaciens*, EHA101 and LBA440 (Hassanein et al. 2005). Many more attempts were made to improve the agronomic traits of geranium such as improvement in oil quality (Saxena et al. 2007). Winkelmann et al. (2005) reported an efficient regeneration system and genetic transformation protocol for *Pelargonium zonale* and *Pelargonium peltatum* hybrids. *Agrobacterium rhizogenes*-mediated genetic transformation of *Pelargonium sidoides* was established for enhancing the content of essential metabolites (Colling et al. 2010). Recently, Singh et al. (2017) showed a modified *Agrobacterium tumefaciens* (LBA4404)-mediated transformation protocol for generation of transgenic plants of *Pelargonium graveolens* (cv. CIM-BIO171).

12.8 Future Aspects

Geranium is an ornamental and medicinally important crop. Traditionally, it is used for treatment of several diseases due to its unique pharmacological properties. Significant work has been undertaken for improvements in its agronomic traits; however, the functional characterization of genes related to its primary as well as secondary metabolite biosynthesis pathways, especially essential oil biosynthesis pathway, is required at a more vigorous pace. Further, their regulatory elements should be identified and isolated so that the pathway machinery could be manipulated in a desired direction. The protocols for in vitro regeneration and plant transformation were well optimized, which are helpful in incorporation of favourable traits in geranium crop. However, the limitation in geranium is that it has so many species and cultivars, which differ at a high level in their morphology and ploidy level, so any technique optimized for one cultivar may or may not work for another cultivar. Therefore, extensive research is required to enhance the market value of a wide range of cultivars. The industrial importance of geranium depends on the quality of essential oil, so efforts are needed to produce such varieties, which can sustain challenges in the marketing route. Therefore, there is a need to produce improved genotypes, either through classical breeding or through genetic engineering approaches, so that plants with improved essential oil profiling and yield, which exhibit resistance to cold seasons and drought, good aroma, large flower size, altered flower colour and disease-resistant properties can be developed. Interspecific hybridization for the pyramiding of desirable genes can be beneficial in obtaining high oil-yielding varieties. Recent biotechnological and synthetic biology avenues have remarkable potential to further improve geranium for its societal importance.

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References

Arrigoni-Blank MD, Almeida SA, Oliveira AC, Blank AF (2011) Micropropagation and acclimatization of geranium (*Pelargonium graveolens* L.). Revista Brasileira de Plantas Medicinais 13(3):271–275

Babu KG, Kaul VK (2005) Variation in essential oil composition of rose scented geranium (*Pelargonium* sp.) distilled by different distillation techniques. Flavour Fragr J 20(2):222–231

Bi YM, Cammue BP, Goodwin PH, KrishnaRaj S, Saxena PK (1999) Resistance to *Botrytis cinerea* in scented geranium transformed with a gene encoding the antimicrobial protein Ace-AMP1. Plant Cell Rep 18(10):835–840

Bigos M, Wasiela M, Kalemba D, Sienkiewicz M (2012) Antimicrobial activity of geranium oil against clinical strains of *Staphylococcus aureus*. Molecules 17(9):10276–10291

Boase MR, Bradley JM, Borst NK (1998) An improved method for transformation of regal pelargonium (*Pelargonium X domesticum* Dubonnet) by *Agrobacterium tumefaciens*. Plant Sci 139(1):59–69

Boukhris M, Nasri-Ayachi MB, Mezghani I, Bouaziz M, Boukhris M, Sayadi S (2013) Trichomes morphology, structure and essential oils of *Pelargonium graveolens* L'Hér.(Geraniaceae). Ind Crop Prod 50:604–610

Bouzenna H, Krichen L (2013) *Pelargonium graveolens* L'Her. and *Artemisia arborescens* L. essential oils: chemical composition, antifungal activity against *Rhizoctonia solani* and insecticidal activity against *Rhyzopertha dominica*. Nat Prod Res 27(9):841–846

Cannon JB, Cantrell CL, Astatkie T, Zheljazkov VD (2013) Modification of yield and composition of essential oils by distillation time. Ind Crop Prod 41:214–220

Carmen G, Hancu G (2014) Antimicrobial and antifungal activity of *Pelargonium roseum* essential oils. Adv Pharm Bull 4(2):511

Cassells AC, Carney BF, McCarthy E, McHugh A, Harmey MA (1987) Problems posed by cultivable bacterial endophytes in the establishment of axenic cultures of *Pelargonium x domesticum* the use of *Xanthomonas pelargonii* specific ELISA, DNA probes and culture indexing in the screening of antibiotic treated and untreated donor plants. Bacterial and bacteria-like contaminants of plant tissue. Cultures 225:153–162

Chang C, Moll BA, Evenson KB, Guiltinan MJ (1996) *In vitro* plantlet regeneration from cotyledon, hypocotyl and root explants of hybrid seed geranium. Plant Cell Tissue Organ Cult 45(1):61–66

Chen M, Poulter CD (2010) Characterization of thermophilic archaeal isopentenyl phosphate kinases. Biochemistry 49:207–217

Colling J, Groenewald JH, Makunga NP (2010) Genetic alterations for increased coumarin production lead to metabolic changes in the medicinally important *Pelargonium sidoides* DC (Geraniaceae). Metab Eng 12(6):561–572

Croke JT, Cassells AC (1997) Dark induction and genetic stability of somatic embryos of zonal geraniums (*Pelargonium x hortorum* Baily). Angew Bot 71(3–4):119–124

Debergh P, Maene L (1977) Rapid clonal propagation of pathogen-free Pelargonium plants starting from shoot tips and apical meristems. Acta Hortic 78:449–454

Debolt S, Melino V, Ford CM (2007) Ascorbate as a biosynthetic precursor in plants. Ann Bot 99:3–8

Demarne FE, Van der Walt JJ (1993) Composition of the essential oil of *Pelargonium citronellum* (Geraniaceae). J Essent Oil Res 5(3):233–238

Desilets H, Desjardins Y, Bélanger RR (1993) Clonal propagation of *Pelargonium x hortorum* through tissue culture: effects of salt dilution and growth regulator concentration. Can J Nurs Res 73(3):871–878

Dhananjay S, Atul K, Mishra BN (2010) Essential oil: economic and herbal importance in aromatherapy. Int J Plant Sci 5(2):431–435

Doyle BM, Lawton DK, Cassells AC (1999) Adventitious regeneration in root culture of a selection of *Pelargonium x hortorum* cultivars: an assessment of caulogenic potential and genetic stability. Acta Hortic 530:225–230

Duchow S, Blaschek W, Classen B (2015) Reproduction of the medicinal plant *Pelargonium sidoides* via somatic embryogenesis. Planta Med 81(12/13):1169–1174

Dudareva N, Pichersky E, Gershenzon J (2004) Biochemistry of plant volatiles. Plant Physiol 135:1893–1902

Dunbar KB (1990) Geranium tissue culture for the development of bacterial blight resistance. Ph.D. Thesis, Michigan State University

Dunbar KB, Stephens CT (1989) Shoot regeneration of hybrid seed geranium (*Pelargonium x hortorum*) and regal geranium (*Pelargonium x domesticum*) from primary callus cultures. Plant Cell Tissue Organ Cult 19(1):13–21

Džamić AM, Soković MD, Ristić MS, Grujić SM, Mileski KS, Marin PD (2014) Chemical composition, antifungal and antioxidant activity of *Pelargonium graveolens* essential oil. J Appl Pharm Sci 4(03):001–005

Edwards-Jones V, Buck R, Shawcross SG, Dawson MM, Dunn K (2004) The effect of essential oils on methicillin-resistant *Staphylococcus aureus* using a dressing model. Burns 30(8):772–777

Eisenreich W, Bacher A, Arigoni D, Rohdich F (2004) Biosynthesis of isoprenoids via the non-mevalonate pathway. Cell Mol Life Sci 61(12):1401–1426

Elmann A, Mordechay S, Rindner M, Ravid U (2010) Anti-neuroinflammatory effects of geranium oil in microglial cells. J Funct Foods 2(1):17–22

Finer JJ (1995) Direct somatic embryogenesis. In: Plant cell tissue organ cult. Springer, Berlin, Heidelberg, pp 91–102

Friedrich H (1976) Phenylpropanoid constituents of essential oils. Lloydia 39(1):1–7

Ghannadi A, Bagherinejad MR, Abedi D, Jalali M, Absalan B, Sadeghi N (2012) Antibacterial activity and composition of essential oils from *Pelargonium graveolens* L'Her and *Vitex agnus-castus* L. Iran J Microbiol 4(4):171

Giongo JL, Vaucher R, Borin DI, Correa MS, Dos Santos VB, Santos RC, Boligon AA, Athayde ML, Bonez PC, Rossi GG, De Campus MM (2015) Antimycobacterial, antimicrobial and antifungal activities of geranium oil loaded nanocapsules. Int J Pharm Sci 7:414–419

Gupta P, Goel R, Pathak S, Srivastava A, Singh SP, Sangwan RS, Asif MH, Trivedi PK (2013) *De novo* assembly, functional annotation and comparative analysis of *Withania somnifera* leaf and root transcriptomes to identify putative genes involved in the withanolides biosynthesis. PLoS One 8(5):e62714

Gupta R, Banerjee S, Mallavarapu GR, Sharma S, Khanuja SP, Shasany AK, Kumar S (2002) Development of a superior somaclone of rose-scented geranium and a protocol for inducing variants. Hort Sci 37(4):632–636

Gupta R, Mallavarapu GR, Banerjee S, Kumar S (2001) Characteristics of an isomenthone-rich somaclonal mutant isolated in a geraniol-rich rose-scented geranium accession of *Pelargonium graveolens*. Flavour Fragr J 16(5):319–324

Haensch KT (2004) Morpho-histological study of somatic embryo-like structures in hypocotyl cultures of *Pelargonium*× *hortorum* bailey. Plant Cell Rep 22(6):376–381

Hakkaart FA, Hartel G (1979) Virus eradication from some *Pelargonium zonale* cultivars by meristem-tip culture. Eur J Plant Pathol 85(2):39–46

Hamdorf G (1976) Propagation of Pelargonium varieties by stem-tip culture. Acta Hortic 59:143–152

Harney PM (1982) Tissue culture propagation of some herbaceous horticultural plants. In: Application of plant cell and tissue culture to agriculture and industry. University of Guelph, Ontario, pp 187–208

Hassanein A, Chevreau E, Dorion N (2005) Highly efficient transformation of zonal (*Pelargonium x hortorum*) and scented (*P. capitatum*) geraniums via *Agrobacterium tumefaciens* using leaf discs. Plant Sci 169(3):532–541

Hayakawa H, Sobue F, Motoyama K, Yoshimura T, Hemmi H (2017) Identification of enzymes involved in the mevalonate pathway of *Flavobacterium johnsoniae*. Biochem Biophys Res Commun 487(3):702–708

Helfer M, Koppensteiner H, Schneider M, Rebensburg S, Forcisi S, Müller C, Schmitt-Kopplin P, Schindler M, Brack-Werner R (2014) The root extract of the medicinal plant *Pelargonium sidoides* is a potent HIV-1 attachment inhibitor. PLoS One 9(1):e87487

Hutchinson MJ, KrishnaRaj S, Saxena PK (1997) Inhibitory effect of GA 3 on the development of thidiazuron-induced somatic embryogenesis in geranium (Pelargonium xhortorum Bailey) hypocotyl cultures. Plant Cell Rep 16(6):435–438

Jadaun JS, Sangwan NS, Narnoliya LK, Singh N, Bansal S, Mishra B, Sangwan RS (2017) Over-expression of DXS gene enhances terpenoidal secondary metabolite accumulation in rose-scented geranium and *Withania somnifera*: active involvement of plastid isoprenogenic pathway in their biosynthesis. Physiol Plant 159(4):381–400

Khosravi AR, Shokri H, Sharifrohani M, Mousavi HE, Moosavi Z (2012) Evaluation of the antifungal activity of *Zataria multiflora*, *Geranium herbarium*, and *Eucalyptus camaldolensis* essential oils on *Saprolegnia parasitica* infected rainbow trout (*Oncorhynchus mykiss*) eggs. Foodborne Pathog Dis 9(7):674–679

Krishna H, Alizadeh M, Singh D, Singh U, Chauhan N, Eftekhari M, Sadh RK (2016) Somaclonal variations and their applications in horticultural crops improvement. 3 Biotech 6(1):54

Krishnan NM, Pattnaik S, Jain P, Gaur P, Choudhary R, Vaidyanathan S, Deepak S, Hariharan AK, Krishna PB, Nair J, Varghese L (2012) A draft of the genome and four transcriptomes of a medicinal and pesticidal angiosperm *Azadirachta indica*. BMC Genomics 13(1):464

KrishnaRaj S, Bi YM, Saxena PK (1997) Somatic embryogenesis and Agrobacterium-mediated transformation system for scented geraniums (*Pelargonium sp.* 'Frensham'). Planta 201(4):434–440

Kulkarni RN, Mallavarapu GR, Baskaran K, Ramesh S, Kumar S (1998) Composition of the essential oils of two isomenthone-rich variants of geranium (*Pelargonium sp.*). Flavour Fragr J 13(6):389–392

Kulkarni SS, Ravindra NS, Srinivas KV, Kulkarni RN (2012) A somaclonal variant of rose-scented geranium (*Pelargonium spp*.) with moderately high content of isomenthone in its essential oil. Nat Prod Commun 7(9):1223–1224

Kulkarni SS, Ravindra NS, Srinivas KV, Kulkarni RN (2014) In vitro chemical mutagenesis in exclusively vegetatively-propagated rose-scented geranium (*Pelargonium spp.*). J Hortic Sci Biotechnol 89(2):173–178

Lichtenthaler HK (1999) The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. Annu Rev Plant Biol 50(1):47–65

Lis BM (1996) A Chemotaxonomic reappraisal of the Section Ciconium Pelargonium (Geraniaceae). S Afr J Bot 62:277–279

Lis-Balchin M, Buchbauer G, Hirtenlehner T, Resch M (1998) Antimicrobial activity of Pelargonium essential oils added to a quiche-filling as a model food system. Lett Appl Microbiol 27(4):207–210

Loews FA (1999) Biosynthesis and metabolism of ascorbic acid in plants and of analogs of ascorbic acid in fungi. Phytochemistry 52:193–210

Marsolais AA, Wilson DP, Tsujita MJ, Senaratna T (1991) Somatic embryogenesis and artificial seed production in Zonal (*Pelargonium× hortorum*) and Regal (*Pelargonium× domesticum*) geranium. Can J Bot 69(6):1188–1193

Michaelis M, Doerr HW, Cinatl J (2011) Investigation of the influence of EPs® 7630, a herbal drug preparation from *Pelargonium sidoides*, on replication of a broad panel of respiratory viruses. Phytomedicine 18(5):384–386

Mithila J, Murch SJ, KrishnaRaj S, Saxena PK (2001) Recent advances in Pelargonium in vitro regeneration systems. Plant Cell Tissue Organ Cult 67(1):1–9

Mohapatra PP, Batra VK (2017) Tissue culture of potato (*Solanum tuberosum* L.): a review. Int J Curr Microbiol App Sci 6(4):489–495

Moyo M, Aremu AO, Gruz J, Šubrtová M, Szüčová L, Doležal K, Van Staden J (2013) Conservation strategy for *Pelargonium sidoides* DC: phenolic profile and pharmacological activity of acclimatized plants derived from tissue culture. J Ethnopharmacol 149(2):557–561

Moyo M, Van Staden J (2014) Medicinal properties and conservation of *Pelargonium sidoides* DC. J Ethnopharmacol 152(2):243–255

Murthy BNS, Vettakkorumakankav N, KrishnaRaj S, Odumeru J, Saxena PK (1999) Characterization of somatic embryogenesis in *Pelargonium x hortorum* mediated by a bacterium. Plant Cell Rep 18:607–613

Nadjib Boukhatem M, Kameli A, Amine Ferhat M, Saidi F, Mekarnia M (2013) Rose geranium essential oil as a source of new and safe anti-inflammatory drugs. Libyan J Med 8(1):22520

Naeini AR, Nazeri M, Shokri H (2011) Antifungal activity of *Zataria multiflora*, *Pelargonium graveolens* and *Cuminum cyminum* essential oils towards three species of Malassezia isolated from patients with pityriasis versicolor. J Mycol Med 21(2):87–91

Nameth ST, Daughtrey ML, Moorman GW, Sulzinski MA (1999) Bacterial blight of geranium: a history of diagnostic challenges. Plant Dis 83(3):204–212

Narnoliya LK, Kaushal G, Singh SP, Sangwan RS (2017) *De novo* transcriptome analysis of rose-scented geranium provides insights into the metabolic specificity of terpene and tartaric acid biosynthesis. BMC Genomics 18(1):74

Narnoliya LK, Rajakani R, Sangwan NS, Gupta V, Sangwan RS (2014) Comparative transcripts profiling of fruit mesocarp and endocarp relevant to secondary metabolism by suppression subtractive hybridization in *Azadirachta indica* (neem). Mol Biol Rep 41(5):3147–3162

Narnoliya LK, Sangwan RS, Singh SP (2018a) Transcriptome mining and in silico structural and functional analysis of ascorbic acid and tartaric acid biosynthesis pathway enzymes in rose-scanted geranium. Mol Biol Rep 45(3):315–326

Narnoliya LK, Jadaun JS, Singh SP (2018b) Synthetic biology advances for enrichment of bioactive molecules inplants. In: Recent trends and techniques in plant metabolic engineering. Springer Nature, Singapore, pp 117–145. https://doi.org/10.1007/978-981-13-2251-8_6. isbn:978-981-13-2251-8

Pellegrineschi A, Damon JP, Valtorta N, Paillard N, Tapfer D (1994) Improvement of ornamental characters and fragrance production in lemon-scented geranium through genetic transformation by *Agrobacterium rhizogenes*. Nature Biotechnol 12(1):64–68

Putievsky E, Ravid U, Dudai N (1990) The effect of water stress on yield components and essential oil of *Pelargonium graveolens* L. J Essent Oil Res 2(3):111–114

Qureshi JA, Saxena PK (1992) Adventitious shoot induction and somatic embryogenesis with intact seedlings of several hybrid seed geranium (*Pelargonium x hortorum* Bailey) varieties. Plant Cell Rep 11(9):443–448

Rajeswara Rao BR, Bhattacharya AK (1997) Yield and chemical composition of the essential oil of rose-scented geranium (*Pelargonium* species) grown in the presence and absence of weeds. Flavour Fragr J 12(3):201–204

Rajeswara Rao BR, Bhattacharya AK, Kaul PN, Chand S, Ramesh SI (1993) Changes in profiles of essential oils of rose-scented geranium (*Pelargonium sp.*) during leaf ontogeny. J Essent Oil Res 5(3):301–304

Rajeswara Rao BR, Sastry KP, Prakasa Rao EV, Ramesh SI (1990) Variation in yields and quality of geranium (*Pelargonium graveolens* L'Hér. ex Aiton) under varied climatic and fertility conditions. J Essent Oil Res 2(2):73–79

Range CM, Rudolph EW, Ajay PS, Michael ER, Jonathan MF, James C, Charles RK (2011) Rare exitary amino acid from flower of zonal geranium responsible for paralyzing the Japanese beetle. PNAS 108(4):1217–1221

Rao BR (2009) Chemical composition and uses of Indian rose-scented Geranium (*Pelargonium species*) essential oil-A review. J Essent Oil Bear Plant 12(4):381–394

Rastogi S, Meena S, Bhattacharya A, Ghosh S, Shukla RK, Sangwan NS, Lal RK, Gupta MM, Lavania UC, Gupta V, Nagegowda DA (2014) *De novo* sequencing and comparative analysis of holy and sweet basil transcriptomes. BMC Genomics 15(1):588

Ravindra NS, Kulkarni RN (2015) Essential oil yield and quality in rose-scented geranium: variation among clones and plant parts. Sci Hort 184:31–35

Reuther G (1982) Propagation of disease-free Pelargonium cultivars by tissue culture. Acta Hortic 131:311–320

Robichon MP, Renou JP, Jalouzot R (1995) Genetic transformation of *Pelargonium X hortorum*. Plant Cell Rep 15(1):63–67

Rath CC, Dash SK, Rao BR (2005) Antifungal activity of rose-scented geranium (Pelargonium species) essential oil and its six constituents. J Essent Oil Bear Plants 8(2):218–222

Ravindra NS, Kulkarni RN, Gayathri MC, Ramesh S (2004) Somaclonal variation for some morphological traits, herbyield, essential oil content and essential oil composition in an Indian cultivar of rose-scented geranium. Plant Breed 123(1):84–86

Sangwan NS, Farooqi AH, Shabih F, Sangwan RS (2001) Regulation of essential oil production in plants. Plant Growth Regul 34(1):3–21

Sangwan RS, Tripathi S, Singh J, Narnoliya LK, Sangwan NS (2013) *De novo* sequencing and assembly of *Centella asiatica* leaf transcriptome for mapping of structural, functional and regulatory genes with special reference to secondary metabolism. Gene 525(1):58–76

Saxena G, Banerjee S, Verma PC, Mallavarapu GR, Kumar S (2007) Rose-scented geranium (*Pelargonium sp*.) generated by *Agrobacterium rhizogenes* mediated Ri-insertion for improved essential oil quality. Plant Cell Tissue Organ Cult 90(2):215–223

Saxena G, Verma PC, Banerjee S, Kumar S (2008a) Field performance of somaclones of rose scented geranium (*Pelargonium graveolens* L'Her Ex Ait.) for evaluation of their essential oil yield and composition. Ind Crop Prod 27(1):86–90

Saxena G, Verma PC, Rahman LU, Banerjee S, Shukla RS, Kumar S (2008b) Selection of leaf blight-resistant *Pelargonium graveolens* plants regenerated from callus resistant to a culture filtrate of *Alternaria alternata*. Crop Prot 27(3):558–565

Sharopov FS, Zhang H, Setzer WN (2014) Composition of geranium (*Pelargonium graveolens*) essential oil from Tajikistan. Am J Essent Oil Nat Prod 2:13–16

Silva NCC, Fernandes AJ (2010) Biological properties of medicinal plants: a review of their antimicrobial activity. J Venom Anim Toxins Incl Trop Dis 16:402–413

Singh M, Singh UB, Ram M, Yadav A, Chanotiya CS (2013) Biomass yield, essential oil yield and quality of geranium (*Pelargonium graveolens* L. Her.) as influenced by intercropping with garlic (*Allium sativum* L.) under subtropical and temperate climate of India. Ind Crop Prod 46:234–237

Smith RH (2013) Plant tissue culture: techniques and experiments. Academic Press, Amsterdam

Sukhumpinij P, Kakihara F, Kato M (2010) *In vitro* regeneration from mature leaf explants of *Pelargonium rapaceum* (L.) L'Hérit. Sci Hortic 126(3):385–389

Swanson JK, Yao J, Tans-Kersten J, Allen C (2005) Behavior of *Ralstonia solanacearum* race 3 biovar 2 during latent and active infection of geranium. Phytopathology 95(2):136–143

Singh P, Khan S, Kumar S, ur Rahman L (2017) Establishment of an efficient Agrobacterium-mediated genetic transformation system in *Pelargonium graveolens*: an important aromatic plant. Plant Cell Tissue Org Cult (PCTOC) 129(1):35–44

Slimmon T, Qureshi JA, Saxena PK (1991) Phenylacetic acid-induced somatic embryogenesis in cultured hypocotylexplants of geranium (Pelargonium x hortorum Bailey). Plant Cell Rep 10(11):587–589

Theisen LL, Muller CP (2012) EPs® 7630 (Umckaloabo®), an extract from *Pelargonium sidoides* roots, exerts anti-influenza virus activity *in vitro* and *in vivo*. Antivir Res 94(2):147–156

Tuleja M, Krupa A, Góralski G, Płachno BJ (2014) Morphological and histological events in the preliminary tissue culture of haploid and diploid *Pelargonium zonale* var.'Kleiner Liebling'. Mod Phytomorphol 6:39–40

Van Zaayen A, Van Eijk C, Versluijs JM (1992) Production of high quality, healthy ornamental crops through meristem culture. Acta Bot Neerl 41(4):425–433

Visser C, Qureshi JA, Gill R, Saxena PK (1992) Morphoregulatory role of thidiazuron substitution of auxin and cytokinin requirement for the induction of somatic embryogenesis in geranium hypocotyl cultures. Plant Physiol 99:1704–1707

Visser-Tenyenhuis C, Murthy BNS, Odumeru J, Saxena PK (1994) Modulation of somatic embryogenesis in hypocotyl-derived cultures of geranium (Pelargonium x hortorum Bailey) cv. Ringo Rose by a bacterium. In Vitro Cell Dev Biol 30P:140–143

Verma RK, Chauhan A, Verma RS, Rahman LU, Bisht A (2013) Improving production potential and resources use efficiency of peppermint (Mentha piperita L.) intercropped with geranium (*Pelargonium graveolens* L. Herit ex Ait) under different plant density. Ind Crop Prod 44:577–582

Verma RK, Verma RS, Rahman LU, Yadav A, Patra DD, Kalra A (2014) Utilization of distillation waste–based vermicompost and other organic and inorganic fertilizers on improving production potential in geranium and soil health. Commun Soil Sci Plant Anal 45(2):141–152

Winkelmann T, Kaviani K, Serek M (2005) Development of a shoot regeneration protocol for genetic transformation in *Pelargonium zonale* and *Pelargonium peltatum* hybrids. Plant Cell Tissue Organ Cult 80(1):33–42

Wojtania A, Gabryszewska E, Marasek A (2004) Regeneration of *Pelargonium*× *hederifolium* 'Bonete' from petiole explants. Acta Physiol Plant 26(3):255–262

Zhang J, Ruhlman TA, Mower JP, Jansen RK (2013) Comparative analyses of two Geraniaceae transcriptomes using next-generation sequencing. BMC Plant Biol 13(1):228

Chapter 13
Biotechnological Production of Aromatic Oils from Plants

Aditya Banerjee and Aryadeep Roychoudhury

13.1 Introduction

Secondary metabolites like aromatic oils have several ethno-medicinal usages. These oils contain a natural essence and are organically biosynthesised in specialised cells of aromatic plants. The oil-producing cells act as natural factories mediating oil biosynthesis, accumulation and volatilisation into the atmosphere. Cells secreting aromatic oils possess diverse morphological features like specialised trichomes, osmophores, etc. It has been observed that the ducts, trichomes, conical-papillate cells and other oil-secreting tissues contain cellular structures dedicated for oil synthesis and maintenance (Rehman et al. 2016).

Biotechnology is one of the best evolved tools in the frontiers of science. Since aromatic oils have a huge ethno-medicinal market, the use of this technology for scaling-up oil production has been of great focus. Eventually, limited amount of aromatic oil is produced and collected from plants via distillation protocols. Thus, caring about the vast demand of such oils in the market, the production requires biotechnological boost. Scarce reports are available on such developments in aromatic oil production, though the field can grant novel opportunities in promoting economic expansions. This book chapter comprehensively discusses the morphological targets and the biotechnological advances in promoting aromatic oil production in plants.

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13.2 Chemistry of Aromatic Oils

Aromatic oils are extracted from plants by distillation processes like hydrodistillation (HD) and steam distillation (SD). Such oils consist of monoterpenes, sesquiterpenes and their oxygenated derivatives like alcohols, aldehydes and ketones (Coelho et al. 2012). Aromatic oils have great impacts in food, cosmetic, and pharmaceutical industries (Table 13.1). However, such traditional techniques have several cons. The thermolabile compounds often get degraded during extraction, thus altering the crude flavour and aromatic profile of the compound. The isolated product thus can be an altered derivative of the actual product. To overcome such limitations, the solvating power of supercritical fluids have been utilised to design an alternative technique called supercritical fluid extraction (SFE) (Fornari et al. 2012). The most environmental-friendly supercritical fluid suitable for aromatic oil extraction from plant tissues is carbon dioxide (CO_2). Supercritical operations can be performed with CO_2 at relatively low pressures and room temperatures. The post-operational extract is also solvent free and ready to be processed. However, this technique has a high initial cost which often stands as a barrier during execution (Coelho et al. 2012). SFE-mediated volatile oil extraction has been successfully performed from aromatic varieties like *Mentha pulegium*, *Foeniculum vulgare*, *Coriandrum sativum*, *Satureja fruticosa*, *Satureja montana, Santolina chamaecyparisus* and *Thymus vulgaris* (Coelho et al. 2012).

13.3 Anatomical Targets of Aromatic Oil Synthesis

Plants have evolved some specialised cells forming glandular trichomes and osmophores which secrete aromatic volatiles and oils. Non-specialised tissues like ducts and cavities secrete terpenes. Figure 13.1 is a schematic representation of aromatic oil production from such tissues.

13.3.1 Glandular Trichomes (GTs)

GTs are epidermal hairs dedicated for secreting abundant quantities of secondary products like mucilage, nectar, acyl lipids and essential oils (Lange and Turner 2013). The cocktail of organic chemicals secreted by GTs in aromatic plants has immense demand in flavour and fragrance industries. The chemistry of the aromatic exudates of GTs is of great interest as its unravelling can promote the potential uses of the product (Lange and Turner 2013). Aromatic monocot members from *Tradescantia*, *Dioscorea* and *Sisyrinchium* contain extensive epidermal GTs (Chwil 2011; Chauveau et al. 2011). The GTs are more prevalent in the dicot families like

Table 13.1 List of popular and important aromatic oils, their active principles, plant sources and economic uses

Aromatic oil (Active principles)	Source	Uses	References
Agar oil (4-phenyl-2-butanone, jinkoheremol, α-guaiene)	*Aquilaria malaccensis*	Priced for perfume	Korinek et al. (2016)
Ajwain oil (35–65% thymol, carvacrol, terpinene, paracymene)	*Carum copticum*	Has anti-inflammatory, antiaflatoxigenic and pharmacological effects	Boskabady et al. (2014)
Anise oil (licorice)	*Pimpinella anisum*	Antimicrobial effects	Gradinaru et al. (2014)
Basil oil (citral, α-terpeneol, linalool)	*Ocimum basilicum*	Aromatherapy and antiseptic effects	Perumalsamy et al. (2014)
Black pepper oil (β-carophyllene, limonene, sabinene, 3-carene, α-pinene, β-pinene)	*Piper nigrum*	Treatment for muscle aches, sprains and indigestion	Bagheri et al. (2014)
Camphor oil (α-pinene, camphene, β-pinene, sabinene, phellandrene, limonene, 1,8-cineole, γ-terpinene, p-cymene, terpinolene, furfural, camphor, linalool, bornyl acetate, terpinen-4-ol, caryophyllene, borneol, piperitone, geraniol, safrole, cinnamaldehyde, methyl cinnamate, eugenol)	*Cinnamomum camphora*	Treatment for cold, cough and arthritis	Fu et al. (2015)
Cardamom oil (1,8-cineole, α-terpineol, DL-limonene, nerolidol, 4-terpineol, δ-terpineol, δ-3-carene, β-myrcene, germacrene D, α-terpinene, longifolenaldehyde)	*Amomum subulatum*	Used in cosmetics and as flavour enhancers	Joshi et al. (2013)
Citronella oil (geraniol, citronellol, limonene, citronellal)	*Cymbopogon winteratus*	Insect repellent	Zamora et al. (2015)
Coconut oil (essential fatty acids)	*Cocos nucifera*	Used in foods and cosmetics	Gunasekaran et al. (2017)
Clove oil (60–80% eugenol, acetyl eugenol, carophyllene)	*Eugenia caryophyllata*	Relieves dental problems; acts as a neuroprotective, antinociceptive and antipyretic agent	Taher et al. (2015)
Davana oil (davanone and related oxygenated sesquiterpenes)	*Artemisia pallens*	Used as a germicide	Bhagavathy et al. (2015)

(continued)

Table 13.1 (continued)

Aromatic oil (Active principles)	Source	Uses	References
Eucalyptus oil (α-pinene, 1,8-cineol, pinocarveol-trans)	*Eucalyptus* spp.	Insect repellent and contains medicinal properties	Sebei et al. (2015)
Fennel seed oil (trans-anethole, α-phellandrene, α-pinene, β-pinene)	*Trachyspermum ammi*	Treatment of colic	Zheljazkov et al. (2013)
Ginger oil (geranyl acetate, zingiberene, geranial)	*Zingiber officinale*	Treatment of nausea and cough	Sasidharan et al. (2012)
Jasmine oil (benzyl acetate, linalool, cis-jasmonate, geraniol, methyl anthranilate)	*Jasminum grandiflorum*	Used in cosmetics and therapeutics	Ye et al. (2015)
Lemon oil (α-pinene, camphene, sabinene, myrcene, α-terpinene, linalool, β-bisabolene, limonene, trans-α-bergamotene, nerol, neral)	*Citrus limonum*	Used as an antiseptic and in cosmetics	Thany et al. (2015)
Mustard oil (allyl isothiocyanate and related derivatives)	*Brassica* spp.	Used in cooking purposes	Wendlinger et al. (2014)
Neem oil (azadirachtin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin, quercetin)	*Azadirachta indica*	Multiple medicinal and antiseptic uses	Scudeler et al. (2017)
Orange oil (90% limonene)	*Citrus sinensis*	Used as fragrance in cleaning products and flavouring foods	Zhao et al. (2015)
Oregano oil (thymol and carvacrol)	*Origanum vulgare*	Fungicide	Mohiti-Asli and Ghanaatparast-Rashti (2015)
Perilla oil (50–60% perillaldehyde)	*Perilla frutescens*	Improves cardiovascular system, inhibits platelet aggregation and thrombus formation	Jang et al. (2014)
Rosemary oil (cineole, camphor, camphene, α-pinene, diterpene lactone, carnosol)	*Rosemarinus officinalis*	Used to soothe muscles and as an anti-microbial agent	Raskovic et al. (2015)
Sandalwood oil (α-santalol, β-santalol)	*Santalum album*	Used in cosmetics	Braun et al. (2014)
Turmeric oil (curcumin)	*Curcuma longa*	Used as a flavouring agent and has anti-cancerous properties	Jacob and Badyal (2014)

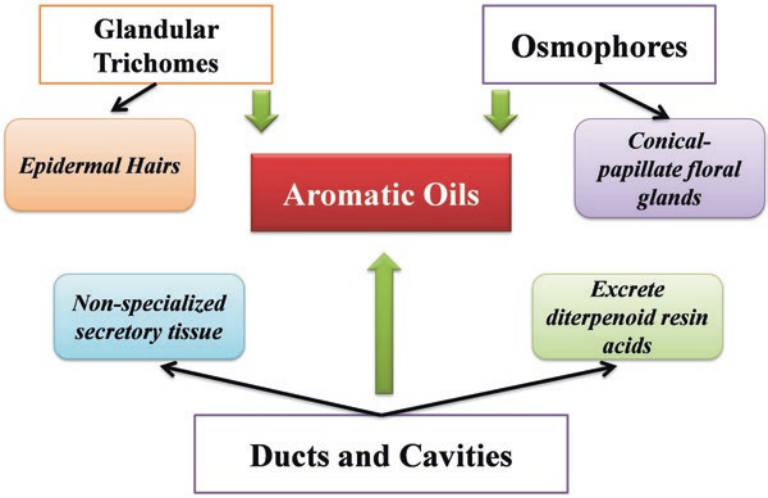

Fig. 13.1 The specialised and non-specialised anatomical sites of aromatic oil production in plants

Lamiaceae, Asteraceae, Sphaerosepalaceae, Carophyllaceae, Cucurbitaceae, Fabaceae, Rosaceae, Sapindaceae, Saxifragaceae and Cannabaceae (Rehman et al. 2016). It has been observed in peppermint that the peltate GTs are often separated by epidermal cells and hence are detected as pairs or clusters. However, the GTs are found in equivalent densities among multiple areas of the leaf (Turner et al. 2000). Lange and Turner (2013) inferred that GT development occurs via an interactome initiated by a network of transcription factors (TFs), which either act as activators or inhibitors. Characterisation of such interactome and biotechnological manipulations can manipulate GT anatomy to synthesise and accommodate higher quantities of aromatic oils.

Analysis of GTs can be performed using the online omics portal, TrichOME (www.planttrichome.org) (Dai et al. 2010). This web platform is a functional omics database containing important and distinct information regarding gene expression and metabolite levels in trichomes (Fig. 13.2). Such data is often misrepresented in regular non-specialised cDNA libraries (Dai et al. 2010).

13.3.2 Osmophores

Osmophores also referred to as the floral fragrance glands are distributed on the sepals and petals to allure potential pollinators. These glands are specialised in scent emission and occur as conical-papillate cell clusters in the floral organ (Anton et al. 2012). Osmophores usually constitute of homogeneously layered glandular epithelium with dense cytoplasm and starch deposition within the mesophyll. The cells of

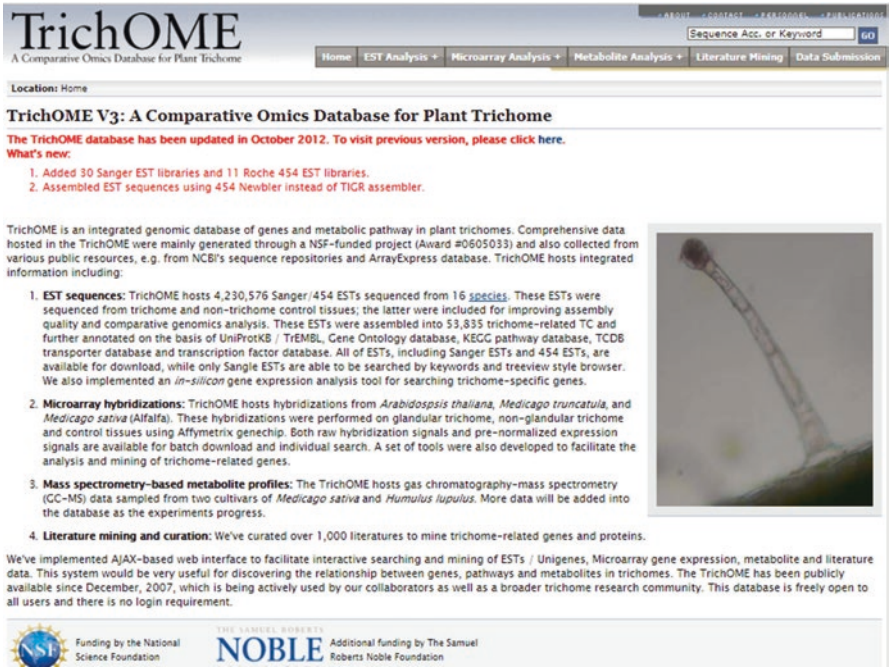

Fig. 13.2 The TrichOME database (http://www.planttrichome.org/trichomedb/) which eases comparative omics studies on plant trichomes. The database is a collection of expressed sequence tags, microarray hybridisation data and mass spectrometry-based metabolic profiles in trichome and non-trichome control tissues across various plant species. The website is a rich source of available literature on plant trichome research

the epidermis lack such deposits and this anatomical feature can be used to distinguish between the emission and production layers (Weryszko-Chmielewska and Chwil 2010). Osmophores have been observed on the petal epidermis in *Stanhopea, Sievekingia*, *Galanthus nivalis* and members belonging to Araceae and Orchidaceae (Rehman et al. 2016).

The anatomical conical shape of osmophores has been accredited to the *MIXTA* gene. Overexpression of this gene from *Antirrhinum majus* (Scrophulariaceae) in tobacco (Solanaceae) resulted in the development of ectopically secreting trichomes throughout the plant. This indicates a correlation between the osmophores and maturation of secreting trichomes (Glover et al. 1998). Floral tissue-specific overexpression of this gene in targeted aromatic species can be performed to scale up the production of industrially important aromatic oils. Production of fragrance is associated with cytoplasmic lipid inclusions and amyloplastidic plastoglobuli, present in plants like *Sievekingia* and *Stanhopea*. Due to the wrinkled surface of osmophores, the remnants of secretion can be identified on the epidermal surface of *Stanhopea graveolens* (Weryszko-Chmielewska and Chwil 2010).

13.3.3 Ducts and Cavities

In coniferous plants, ducts and cavities are used to excrete diterpenoid resin acids dissolved in volatile turpentine. Upon pathogen infestation, the turpentine evaporates leading to pathogen entrapment within the crystallised resin mass (Berton 2007). Several members belonging to families like Asteraceae, Apiaceae, Rutaceae, Helianthaceae and Rubiaceae possess ducts and cavities to excrete out aromatic exudates which have immense industrial uses (Bombo et al. 2012). Budel et al. (2012) showed the presence of secretory ducts and non-GTs in the leaves and stems of members of the *Baccharis* genus. Plants belonging to Brassicaceae release volatile monoterpenoids and sesquiterpenoids from leaves after injury. Such emission is mediated without the help of any specialised secretory tissue (Ahuja et al. 2010). Oil bodies present in liverworts are single membrane encapsulated intracytoplasmic secretory bodies which are derived from the dilation of the cisternae of the endoplastic reticulum (Marinho et al. 2014). Characterisation of candidate genes regulating such anatomical demarcations of oil production is yet to be performed.

13.4 Biotechnology and Aromatic Oil Production

As discussed, aromatic oils are of high industrial demand but are naturally produced in minute amounts in the plant system. Several chemical protocols have been utilised to refine the quality of oils for economic marketing. However, these procedures have not been beneficial for boosting the inherent production of oils within the system. Current advances in this field indicate that a biotechnological aptitude involving metabolic engineering, transgenic approaches and enzymatic technologies can improve the production and refine natural quality of aromatic oils (Fig. 13.3).

For enhancing the levels of S-linalool, the *linalool synthase* (*LIS*) gene from *Clarkia breweri* was overexpressed in *Lavandula latifolia* (spike lavender). The linalool content increased by 1000% in the youngest leaves of the transgenic plants (Mendoza-Poudereux et al. 2014). The transgenic plants were cross-pollinated to generate double transgenics expressing *1-deoxy-d-xylulose-5-P synthase* (encodes DXS, the first enzyme of methyl-d-erythritol-4-phosphate pathway) and *LIS*. Interestingly, the double transgenics exhibited lower linalool content in the aromatic oils than the parental lines possibly due to co-suppression effects associated with the construct structure used (Mendoza-Poudereux et al. 2014). Overexpression of *DXS* from *Arabidopsis thaliana* in spike lavender plants increased the aromatic oil content by 101.5–359% when compared with the control plants (Muñoz-Bertomeu et al. 2006). The transgenic plants showed no deterioration in chlorophyll and carotenoid contents, morphology, growth, development, flowering and seed germination with respect to those of control plants devoid of the transgene. Thus, *DXS*

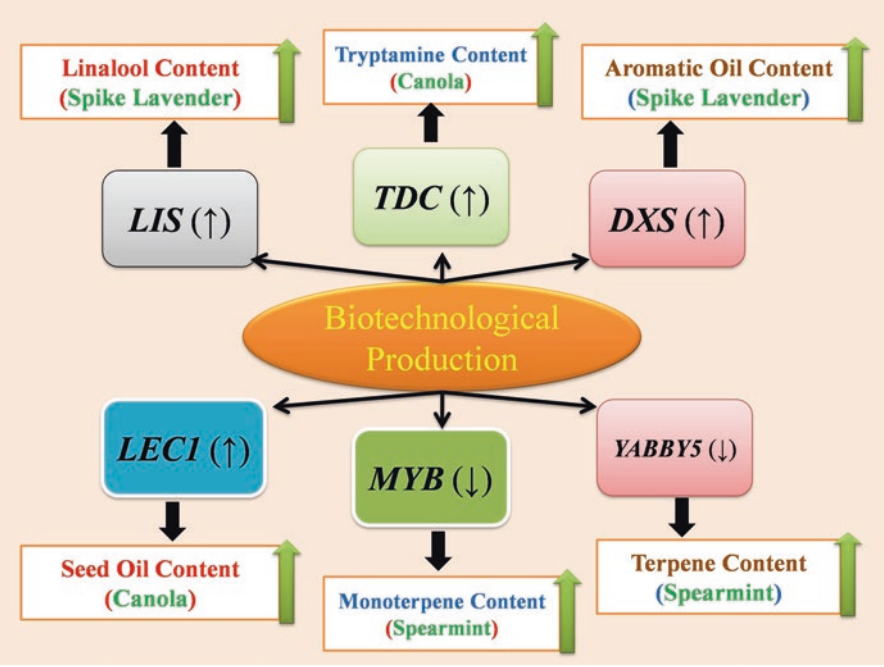

Fig. 13.3 Biotechnological increase of plant aromatic oil content is mediated by overexpressing positive regulatory genes like *LIS*, *TDC* and *DXS* which encode crucial enzymes participating in oil biosynthesis. Overexpression of *LEC1* increases the seed oil content in canola by accelerating fatty acid biosynthesis. The transcription factors like MYB and YABBY5 negatively regulate terpene synthesis in spearmint plants. Hence the RNAi-mediated knock-in lines exhibited high accumulation of terpenes

overexpression greatly increased the aromatic oil content in lavenders without detrimentally affecting plant development and fitness (Muñoz-Bertomeu et al. 2006).

Mustard oil glycosides are derivatives of methionine, phenylalanine or tryptophan. Overexpression of *tryptophan decarboxylase* (*TDC*) in *Brassica napus* (canola) plants reprogrammed the metabolic pathway leading to higher accumulation of tryptamine instead of indole glucosinolates (Chavadej et al. 1994). Interestingly, the indole glucosinolate content in the transgenic plants was only 3% of that in the control plants, showing the efficient diversion of metabolites towards biotechnologically created metabolic sinks (Chavadej et al. 1994). It is known that the expression of fatty acid (FA) synthetic genes in *Arabidopsis* is enhanced by the embryo developmental regulator, LEAFY COTYLEDON 1 (LEC1) (Banerjee and Roychoudhury 2014). The canola seed oil content increased by 7–16% in the transgenic lines overexpressing *BnLEC1*. This was due to increased carbon flux towards FA biosynthesis via alterations in the activities of enzymes involved in sucrose metabolism, glycolysis and FA anabolism (Elahi et al. 2016).

Mentha spicata (spearmint) plants produce aromatic oils in peltate glandular trichomes (PGTs). Reddy et al. (2017) recently identified a PGT-specific R2R3-MYB gene, *MsMYB*, from comparative RNA-Seq data analysis in spearmint. The *MsMYB-RNAi* knock-in lines exhibited elevated levels of monoterpenes, the precursors of aromatic oil in spearmint. It was also observed that ectopic expression of *MsMYB* in sweet basil and tobacco negatively regulated the sesquiterpene- and diterpene-derived metabolite synthesis (Reddy et al. 2017). Though phylogenetic analyses revealed that *MsMYB* could regulate the phenylpropanoid pathway, it was experimentally revealed that this TF was more specific to the terpene biosynthetic pathway (Reddy et al. 2017). Wang et al. (2016) functionally characterised a PGT-specific novel *MsYABBY5* gene in spearmint. The knock-in lines produced by *MsYABB5-RNAi* led to higher accumulation of terpenes, showing that MsYABBY5 is a negative regulator of terpene biosynthesis (Wang et al. 2016). Such RNAi lines can be developed on large scales to enhance the production of aromatic oils from spearmint plants. Jin et al. (2014) performed next generation sequencing (NGS; Illumina paired end sequencing) of spearmint RNAs from PGT, leaf and leaf stripped of PGTs. The elaborate transcriptome analysis characterised the potential differentially expressed genes in spearmint which can be targeted for metabolic engineering for sustainable production of aromatic oils (Jin et al. 2014). Enzymes have also been utilised in the production of aromatic oils. It was observed that cutinase-catalysed hydrolysis of oil palm empty fruit bunch fibre (OPEFBF) lignin was more efficient than the manganese peroxidase-induced oxidation. The level and quality of aromatic compounds were also conserved in the enzyme-treated product (Tang et al. 2015).

13.5 Conclusion and Future Perspectives

The concept of biotechnology and aromatic oil production is a new but rapidly evolving field. Investigations which have been highlighted in this chapter clearly indicate at the potential of metabolic engineering in boosting aromatic oil production and quality at the industrial scale. The aromatic oils are produced in specialised structures like the glandular trichomes, osmophores, ducts and cavities. These oils usually are the derivatives of terpenes. This field encompasses several future perspectives. The genes regulating the anatomical niche of the specialised structures can be targeted to enlarge the oil-producing units of the plants. The aromatic plants can be converted into specialised oil-producing bio-factories by overexpressing candidate rate-limiting enzyme-encoding genes and by downregulating the TF-encoding genes which negatively regulate the oil-producing pathway. An exhaustive analysis at the transcriptomic and metabolomic levels is quintessential for identification of such candidate loci.

References

Ahuja I, Rohloff J, Bones AM (2010) Defence mechanisms of Brassicaceae: implications for plant-insect interactions and potential for integrated pest management: a review. Agron Sustain Dev 30:311–348

Anton S, Kaminska M, Stpiczynska M (2012) Comparative structure of the osmophores in the flowers of *Stanhopea graveolens* Lindley and *Cycnoches chlorochilon* Klotzsch (Orchidaceae). Acta Agrobot 65:11–22

Bagheri H, Abdul Manap MY, Solati Z (2014) Antioxidant activity of *Piper nigrum* L. essential oil extracted by supercritical CO_2 extraction and hydro-distillation. Talanta 121:220–228

Banerjee A, Roychoudhury A (2014) Metabolic engineering of lipids in plants. J Plant Sci Res 1:1–20

Berton LP (2007) Chemical engineering research. Trends Nova Publishers, New York

Bhagavathy GV, Velazquez Nieves GM, Webb MZ, Chauhan KR (2015) Arthropod deterrents from *Artemisia pallens* (davana oil) components. Nat Prod Commun 10:1335–1336

Bombo AB, De Oliveira TS, De Oliveira ADSS, Rehder VLG, Magenta MAG (2012) Anatomy and essential oils from aerial organs in three species of Aldama (Asteraceae–Heliantheae) that have a difficult delimitation. Aus J Bot 60:632–642

Boskabady MH, Alitaneh S, Alavinezhad A (2014) *Carum copticum* L.: a herbal medicine with various pharmacological effects. Biomed Res Int 2014:569087

Braun NA, Sim S, Kohlenberg B, Lawrence BM (2014) Hawaiian sandalwood: oil composition of *Santalum paniculatum* and comparison with other sandal species. Nat Prod Commun 9:1365–1368

Budel JM, Duarte MR, Döll-Boscardin PM (2012) Composition of essential oils and secretory structures of *Baccharis anomala*, *B. megapotamica* and *B. ochracea*. J Essent Oil Res 24:19–24

Chauveau O, Eggers L, Raquin C, Silvério A, Brown S et al (2011) Evolution of oil-producing trichomes in *Sisyrinchium* (Iridaceae): insights from the first comprehensive phylogenetic analysis of the genus. Ann Bot 107:1287–1312

Chavadej S, Brisson N, McNeil JN, De Luca V (1994) Redirection of tryptophan leads to production of low indole glucosinolate canola. Proc Natl Acad Sci U S A 91:2166–2170

Chwil M (2011) Micromorphology and anatomy of the floral elements of *Tradescantia* x *andersoniana* W Ludw Rohweder. Acta Agrobot 64:15–24

Coelho JP, Cristino AF, Matos PG, Rauter AP, Nobre BP et al (2012) Extraction of volatile oil from aromatic plants with supercritical carbon dioxide: experiments and modelling. Molecules 17:10550–10573

Dai X, Wang G, Yang DS, Tang Y, Broun P et al (2010) TrichOME: a comparative omics database for plant trichomes. Plant Physiol 152:44–54

Elahi N, Duncan RW, Stasolla C (2016) Modification of oil and glucosinolate content in canola seeds with altered expression of *Brassica napus LEAFY COTYLEDON1*. Plant Physiol Biochem 100:52–63

Fornari T, Vicente G, Vázquez E, García-Risco MR, Reglero G (2012) Isolation of essential oil from different plants and herbs by supercritical fluid extraction. J Chromatogr A 1250:34–48

Fu JT, Tang L, Li WS, Wang K, Cheng DM, Zhang ZX (2015) Fumigant toxicity and repellence activity of camphor essential oil from *Cinnamonum camphora* Siebold against *Solenopsis invicta* workers (Hymenoptera: Formicidae). J Insect Sci 15:129

Glover BJ, Perez-Rodriguez M, Martin C (1998) Development of several epidermal cell types can be specified by the same MYB-related plant transcription factor. Development 125:3497–3508

Gradinaru AC, Miron A, Trifan A, Spac A, Brebu M, Aprotosoaie AC (2014) Screening of antibacterial effects of anise essential oil alone and in combination with conventional antibiotics against *Streptococcus pneumoniae* clinical isolates. Rev Med Chir Soc Med Nat Iasi 118:537–543

Gunasekaran R, Shaker MR, Mohd-Zin SW, Abdullah A, Ahmad-Annuar A, Abdul-Aziz NM (2017) Maternal intake of dietary virgin coconut oil modifies essential fatty acids and causes low body weight and spiky fur in mice. BMC Complement Altern Med 17:79

Jacob JN, Badyal DK (2014) Biological studies of turmeric oil, part 3: anti-inflammatory and analgesic properties of turmeric oil and fish oil in comparison with aspirin. Nat Prod Commun 9:225–228

Jang JY, Kim TS, Cai J, Kim J, Kim Y et al (2014) Perilla oil improves blood flow through inhibition of platelet aggregation and thrombus formation. Lab Anim Res 30:21–27

Jin J, Panicker D, Wang Q, Kim MJ, Liu J et al (2014) Next generation sequencing unravels the biosynthetic ability of spearmint (*Mentha spicata*) peltate glandular trichomes through comparative transcriptomics. BMC Plant Biol 14:292

Joshi R, Sharma P, Sharma V, Prasad R, Sud RK, Gulati A (2013) Analysis of the essential oil of large cardamom (*Amomum subulatum* Roxb.) growing in different agro-climatic zones of Himachal Pradesh, India. J Sci Food Agric 93:1303–1309

Korinek M, Wagh VD, Lo IW, Hsu YM, Hsu HY et al (2016) Antiallergic phorbol ester from the seeds of *Aquilaria malaccensis*. Int J Mol Sci 17:398

Lange BM, Turner GW (2013) Terpenoid biosynthesis in trichomes--current status and future opportunities. Plant Biotechnol J 11:2–22

Marinho CR, Souza CD, Barros TC, Teixeira SP (2014) Scent glands in legume flowers. Plant Biol (Stuttg) 16:215–226

Mendoza-Poudereux I, Muñoz-Bertomeu J, Navarro A, Arrillaga I, Segura J (2014) Enhanced levels of S-linalool by metabolic engineering of the terpenoid pathway in spike lavender leaves. Metab Eng 23:136–144

Mohiti-Asli M, Ghanaatparast-Rashti M (2015) Dietary oregano essential oil alleviates experimentally induced coccidiosis in broilers. Prev Vet Med 120:195–202

Muñoz-Bertomeu J, Arrillaga I, Ros R, Segura J (2006) Up-regulation of *1-deoxy-D-5-phosphate synthase* enhances production of essential oils in transgenic spike lavender. Plant Physiol 142:890–900

Perumalsamy H, Kim JY, Kim JR, Hwang KN, Ahn YJ (2014) Toxicity of basil oil constituents and related compounds and the efficacy of spray formulations to *Dermatophagoides farinae* (Acari: Pyroglyphidae). J Med Entomol 51:650–657

Raskovic A, Milanovic I, Pavlovic N, Milijasevic B, Ubavic M, Mikov M (2015) Analgesic effects of rosemary essential oil and its interactions with codeine and paracetamol in mice. Eur Rev Med Pharmacol Sci 19:165–172

Reddy VA, Wang Q, Dhar N, Kumar N, Venkatesh PN et al (2017) Spearmint R2R3-MYB transcription factor MsMYB negatively regulates monoterpene production and suppresses the expression of geranyl diphosphate synthase large subunit (MsGPPS.LSU). Plant Biotechnol J 15:1105–1119

Rehman R, Hanif MA, Mushtaq Z, Mochona B, Qi X (2016) Biosynthetic factories of essential oils: the aromatic plants. Nat Prod Chem Res 4:227

Sasidharan I, Venugopal VV, Menon AN (2012) Essential oil composition of two unique ginger (*Zingiber officinale* Roscoe) cultivars from Sikkim. Nat Prod Res 26:1759–1764

Scudeler EL, Garcia AS, Pinheiro PF, Santos DC (2017) Neem oil (*Azadirachta indica* A. Juss) affects the ultrastructure of the midgut muscle of *Ceraeochrysa claveri* (Navás, 1911) (Neuroptera: Chrysopidae). Acta Histochem 119:84–91

Sebei K, Sakouhi F, Herchi W, Khouja ML, Boukhchina S (2015) Chemical composition and antibacterial activities of seven *Eucalyptus* species essential oils leaves. Biol Res 48:7

Taher YA, Samud AM, El-Taher FE, ben-Hussin G, Elmezogi JS et al (2015) Experimental evaluation of anti-inflammatory, antinociceptive and antipyretic activities of clove oil in mice. Libyan J Med 10:28685

Tang PL, Hassan O, Maskat MY, Badri K (2015) Production of monomeric aromatic compounds from oil palm empty fruit bunch fiber lignin by chemical and enzymatic methods. Biomed Res Int 2015:891539

Thany SH, Tong F, Bloomquist JR (2015) Pre-treatment of *Stegomyia aegypti* mosquitoes with a sublethal dose of imidacloprid impairs behavioural avoidance induced by lemon oil and DEET. Med Vet Entomol 29:99–103

Turner GW, Gershenzon J, Croteau RB (2000) Distribution of peltate glandular trichomes on developing leaves of peppermint. Plant Physiol 124:655–664

Wang Q, Reddy VA, Panicker D, Mao HZ, Kumar N et al (2016) Metabolic engineering of terpene biosynthesis in plants using a trichome-specific transcription factor MsYABBY5 from spearmint (*Mentha spicata*). Plant Biotechnol J 14:1619–1632

Wendlinger C, Hammann S, Vetter W (2014) Various concentrations of erucic acid in mustard oil and mustard. Food Chem 153:393–397

Weryszko-Chmielewska E, Chwil M (2010) Ecological adaptations of the floral structures of *Galanthus nivalis* L. Acta Agrobot 63:41–49

Ye Q, Jin X, Zhu X, Lin T, Hao Z, Yang Q (2015) An efficient extraction method for fragrant volatiles from *Jasminum sambac* (L.) Ait. J Oleo Sci 64:645–652

Zamora D, Klotz SA, Meister EA, Schmidt JO (2015) Repellency of the components of the essential oil, *Citronella*, to *Triatoma rubida*, *Triatoma protracta*, and *Triatoma recurva* (Hemiptera: Reduviidae: Triatominae). J Med Entomol 52:719–721

Zhao X, Liu F, Ma C, Yuan F, Gao Y (2015) Effect of carrier oils on the physicochemical properties of orange oil beverage emulsions. Food Res Int 74:260–268

Zheljazkov VD, Horgan T, Astatkie T, Schlegel V (2013) Distillation time modifies essential oil yield, composition, and antioxidant capacity of fennel (*Foeniculum vulgare* Mill). J Oleo Sci 62:665–672

Chapter 14
The Role of Biotechnology in Essential Oil Production from Non-herbaceous Plants

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14.1 Introduction

The plant essential oils are mixtures of compounds, most of them characterized as secondary metabolites, but in certain cases, they can be compounds resulting from primary metabolism, such as fatty acids. The most common classes of secondary metabolites in essential oils are terpenoids, followed by phenolics. A few of these compounds predominate, whereas many more might be present in lesser amounts in the oils, usually at 0.1–5%. Terpenoids comprise the largest and structurally most varied class of secondary metabolites, numbering over 40,000 different molecules. Members of the 10-carbon group of terpenoids, the monoterpenoids, are the main constituents of plant essential oils. These essential oils can also contain sesquiterpenoids, phenylpropanoids, and benzenoids. In addition, plant tissues can produce volatile aldehydes, their corresponding alcohols and acids, as well as volatile ketones. These compounds can be occasionally found in essential oils, but they are usually formed only in certain plant tissues and under specific physiological conditions that favor catabolic reactions. They can be considered to belong to the primary metabolites, although they can have useful flavoring or medicinal applications.

The volatility of the essential oils reflects their composition. Volatility is determined mainly by the molecule's ability to form hydrogen bonds. Of the terpenoids, the only members of the mono- (C10) and sesquiterpenoid (C15) classes are sufficiently volatile. Monoterpenoid molecules made of carbons and hydrogen only are

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very volatile. Those having one hydroxyl, keto, peroxy, or epoxy group are still volatile, but those with more hydroxyls are either only slightly volatile or not volatile at all. In sesquiterpenoids, the presence of one hydroxyl group is the maximum level of tolerance for volatility. Triterpenoids and higher-order terpenoids are not volatile. Phenylpropanoids and benzenoids with one hydroxyl and no carboxyl group are volatile. The simultaneous presence of a keto group does not abolish these molecules' volatility. However, sporting more hydroxyl groups drastically reduces or even completely abolishes their volatility. (Hydroxy)-cinnamic acids are not sufficiently volatile, due to the presence of the carboxyl group, unless it is esterified with a volatile alcohol. Aliphatic and olefinic aldehydes, monoalcohols, and monoketones are volatile for at least up to 12-carbon sizes. As the molecule becomes smaller than five carbons, even the acids become volatile. Organic monocarboxylic acids, esterified with volatile alcohols, are also volatile.

There is intense commercial interest on essential oils because of their pharmaceutical properties. Moreover, essential oils have been also used in cosmetics, as food additives and as flavoring compounds. Fresh plant seeds, flowers, stems, and roots usually contain 0.1–10% v/w of essential oil, but this can range from <0.1% up to 20% v/w in some cases—and even higher have also been known. Essential oils are often produced from specific plant tissues, such as the seeds or flowers, whose total mass from a single harvesting season is only a small percentage of the whole weight of the plant. This is the reason why obtaining useful volatile essential oils from cultivated plants can be so expensive. Chemical manufacture is often so much cheaper that the natural products correspond only to a small percentage of the market. However, consumers often prefer the natural product because they believe it to be free of manufacturing artifacts and leftovers. Also, the chemical synthesis often results in racemic mixtures of the product, resulting in only an approximation of the natural flavor qualities. Thus, there is a need to reduce the cost of the natural product.

Biotechnology attempts to facilitate the production and, therefore, to reduce the market cost of essential oils. Some of the approaches attempt to produce them in bioreactor facilities either by the action of cultured plant cell and tissues or by the use of bacterial and fungal biotransformers. Semi-synthetic methods, in which a precursor molecule is transformed into a useful product by isolated (crude or purified) enzyme preparations in vitro, have also been attempted. Along a different approach, plants have been genetically engineered to overproduce the desired essential oil. Recently, such efforts have involved such metabolic engineering of the biosynthetic pathways leading to the synthesis of the desired essential oil compound.

We are accustomed of connecting essential oils with herbs. Yet, essential oils can also be produced from trees, shrubs, and vines. Research on the biotechnology of producing essential oils from such plants is not as intense as it is for herbaceous species. It is the purpose of the present chapter to summarize the current progress in biotechnological development for essential oil production from non-herbaceous plants and, also, to suggest promising future directions of economically viable approaches for "natural" essential oil production.

14.2 Essential Oils from Non-herbaceous Plants

A list of the trees, shrubs, and vines known to produce useful essential oils is given in Table 14.1. In addition to the references provided in Table 14.1, information on their essential oil yield and composition, as well as on their cultivation methods and uses, is provided by Guenther (1982) and Atal and Kapur (1982). This list is not exhaustive as it is expected that more plant species will be added in the future, as many other, so far unexamined, essential oils become known by ethnobotanists exploring isolated human communities.

The extraction methods, plant parts used, and resulting essential oil yields can vary widely. Wintergreen oil yield is 0.66% of the fresh weight of leaves and stems; it contains 96–99% methyl salicylate and is used as a flavoring agent. Methyl salicylate is also the major component of birch oil, obtained by the distillation of chipped birch bark that yields 950–1450 ml of oil per ton of wood. To produce labdanum essential oil, twigs of the perennial shrub *Cistus ladaniferus* are boiled for some hours, and the resin rising to the surface is collected and dried. The dry resin is then steam-distilled to yield about 1–2% of essential oil. Direct distillation of fresh stems is also possible, producing about 0.06% of essential oil. The oil pressure extracted from coconut nuts is treated with superheated steam to obtain a volatile oil, still of disagreeable odor and also containing several carbinols and ketones. Both oils cannot be used as such in the perfume industry but can serve as starting materials for the preparation of synthetic aromatics. Fir oil is produced at a yield of 0.65–1.4%, by the distillation of young branches and leaves of *Abies balsamea*. By breaking the vesicles beneath the bark, turpentine can be collected, which is then distilled to get 15–25% of essential oil. Distillation of pine needles for 5 h yields 0.25–0.35% of pine oil, whose composition depends largely on the geographic origin of the *Pinus sylvestris*. This oil is used in perfume preparations. The essential oil of *Thuja plicata*, obtained by distillation, in addition to thujone, contains also the very poisonous γ-thujaplicin. The cypress oil is most valuable in the perfume industry. Distillation for 17 h of young branches and leaves of *Cupressus sempervirens* produces about 0.2% of oil, yet the most valuable portion—the one having the ambergris and labdanum-like odor—is extracted after the first 3 h of distillation. Chips of *Juniperus virginiana* heartwood yield on distillation 2–2.5% of cedarwood oil, containing 80% of cedrene. However, cedrene and cedrol are found only in cut and aged heartwood of *Juniperus* and not in live tissues of the plant. *Juniperus communis* berries contain 0.5–2% of essential oil used for flavoring beverages and liquors. The oil composition can change if fermentation precedes distillation.

It becomes clear from the above discussion that essential oil extraction needs considerable expertise in order to be successful. One has to choose not only the right plant location and tissues but also the appropriate extraction process and then secure the removal of compounds of disagreeable odor or even poisonous, as well as prevent the formation of unwanted artifacts. Biotechnology could potentially remove some of the complications as well as increase the yield and facilitate the isolation of essential oils or of their individual components.

Table 14.1 Trees, shrubs, and vines producing essential oils

	Plant	Essential oil	Main ingredients in order of decreasing percentage	References
	Trees			
1	*Abies balsamea* (fir)	Fir oil	β-Pinene, δ-3-carene, bornyl acetate	Régimbal and Collin (1994)
2	*Aniba rosaeodora* (Brazilian rosewood)	Rosewood oil	Linalool, α-terpineol	Fidelis et al. (2012)
3	*Aquilaria malaccensis* (agarwood)	Agar oil	Agarospirol, tetradecanal, pentadecanal	Ab Rahman (2009)
4	*Azadirachta indica* (neem)	Neem oil	*In leaves:* β-Elemene, γ-elemene, germacrene D, caryophyllene, bicyclogermacrene *In flowers:* pentacosane, tetracosane, β-germacrene, β- caryophyllene, dodecene, octadecanol, verdiflorol	El Hawary et al. (2013)
5	*Betula* spp. (birch)	Birch oil	14-Hydroxy-β-caryophyllene, 14-hydroxy-4,5-dihydro-β-caryophyllene, methyl salicylate, 14-acetoxy-β-caryophyllene	Başer and Demirci (2007)
6	*Boswellia sacra* (frankincense)	Frankincense	*E*-β-ocimene, myrcene, α-thujene, α- pinene	Al Harrasi and Al Saidi (2008)
7	*Bursera graveolens* (palo santo)	Palo santo oil	Limonene, β-elemene, β-ocimene, menthofuran	Monzotea et al. (2012)
8	*Cananga odorata* (cananga tree)	Ylang-ylang oil	Benzyl acetate, linalool, methyl benzoate, 3-methyl-2-butenyl acetate, neryl acetate	Brokl et al. (2013)
9	*Canarium luzonicum* (elemi)	Elemi oil	Limonene, α-phellandrene, elemol, α-terpinolene, elemicine	Villanueva et al. (1993)
10	*Carya* spp. (Hickory)	Hickory nut oil	Oleic acid, linoleic acid, palmitic acid	Chow (2007)
11	*Cinnamomum camphora* (camphor tree)	Camphor oil	Linalool, D-camphor, 1,8-cineole, α-terpineol	Guo et al. (2016)
12	*Cinnamomum verum* (Ceylon cinnamon tree)	Cinnamon oil	*trans*-cinnamaldehyde	Li et al. 2013
13	*Citrofortunella microcarpa* (calamondin)	Calamondin oil	*In peel oil*: limonene *In the leaf oil*: elemol, α-eudesmol	Cuevas-Glory et al. (2009)
14	*Citrus aurantifolia* (Lime)	Lime oil	Limonene, beta-pinene, γ-terpinene, citral	Spadaro et al. (2012)

(continued)

Table 14.1 (continued)

	Plant	Essential oil	Main ingredients in order of decreasing percentage	References
15	*Citrus aurantium* subsp. *amara* (bitter orange)	Neroli oil	Limonene, (*E*)-nerolidol, α-terpineol, α-terpinyl acetate, (*E, E*)-farnesol	Ammar et al. (2012)
16	*Citrus bergamia* (bergamot orange)	Bergamot essential oil	Limonene, linalyl acetate, linalool, γ-terpinene, β-pinene	Sawamura et al. (2006)
17	*Citrus limon* (lemon tree)	Lemon oil	Limonene, γ-terpinene, β-pinene	Gök et al. (2015)
18	*Citrus medica* (citron)	Citron oil	*In leaves*: erucylamide, limonene, citral *In peel*: isolimonene, citral, limonene	Bhuiyan et al. (2009)
19	*Citrus reticulata* (mandarin orange)	Mandarin oil	Limonene, γ-terpinene, α-Pinene	Boughendjioua and Boughendjioua (2017)
20	*Citrus sinensis* (orange tree)	Orange oil	Limonene, myrcene, sabinene	Azar et al. (2011)
21	*Citrus tangerine* (tangerine tree)	Tangerine oil	Limonene, γ-terpinene, myrcene, α-pinene	Njoroge et al. (2006)
22	*Citrus paradisi* (grapefruit)	Grapefruit oil	Limonene, α-terpinene, α-pinene	Njoroge et al. (2005)
23	*Cocos nucifera* (coconut tree)	Coconut oil	Methyl laurate, methyl myristate	da Fonseca et al. (2014)
24	*Coffea arabica* (coffee tree)	Coffee oil	Dextrins, chlorogenic acid, caffeine	Nogaim et al. (2013)
25	*Commiphora myrrha* (myrrh)	Myrrh oil	n-Octyl acetate, 4-ethynyl-4-hydroxy-3,5,5-trimethyl 2-cyclohexen-1-one, nerolidol isobutyrate, β-elemene, copaene	Chen et al. (2013)
26	*Cupressus* spp. *(cypress)*	Cypress oil	α-Pinene, δ-3 carene, sabinene, α-cadinol, terpinen-4-ol, limonene	Pierre-Leandri et al. (2003)
27	*Eucalyptus globulus* (eucalyptus)	Eucalyptus oil	*In leaves*: 1,8-cineole, α-pinene, p-cymene, cryptone, spathulenol *In fruits*: aromadendrene, 1,8-cineole *In buds*: aromadendrene 1,8-cineole, α-thujene *In branches*: 1,8-cineole, aromadendrene	Chalchat et al. (1995)
28	*Illicium verum* (star anise)	Star anise oil	*Trans*-anethole	Wei et al. (2014)
29	*Juniperus communis* (Juniper)	Juniper berry oil	α-Pinene, myrcene, sabinene, limonene, β-pinene	Höferl et al. (2014)

(continued)

Table 14.1 (continued)

	Plant	Essential oil	Main ingredients in order of decreasing percentage	References
30	*Juniperus virginiana* (red cedar)	Cedarwood oil	Cedrene, α-Pinene, β-pinene, phelandrene	Stewart et al. (2014)
31	*Laurus nobilis* (bay)	Bay oil	1,8-Cineole, α-terpinyl acetate, α-pinene, terpinen-4-ol, sabinene, methyl eugenol, eugenol	Kivrak et al. (2017)
32	*Lawsonia inermis* (henna tree)	Henna oil	Eugenol, ethyl hexadecanoate, (*E*)-methyl cinnamate, isocaryophyllene, (*E*)-β-ionone, methyl linolenate	Adebola et al. (2005)
33	*Litsea cubeba* (may change)	Litsea cubeba oil	Geranial, neral, D-limonene	Si et al. (2012)
34	*Melaleuca alternifolia* (tea tree)	Tea tree oil	*Leaves:* Terpinen-4-ol, γ-terpinene, α-terpinene, 1,8-cineole, terpinolene	Carson et al. 2006
35	*Moringa oleifera* (drumstick tree)	Moringa seed oil	Oleic acid, palmitic acid, stearic acid, behenic acid	Ghazali and Abdulkarim (2011)
36	*Murraya koenigii* (curry tree)	Curry leaf oil	Sabinene, α-pinene, phellandrene, terpinen-4-ol, β-pinene, γ-terpinene	Mallavarapu et al. (1999)
37	*Myristica fragrans* (nutmeg)	Nutmeg seed oil	Sabinene, α-pinene, α-phellandrene, terpinen-4-ol	Ogunwande et al. (2003)
38	*Myroxylon balsamum* (L.) (Myroxylon)	Balsam of Peru	Benzyl benzoate	Swift (1997)
39	*Picea* spp. (spruce)	Spruce oil	β-Pinene, camphor, α-pinene, bornyl acetate Bornyl acetate is the main compound in Black Spruce	Garneau et al. (2012)
40	*Pinus sylvestris* (pine)	Pine oil	*In branches*: α-pinene, sabinene, β-pinene, limonene *In needles*: α-pinene, camphene, β-pinene, sabinene	Zafra and García-Peregrín (1976)
41	*Ravensara aromatica* (clove nutmeg)	Raversara oil	Methyl chavicol, methyl eugenol, α-terpinene, limonene, sabinene, linalool, terpinen-4-ol	Andrianoelisoa et al. (2006)
42	*Santalum paniculatum* or *S. album* (sandalwood)	Sandalwood oil	α-Santalol, β-sandalol, α-bergamotol, (*E*)-nuciferol, germacrene B, teresantalol	Kusuma and Mahfud (2016)
43	*Sassafras* spp. (sassafras)	Sassafras oil	Safrole, camphor *In root bark*: safrole, 5-methoxyeugenol, asaron, piperonylacrolein, coniferaldehyde, camphor	Sethi et al. (1976)

(continued)

Table 14.1 (continued)

	Plant	Essential oil	Main ingredients in order of decreasing percentage	References
44	*Thuja plicata* (western red cedar)	Western red cedar oil	α-Thujone (*cis*), sabinene, β-thujone (*trans*), terpinen-4-ol	Tsiri et al. (2009)
45	*Tsuga canadensis* (tsuga, Canadian hemlock)	Tsuga oil	α-Pinene, camphene, limonene	Kılıç and Kocak (2014)
	Shrubs			
46	*Cistus ladaniferus* (labdanum)	Labdanum	α-Pinene, *trans*-pinocarveol, viridiflorol, ledol	Mariotti et al. (1997)
47	*Gaultheria procumbens* (American wintergreen)	Wintergreen oil	Methyl salicylate, limonene	Nikolić et al. (2013)
48	*Jasminum officinale* (jasmine)	Jasmine oil	*In flowers*: 3,7,11,15- tetramethyl-2-hexadecen-1-ol(phytol), 3,7,11- trimethyldodeca -1,6,10-trien-3-ol, 3,7,11,15-tetramethyl −1-Hexadecen-3-ol	We et al. (2015)
49	*Myrtus communis* (myrtle)	Myrtle oil	α-Pinene, 1,8-cincole, α-limonène, linalool, myrtenyl acetate	Mulas and Melis (2011)
50	*Pelargonium graveolens* (rose geranium)	Geranium oil	Citronellol (48.44%), α- pinene, octen-1-o1, geraniol, p-menthone, β-caryophyllene	Mousavi et al. (2014)
51	*Rhododendron tomentosum* (*syn. Ledum palustre*) (wild rosemary)	Ledum oil	*In leaves and shoots*: γ-terpineol *In stem*: β-myrcene	Gretšušnikova et al. (2010)
52	*Rosa damascena* or *Rosa centifolia* (rose)	Rose oil	Citronelol, geraniol, nonadecane, nerol	Dobreva et al. (2013)
53	*Rosa rubiginosa* or *Rosa mosqueta* (rose)	Rose hip oil	Vitispiran, α-*E*-acaridial, dodecanoic acid, hexadecanoic acid, docosane (C22), β-ionone, 6-methyl-5-hepten-2-one, 2-heptanone, heptanal, myristic acid and linolic acid	Nowak (2005)
54	*Vaccinium oxycoccos* (cranberry)	Cranberry seed oil	Palmitic acid, linoleic acid, oleic acid, linolenic acid	Bhagdeo (2004)
	Vines			
55	*Piper nigrum* (black pepper)	Black pepper oil	β-Caryophyllene, α-copaene, sabinene, cubenol	Rmili et al. (2014)
56	*Schisandra chinensis* (magnolia-vine)	Schisandra oil	Ylangene, β-himachalene α-bergamotene, β-chamigrene	Chen et al. (2011)

14.3 Synthesis of the Essential Oil Constituents

In order to genetically manipulate the essential oil yield and composition, the thorough understanding of their biosynthesis is required. The monoterpenoids are produced by the plastidic methyl–erythritol–phosphate (MEP) path, whose sequence and enzymatic properties have been explored (Estevez et al. 2001; Gao et al. 2006; Julsing et al. 2007; Proteau 2004; Rohdich et al. 2000; Rohdich et al. 2001; Rohdich et al. 2003; Wolfertz et al. 2004). The rate-limiting step is catalyzed by 1-deoxy-D-xylulose 5-phosphate synthase (DXS), an enzyme consuming NADPH, CTP, and ATP. The condensation of the produced dimethylallyl diphosphate (DMADP) by use of isopentenyl diphosphate (IDP), in order to form the monoterpenoid precursor geranyl diphosphate (GDP), is catalyzed by geranyl diphosphate synthase, a slow catalyst. Subsequent cyclization of GDP in addition to slow cyclases, which are membrane-bound enzymes in the plastids and endoplasmic reticulum, leads to the synthesis of various monoterpenes. The hydroxylations of the linear or cyclic monoterpenes are catalyzed by NADPH-consuming, cyt450-dependent monooxygenases, utilizing molecular oxygen or hydrogen peroxide. These hydroxylases are inducible by a variety of biotic or abiotic stress factors. Sesquiterpenoids are considered to be synthesized in cytosol from farnesyl diphosphate (FDP), derived from the mevalonic acid pathway. Two NADPH and three ATP molecules are consumed for the FDP synthesis, and the rate-limiting step is catalyzed by the 3-hydroxymethylglutaryl coenzyme A reductase (HMGR). Sesquiterpene cyclases act on FDP to produce at least 200 types of cyclic sesquiterpenoids.

The phenylpropanoid constituents of essential oils are produced from phenylalanine and tyrosine of the shikimic acid pathway (Goodwin and Mercer 1972; Weisshaar and Jenkins 1998). The pathway is tightly regulated by feedback inhibition at several of its steps as well as by the need for NADPH and ATP up to the synthesis of tyrosine and phenylalanine, with the requirement for NADPH being even greater in the transformation of Phe and Tyr into phenylpropanoids. NADPH is required for the reductive elimination of the carboxyl group of the propenyl side by successive reductions that form the corresponding volatile aldehydes, alcohols, and phenylpropenes. NADPH is also required for the hydroxylations of the aromatic ring. Benzoic and phenolic acids come from the corresponding hydroxycinnamic acids by β-oxidation of the propenyl chain, followed by oxidative decarboxylation. This process is tightly regulated by feedback inhibition. Volatile derivatives are then formed by the reduction of the carboxyl group, as in phenylpropanoids.

Non-branched volatile aldehydes and their corresponding alcohols can be derived by degradation of unsaturated fatty acids, mainly linoleic and linolenic acid (Combet et al. 2006; Feussner and Wasternack 2002) by the sequential action of lipoxygenases (LOX), hydroperoxide lyases (HPL), and aldehydes dehydrogenase (ADH). The initial introduction of molecular oxygen into the carbon–carbon double bonds also requires NADPH. Methyl-branched volatiles are produced by the catabolism of lineal terpenoids and of leucine-derived 3-methyl-crotonyl-CoA, via degradation of the produced 3-methyl glutaryl-CoA (Hoschle et al. 2005). Methyl-branched compounds, such as isovaleric and isobutyric acid, are also derived from

valine catabolism. Isovaleric acid could potentially be produced from DMADP of the terpenoid synthesis pathways. In bacteria, they could also be produced by the catabolism of linear monoterpenoids and sesquiterpenoids (Aguilar et al. 2006; Forster-Fromme et al. 2006). Unlike the LOX path, the degradations of leucine, valine, and monoterpenoids and DMADP, leading to volatile aldehyde, alcohol, and acid production, do not need reductive equivalents but rather produce them.

It should have become obvious from the above that for secondary metabolite production to be successfully completed, the need for enough available NADH must be satisfied. Thus, any genetic engineering approaches aiming to increase the production of secondary metabolites in transgenics should consider this requirement. Predictably, in order to enhance the yield of a particular metabolite, it would not be enough to increase the intracellular amount of key regulatory enzymes of their synthesis, if the coenzymes are in limited supply.

14.4 Tissue or Cell Cultures

Callus or cell culturing has been achieved for almost all of the plants presented in Table 14.1. However, most of these efforts lead to the genetic transformation of the plant by focusing on genes not directly involved in secondary metabolism. There was no reason to analyze the effect of in vitro culturing on the yield and composition of the essential oils in the untransformed or genetically transformed plants. Table 14.2 presents the cases in which the essential oil or the production of a specific secondary metabolite was analyzed.

A comparison with Table 14.1 shows that the secondary metabolite profile of in vitro cultured cells and calli is different from that of intact plants. As expected, taking into account the results of many previous studies with various plant species, the amounts were much reduced and, in some cases, no secondary metabolites were detected at all. Application of ethylene-producing agents or pathogen elicitors stimulates secondary metabolite synthesis. The exceptions are the alkaloids—caffeine and trigonelline—that are synthesized at amounts greater and comparable (respectively), compared to those in the intact plant tissues. Also the phenolic schisandrin A was produced at half the yield recorded from plant tissues.

Plants used as sources of secondary metabolites are often rare or slow growing or inhabiting difficult-to-approach localities and could be difficult to cultivate as well. Tissue or cell culturing overcomes most of these problems. Unfortunately, in vitro cultures are poor producers of secondary metabolites (Scragg 1995). The ability of cultured plant tissue and cells to produce secondary metabolites is inducible by a variety of chemical and physical factors (Olivoto et al. 2017; Shanker and Shanker 2016). Cases of higher production from in vitro cultures, compared to intact plant tissue, are also known. For example, *Ocimum sanctum* calli have less total phenolics but more total terpenoids than intact plant tissues (Mathew and Sankari 2014), although the monoterpene and sesquiterpene content has not been separately examined. Callus cultures of *Genista* species produce more isoflavones,

Table 14.2 Secondary metabolites produced by in vitro cultured tissues or cells of non-herbaceous plant species

Plant	Result/product	Reference
Trees		
Aquilaria malaccensis (agarwood)	Some essential oil was produced by callus or cell suspension cultures. More fatty acid derivatives formed by elicitation with *Trichoderma*	Jayaraman and Mohamed (2015)
Azadirachta indica (neem)	380 mg·L^{-1} azadirachtin in the cell suspension culture medium	Srivastava and Prakash (2010)
Betula spp. (birch)	Betulinic acid 2.01 g·kg^{-1} dry weight in callus. Betulin in cell suspension cultures was 0.96 g·kg^{-1} dry weight. Betulin forms 30% of intact birch bark dry weight	Hajati et al. (2016)
Cananga odorata (cananga tree)	Calli from petal explants. Only linalool and benzyl acetate were detected. Less linalool and more benzyl acetate than in intact flowers. More linalool under light conditions and more benzyl acetate in the dark	Lindain et al. (2008)
Citrus aurantifolia (Lime)	*C. aurantifolia* callus yielded limonene only, 4.4 mg·kg^{-1} fresh weight. Grapefruit peel has 2500 mg·kg^{-1}	Reila and Bergerh (1996)
Citrus aurantium subsp. *amara* (bitter orange)	No essential oil compound was detected in calli by Kriaa (2012), but del Rio et al. (1991) detected valencene at 0.060 mg·kg^{-1} and nootkatone at 0.16 mg·kg^{-1} callus fresh weight. The corresponding values in fruit pericarp are 3 and 20 mg·kg^{-1}, respectively	Kriaa (2012); del Rio et al. (1991)
Citrus Limon (lemon tree)	Valencene accumulated at 0.09 mg·kg^{-1} of callus fresh weight. The corresponding values in fruit pericarp are 47 and 131 mg·kg^{-1}	del Rio et al. (1991)
Citrus sinensis (orange tree)	Embryogenic calli yielded 5.4 μg·kg^{-1} essential oil of which 3.6 μg was 3-hydroxy-2-butanone	Niedz et al. (1997)
Citrus paradisi (grapefruit)	Valencene accumulated at 0.080 mg·kg^{-1} and nootkatone at 1.6 mg·kg^{-1} callus fresh weight. The corresponding values in fruit pericarp are 47 and 123 mg·kg^{-1}, respectively	del Rio et al. (1991)
Coffea arabica (coffee tree)	50- to 90-fold more caffeine in calli (4.5–10 kg per kg of callus) comparing to explant, calculated on a tissue dry weight basis. Most of the caffeine was extracellular	Waller et al. (1983)
	Calli yielded intracellularly 2 g caffeine and 12 g chlorogenic acid per kg of callus dry weight. Explants have 90 mg caffeine per kg dry weight	Baumann and Rohrig (1989)
Cupressus spp. *(cypress)*	*Cupressus lusitanica* suspension cells accumulated 22 mg of β-thujaplicin per g dry cell weight in 3 days after elicitation by insufficient inorganic nutrients and excess Fe	Itose and Sakai (1997)
	Monoterpenes and lignin were produced by *Cupressus lusitanica* suspension cells induced by fungal elicitor or mechanical stress	de Alwis et al. (2009)
Illicium verum (star anise)	Calli produce anethole and foeniculin	Kohda et al. (1997)
Juniperus virginiana (red cedar)	Suspension cultures provided with phenylalanine synthesized podophyllotoxin (0.56 g·kg^{-1} tissue dry weight), 20 times less than in the intact plant tissues	Kašparová et al. (2017)

(continued)

Table 14.2 (continued)

Plant	Result/product	Reference
Laurus nobilis (bay)	Calli produce 1,8-cineole and α-pinene	Rady and Youssef (1999)
Lawsonia inermis (henna tree)	Lawsone (0.13% dry weight) was observed in hairy root tissues incubated in the dark	Bakkali et al. (1997)
Melaleuca alternifolia (tea tree)	Total polyphenolics in calli were 13 μg (as gallic acid equivalents) per g of fresh callus tissue	Jeyakani Santhosh and Rajalakshmi (2016)
Moringa oleifera (drumstick tree)	Callus cultures had 2.38 g kg^{-1} trigonelline, comparing to that in pods (3.55 g·kg^{-1}), leaves (2.60 g·kg^{-1}), roots (2.15 g·kg^{-1}), stem (1.90 g·kg^{-1}), and flowers (1.60 g·kg^{-1})	Mathur and Kamal (2012)
Myristica fragrans (nutmeg)	β-Pinene, myristicin, safrole, methyl eugenol, and betasitosterol were detected in embryogenic callus	Indira-Iyer et al. (2009)
Santalum paniculatum or *S. album* (sandalwood)	Various sesquiterpenes, including beta-santalol and betasantalene, were synthesized by *S. album* calli treated with 1-aminocyclopropane-1-carboxylic acid (ACC)	Crovadore et al. (2012)
	No essential oils, but phenolics are produced by stressed sandalwood cell cultures	Valluri (2009)
	Santalols (5.2 mg·L^{-1}) and phenolics (31 mg·L^{-1}) are produced by somatic embryos	Misra and Dey (2013)
Thuja plicata (western red cedar)	Elicitation of calli by yeast extract results in accumulation of tropolones in about 6.5 g·kg^{-1} cell fresh weight. In a medium supplemented with glucose, tropolone accumulates to 8.1 g·kg^{-1} with elicitation and 3.15 g·kg^{-1} without elicitation	Haluk and Roussel-Bousta (2003)
Shrubs		
Jasminum officinale (jasmine)	*Jasminum officinale* callus accumulated traces of several monoterpenes (<0.1% the amount in petals)	Banthorpe et al. (1986)
Pelargonium graveolens (rose geranium)	0.54% oil yield by cell suspensions	Aly and Hanafy (2008)
Rosa damascena (rose)	Only traces, if at all, of some essential oil components in callus or cell cultures of *R. damascena*	Banthorpe and Barrow (1983)
Vines		
Piper nigrum (black pepper)	Piperine 0.852 g·kg^{-1} dry weight of callus. Plantlets had 4.16 g·kg^{-1} dry weight	Ahmad et al. (2013)
Schisandra chinensis (magnolia-vine)	Lignans at 244.8 g·kg^{-1} accumulated in the calli. The lignans were not detected in the media	Szopa et al. (2016)
	Schisandrin A at 0.251 g·kg^{-1}, 0.118 g·kg^{-1}, and 0.115 g·kg^{-1} was found in seeds, callus, and suspension cells, respectively. Schisandrin B was 0.142 g·kg^{-1}, 0.086 mg·kg^{-1}, and 0.05 mg·kg^{-1}, respectively	Zhou et al. (2017)
	Schisandrin 67.70 g, deoxyschisandrin 55.19 g, gomisin A 36.97 g, chlorogenic acid 15.33 g, and protocatechuic acid 13.11 g accumulated per kg dry weight of callus, under blue light	Szopa and Ekiert (2016)

whereas callus and cell cultures of *Maclura pomifera* produce more flavones and flavanones than the parent herbs (Filová 2014). Isoflavones and all flavonoids are synthesized from phenylalanine in the cytoplasm (Winkel-Shirley 2001). From Table 14.2, it can be seen that caffeine in *Coffea arabica* calli is higher than in explants.

The reasons for the reduced ability of the in vitro culture to produce volatiles, and secondary metabolites in general, are not known with certainty. The cultured cells and callus seem to have some enzymatic activity for terpenoid production (Banthorpe and Barrow 1983; Banthorpe et al. 1986; Soler et al. 1992; Zito et al. 1991). In the synthesis of phenylpropanoid, the enzymatic activities of phenylalanine ammonia lyase, shikimate dehydrogenase, cinnamic acid-4-hydroxylase, *p*-coumaric acid-3 hydroxylase, cinnamoyl-CoA reductase, 4-coumarate:CoA ligase, 4 hydroxycinnamate:CoA ligase, cinnamyl alcohol dehydrogenase, and caffeic acid O-methyltransferase in callus or cell suspensions have been found to often be equal to those of intact plant tissues (Ali et al. 2006; Anterola et al. 2002; Karyagina et al. 2007; Möller et al. 2006; Seidel et al. 2002).

An often-cited observation is that *some degree of differentiation is required* for secondary metabolite synthesis by cell and callus cultures. For example, fine hair-like structures (hairy roots) arise from plant tissues transformed with the transfer DNA (T-DNA) regions of the Ri plasmid of *Agrobacterium rhizogene.* Their culture methods, their morphological and biochemical characteristics, as well as their secondary metabolite production potential are all known (Figueiredo et al. 2006; Georgiev et al. 2007; Srivastava and Srivastava 2007). Hairy roots lack geotropism, and they are highly branched and can be cultured in bioreactor facilities requiring no plant growth regulators, since the inserted T-DNA carries genes for auxin synthesis. They grow as fast, or faster, than normal roots, with meristem cell cycles averaging 10 h. They produce secondary metabolites at levels and patterns similar to those of normal roots but also metabolites produced in aerial parts of the plant. Often novel compounds are also produced. Unlike cell or callus cultures, hairy roots are biochemically stable, and the T-DNA is reliably integrated.

The induction of essential oil production upon elicitation of cultured cells suggests that the transcription level for the necessary enzymes is lower than in the intact plant. In *Croton stellatopilosus*, for example, 1-deoxy-D-xylulose-5-phosphate synthase (*DXS*), 2*C*-methyl-D-erythritol 4-phosphate synthase (*MEPS*), and geranylgeranyl diphosphate synthase (*GGPPS*) were highly expressed in the cells from leaves and of the green callus culture but not in suspension cells, and this was in agreement with the terpenoid profiling (Kongduang et al. 2014). DXS is not the only regulatory step in plastidic terpenoid synthesis. The 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR) and 1-hydroxy-2-methyl-2-butenyl 4-diphosphate reductase (HDR) are also regulatory enzymes. Besides, back-inhibition by the product DMADP regulates DXR. To enhance essential oil production by calli and cell suspensions, it would perhaps be worthy to attempt simultaneous transformation of plastids by all genes for the above three enzymes.

However, even the best results of inducing secondary metabolite synthesis by genetically transformed callus or cell cultures cannot reach the levels of these compounds in intact tissues. It seems that the availability of the necessary biosynthetic

enzymes is not the only limiting factor. It must be considered that the synthesis of monoterpenoids, at least, is primarily carried out in the plastids and requires a lot of NADPH. Auxins used in tissue culture inactivate the chloroplast photosynthetic apparatus (Buggeln and Bal 1977; Volfová et al. 1978; Zubo et al. 2011). Photosynthesis is also inactivated in these cases by the dark culturing regime used. Under these conditions, plastids will have to rely on the oxaloacetate/malate shuttle (Anderson and House 1979) for obtaining NADPH from the cytoplasm. The published literature did not provide with any report on the concentration of NADPH or on the functionality of the oxaloacetate/malate shuttle in callus plastids. We cannot expect plastids, even with over-expressed regulatory enzymes of the MEP pathway, to reach the levels of terpenoid synthesis of intact photosynthesizing plant tissues, if the availability of intra-plastidic reducing equivalents is, indeed, the limiting factor.

14.5 Transgenic Plant Species

The cases of genetic engineering efforts to alter the essential oil production and constituency of non-herbaceous plants are very limited (Table 14.3). Hairy roots of neem tree have been made to produce 7 mg of the furanolactone tertanor-triterpenoid (limonoid) azadirachtin per kg of weight, but azadirachtin is primarily produced in the plant's seed kernels at amounts of 4–6 $g \cdot kg^{-1}$ (Mordue and Nisbet 2000). This compound is not the prevalent one in the corresponding essential oil, but it is the one endowing it with its pesticidal properties. Henna hairy roots produce the 2-hydroxy-1,4-naphtho-quinone lawsone at amounts of 0.13% of dry weight of tissue, whereas natural plant leaves contain these at 0.5–2% (Sabra et al. 2015). Two of several regenerated rose geranium plants, also carrying the Ri plasmid, produced even more geraniol and its esters. Since this was not a general characteristic of the majority of regenerated transgenics, it is not known if it is due to the plasmid or to a variation generated during the callus culturing and regeneration stage.

In black pepper, the isopentenyl pyrophosphate transferase gene reduced the total monoterpenoids in favor of sesquiterpenoids, as expected by the shunting of isopentenyl pyrophosphate to larger terpenoid synthesis. Again as expected, the transgene for limonene synthase determined the levels of limonene and other monoterpenes in eucalyptus and orange oil. Both calli and hairy roots synthesize it at levels higher than the plant roots but still at 1000-fold lower amounts than in the seed kernels (Mordue and Nisbet 2000). Finally, transformation with sense or antisense theobromine synthase and xanthosine-N^7-methyltransferase affected the levels of caffeine and theobromine in coffee plants.

Despite the small number of cases in which the essential oil or some of its components were examined, transgenic trees, shrubs, and vines, such plant species genetically transformed with a variety of genes exist, and it should not be difficult to analyze the effects of genetic transformation on oil composition. For example, transgenic birch with downregulated 4-coumaroyl-CoA-ligase gene has been created, and its lignin, but not the essential oil, has been examined (Shestibratov et al. 2011). Similarly, only the lignin was examined in birch (Zhang et al. 2015) and

Table 14.3 Effect of transgenes on non-herbaceous plant essential oils

Plant/oil	Transformation/transgene	Effect	References
Trees			
Azadirachta indica (neem) Neem oil	Ri plasmid of *A. rhizogenes*	Hairy roots with a tenfold greater amount of azadirachtin compared to the callus line that contains 7 ppm azadirachtin and approximately 30 times more than that detected in natural, plant roots	Allan et al. (2002)
Citrus sinensis (orange tree) Orange oil	Downregulated limonene synthase by antisense constructs	Less limonene in peel. Lower infection by *Penicillium digitatum*, the bacterium *Xanthomonas citri* subsp. *Citri*, and the pest medfly *Ceratitis capitata*	Rodríguez et al. (2011)
Coffea arabica (coffee tree) Coffee oil	Inhibition of a theobromine synthase gene (*CaMXMT1*) using RNAi technology	Lower caffeine and theobromine	Ogita et al. (2003, 2004)
	Transformation with sense or antisense xanthosine-N^7-methyltransferase (XMT)	Lower caffeine	Stiles et al. (2000)
	Transformation with the theobromine synthase gene	More theobromine	Sano et al. (2002)
Eucalyptus spp. (eucalyptus) Eucalyptus oil	*Eucalyptus camaldulensis* carrying the *Perilla frutescens* limonene synthase (PFLS)	2.6 and 4.5 times more limonene, more 1,8-cineole and α-pinene	Ohara et al. (2010)
Lawsonia inermis (henna tree) Henna oil	Co-culture of leaf segments with *Agrobacterium rhizogenes* NCIB 8196	Hairy root cultures accumulated lawsone (0.13% dry weight) in the dark	Bakkali et al. (1997)
Shrubs			
Pelargonium graveolens (rose geranium) Geranium oil	Transformed by *Agrobacterium rhizogenes* (Ri-insertion)	Two transgenic plants showed increase in concentrations of geraniol and geranyl esters	Saxena et al. (2007)
Vines			
Piper nigrum	Transgenics carrying the isopentenyl pyrophosphate transferase (IPT) gene	21.1 to and 15.8% more oxygenated sesquiterpenoids and 14.9–4.0% less monoterpenoids	Zeng and Zhao (2016)

spruce (Wadenbäck 2006) transformed with over- or under-expressing genes for cinnamoyl-CoA reductase. *Citrus* species transformed with pathogen-derived genes or with abiotic stress resistance genes are known (Febres et al. 2011), and the

connection between abiotic or biotic stress and secondary metabolite synthesis has also been well documented, yet the examination of the effect on the essential oil was not included in the researchers' immediate interests at the time.

14.6 Biotransformations of Essential Oils and Their Constituents

Essential oils are very rarely used in their entirety in biotransformations. Although the reasons for this are more empirical than researched, essential oils are usually inhibitory to bacterial or fungal cell growth. Some cases of the use of the entire essential oils in bioconversions are presented in Table 14.4. The general tendency is for the essential oil components to be transformed into more oxygenated products of the same or lower number of carbon atoms. For example, citron oil, consisting primarily of the aliphatic monoterpenoids limonene, terpinene, and isolimonene and citral, produced five-carbon valerolactones, octanol, the nine-carbon ketone cryptone, and the ten-carbon alcohols hydroxycitronelol and cuminol. The 12-carbon γ-dodecalactone was also produced. This valuable flavoring compound is a product of fatty acid catabolism (An et al. 2013). Similarly, hydroxylated monoterpenoids were produced from biotransformed pinenes of spruce turpentines. Interestingly, the non-oxygenated monoterpenes of mandarin oil were transformed into triterpenoids by the fungus *Antrodia cinnamomea*, indicating that anabolic processes could also utilize monoterpenes as substrates.

By far, the most successful biotechnology approaches are those using microorganisms to bioconvert isolated compounds of essential oils. An extensive list has been reported before (Gounaris 2010). However, Table 14.5 provides a more focused and updated list for the major essential oil components from non-herbaceous plants. The bioconversions can be achieved by bacterial, fungal, or plant cell suspensions, or by isolated enzymes. Almost all transformations are catabolic oxygenations

Table 14.4 Cases of use of the entire essential oil from non-herbaceous plants in biotransformation studies

Origin of the essential oil	Result	Reference
Citrus medica (citron)	Peel oil was transformed by *Enterobacter agglomerans* into trans-2-decenal, octanol, δ-valerolactone, γ-valerolactone, cryptone, hydroxycitronellol, cuminol, and γ-dodecalactone	Park et al. (2004)
Citrus reticulata (mandarin orange)	4% peel extract was transformed by the mushroom *Antrodia cinnamomea* into triterpenoids	Ma et al. (2014)
Picea spp. (spruce)	*P. abies* turpentines (primarily pinenes) were transformed by suspension cultures of *P. abies* primarily into pinocarveol and myrtenol	Dvořaková et al. (2011)

Table 14.5 Bioconversions of non-herbaceous plant essential oil constituents

Substrate	Products	Transforming organism	Reference	Usual substrate % in potential plant source*
1,8-cineole	13.3 g·L^{-1} (*1R*)-6b-hydroxy-1,8-cineole in 89 h in a bioreactor. Yield 79%	*Pseudomonas putida* carrying the cytochrome P450 monooxygenase CYP176A1 (P450cin) and its native redox partner cindoxin (CinC) from *Citrobacter braakii*	Mi et al. (2016)	Eucalyptus oil (up to 50%); bay oil (up to 46%); and
	(*1R*)-6b-hydroxy-1,8-cineole	*E. coli* carrying the cytochrome P450 monooxygenases from *Sphingobium yanoikuyae* B2	Unterweger et al. (2016)	myrtle oil (10–18%)
	2-exo-hydroxy-1,8-cineole	*Mucor ramannianus* and *Aspergillus niger* (fungi)	de Souza et al. (2015)	
	2-exo-hydroxy-1,8-cineole, 2-endohydroxy-1,8-cineole, 3-exo-hydroxy-1,8-cineole, and 3-endo-hydroxy-1,8-cineole	*Aspergillus terreus* (fungus)	García et al. (2009)	
	2-endo-hydroxy-1,8-cineole, 2-exo-hydroxy-1,8-cineole and 2-oxo-1,8-cineole	*Rhodococcus* sp. (bacterium)	Rodríguez et al. (2006)	
Anethole	Anethole epoxide, *syn*- and *anti*-anethole-diol, p-anisaldehyde, p-anisic acid, p-anisic alcohol, 3-(4-methoxyphenyl)-1-propanol, 1-(4-methoxyphenyl)-2-propanol, and ethyl ester of anisic acid	*Colletotrichum acutatum* (fungus)	Velasco-Bucheli et al. (2015)	Star anise oil (10%); pine oil (14%)
	57.33% molar conversion rate to anisic acid	*Burkholderia* sp. (bacterium)	Shen et al. (2014)	
	36.1% molar transformation ratio to anisic acid	*Pseudomonas* sp. (bacterium)	Su et al. (2011)	

	syn- and *anti*-anethole epoxides, p-anisic acid, and p-hydroxybenzoic acid	*Pseudomonas putida* (bacterium)	Ryu et al. (2005)	
Aromadendrene	Was converted to (−)-(*10S,11S*)-10,13,14-trihydroxyaromadendrane by hydroxylation at C-10, C-13, and C-14	*Aspergillus wentii* (fungus)	Miyazawa et al. (2008)	Eucalyptus fruit or branch oil (8–23%)
	Hydroxylated at C-10, C-13, and C-14 by oxidation of the double bond and the germinal methyl group	*Glomerella cingulata* (fungus)	Miyazawa et al. (1995)	
Benzyl acetate	Hydrolyzed to benzyl alcohol	*Spirodela oligorrhiza* (aquatic plant)	Pawlowicz and Stewinski (1987)	Ylang-ylang oil (27%)
Bornyl acetate	Hydroxylation and ketonization, as well as of the borneol derived from it by hydrolysis	*Collybia velutipes*, *Trametes hirsute*, *Ganoderma applanatum* (all basidiomycetes)	Nano et al. (2005)	*Picea mariana* (black spruce) oil (34%)
	Hydrolysis to isoborneol and endo-borneol	*Glycyrrhiza glabra* L. cell suspension	Shams-Ardakani et al. (2005)	
	Hydroxylated to (+)- and (−)-5-*exo*-hydroxybornyl acetate, (+)- and (−)-5-oxobornyl acetate that hydrolyzed to (+)- and (−)-borneol, respectively	*Glomerella cingulata* (fungus)	Miyazawa and Miyasato (2001)	
	Hydrolysis to borneol	*Nicotiana tabacum* cell suspension	Suga et al. (1986)	
	Hydrolysis to borneol	*Spirodela oligorrhiza* (aquatic plant)	Pawlowicz et al. (1988)	
Caffeine	Demethylation to theophylline	*Fusarium solani* (fungus)	Nanjundaiah et al. (2017)	Coffee oil (1.5%)
	Demethylation to theobromine	*Salinivibrio costicola* (bacterium)	Ashengroph (2017a)	
	Demethylation to theophylline	*Aureobasidium* sp. (fungus)	Ashengroph (2017b).	

(continued)

Table 14.5 (continued)

Substrate	Products	Transforming organism	Reference	Usual substrate % in potential plant source*
	Demethylation to theophylline and 1,7-dimethylxanthines	*Paecilomyces gunnii* (fungus)	Zheng et al. (2016)	
Camphor	Hydroxylated to 5-exo- and 8-hydroxy-camphor	*Salmonella typhimurium* expressing human cytochrome P450 and NADPH-P450 reductase	Nakahashi and Miyazawa (2011)	Camphor oil (51%); spruce oil
	Hydroxylated to 6-, 5-, 3-, and 8-hydroxycamphor	*Botryosphaeria* sp. CBMAI 1197 (fungus)	de Jesus et al. (2017)	(20%); sassafras oil (3%)
Caryophyllene	Epoxidation and hydroxylation to 4,5-epoxy-caryophyllene-7,12-diol and clovanes	*Chaetomium cochliodes* (fungus)	Abraham et al. (1990a)	Cypress oil (10%)
	Various epoxidate and hydroxylated derivatives	*Diplodia gossypina* (fungus)	Abraham et al. (1990b)	
	Epoxidation to (−)-β-caryophyllene oxide	*Nemania aenea* (fungus)	Oda et al. (2011)	
Chavicol	Hydroxylation of the first carbon of the side chain to produce 1-(4′-hydroxyphenyl) -2-propen-1-ol	4-Ethylphenol methylenehydroxylase from *Pseudomonas putida* JD1 (bacterial enzyme)	Hopper and Cottrell (2003)	Raversara oil (80%)
Chlorogenic acid	Catabolized to caffeic acid, shikimic acid, and 3,4-dihydroxybenzoic acid	*Sphingomonas* sp. (bacterium)	Ma et al. (2016)	Coffee oil (7%)
	Catabolized to hydroxychlorogenic, protocatechuic, and caffeic acid	*Fusarium graminearum* (fungus)	Gauthier et al. (2016)	
	Hydrolysis to caffeic acid	*Bifidobacterium animalis* (anaerobic bacterium)	Raimondi et al. (2015)	
Cinnamaldehyde	Reduction, esterification and decarboxylation to 3-phenyl-1-propanol, cinnamyl alcohol, 3-phenylpropanal, 3-phenylpropyl acetate, cinnamyl acetate, benzylic alcohol, 1-phenylethanol, and 2-phenylethanol	*Aspergillus* sp. (fungus)	Rodrigo et al. (2010)	Cinnamon oil (60–80%)

	Conversion to cinnamic acid	*Streptomyces viridosporus* aldehyde oxidase (bacterial enzyme)	Wiklof et al. (1984)	
	Conversion to 3-phenylpropanol and cinnamyl alcohol	*Euglena gracilis* (algae)	Noma et al. (1991)	
	Reduction to hydrocinnamyl alcohol	Cell suspension culture of *Glycyrrhiza glabra*	Shams-Ardakani et al. (2005)	
	100% conversion to cinnamyl alcohol	Yeast alcohol dehydrogenase (fungal enzyme)	Zucca et al. (2009)	
	Conversion (reduction) to cinnamyl alcohol	*Mucor* sp. (mold, fungus)	Ma et al. (2011)	
Citral	Converted to thymol (21.5%), geranial (18.6%), and nerol (13.7%)	*Penicillium* sp. (fungus)	Esmaeili and Tavassoli (2010)	Citron peel oil (23%); lime oil (4%)
	Primarily citronellol (48.5%)	*Saccharomyces cerevisiae* (yeast, fungus)	Esmaeili et al. (2012)	
	1 mM citronellal	*Zymomonas mobilis* and *Citrobacter freundii* (anaerobic bacteria)	Müller et al. (2006)	
	80% conversion to geraniol and nerol	Cell suspensions of *Vitis vinifera*	Ambid et al. (1982)	
Citronellol	Cyclization to 25–26 $g \cdot l^{-1}$ rose oxide	*Penicillium* sp. (fungus)	Maróstica Jr and Pastore (2006); Pimentel et al. (2012)	*Rosa damascena* oil (up to 48%)
	Oxidativelly halogenated by 51% to 6-bromo-3,7-dimethyloctane-1,7-diol	Chloroperoxidase from *Caldariomyces fumago* (fungal enzyme)	Piantini et al. (2011)	
	Cyclization by 75% to rose oxide and by concomitant oxidation to 12% nerol oxide	*Aspergillus niger* (fungus)	Demyttenaere et al. (2004)	
	8- and 10-hydroxy citronellol in 13.0 and 50.0% yield, respectively	Suspension cells of *Catharanthus roseus*	Hamada et al. (2001)	

(continued)

Table 14.5 (continued)

Substrate	Products	Transforming organism	Reference	Usual substrate % in potential plant source*
	52% conversion to 3,7-dimethyl-1,6,7-octanetriol	*Cystoderma carcharias* (basidiomycete)	Onken and Berger (1999)	
	Oxidation by 70–80% to citronellal	*Rhodococcus equi* (bacterium)	Oda et al. (1996)	
	2,6-dimethyl-l,8-octandiol and (*E*)-2,6-dimethyl-2-octen-l,8-diol	*Botrytis cinerea* (fungus)	Bruneriel et al. (1987)	
Coniferaldehyde	100% conversion to cinnamic acids	*Saccharomyces cerevisia* carrying the *Pseudomonas* acetaldehyde dehydrogenase gene	Adeboye et al. (2016)	Sassafras oil (15%).
Isolimonene	Isomerization to isoterpinolene	*Alcaligenes defragrans* (bacterium)	Heyen and Harder (1998)	Citron oil (40%).
Limonene	Hydroxylated and ketonized with percentage yields 1.53 for limonene-1,2-diol, 2.63 for p-mentha-2,8-diene-1-ol-trans, 6.16 for carveol, and 6.80 for carvone	Immobilized cells of *Nigella sativa*	Rasheed-Uz-Zafar and Kausar (2013)	Grapefruit oil (90–95%); orange oil (60–90%); tangerine oil (85%); calamondin peel oil (77%); lemon oil (72%); mandarin oil (67%); lime oil (58%); elemi oil (56%); bergamot oil (37%); neroli oil (27%); Palo santo oil (26%); citron oil (18%); pine oil (17%)
	Oxidized to 34 $g \cdot L^{-1} \cdot day^{-1}$ perillic acid	*Pseudomonas putida* GS1 (bacterium)	Willrodt et al. (2017)	
	0.536 $g \cdot L^{-1}$carvone and 2.08 $g \cdot L^{-1}$ limonene-1,2-diol. Also, some terpinen-4-ol, menthol, and carveol	*Phomopsis* sp. (fungus)	Bier et al. (2017)	
	833.93 $mg \cdot L^{-1}$ α-terpineol	*Penicillium digitatum* DSM 62840 (fungus)	Tai et al. (2016)	
	40% conversion to carveol	*Escherichia coli* carrying a *Pseudomonas putida* S12 oxygenase	Groeneveld et al. (2016)	
	3.9 $g \cdot L^{-1}$ α-terpineol	*Fusarium oxysporum* 152B (fungus)	Molina et al. (2015)	

	Oxidation to 855 mg·L^{-1} perillic acid	*Yarrowia lipolytica* (fungus, yeast)	Ferrara et al. (2013)	
Linalool	Epoxidation to 4.6 g·L^{-1} linalool oxides	*Corynespora cassiicola* DSM 62475 (fungus)	Bormann et al. (2012)	Camphor oil (95%); rosewood oil (82%); pine oil (24%); myrtle oil (16%); bergamot oil (9%); and ylang-ylang (9%)
	Epoxidation and hydroxylation/ carbonylation to 150 μg·L^{-1} lilac aldehyde and lilac alcohol	*Botrytis cinerea* (fungus)	Mirata et al. (2006)	
	Cis- and *trans*-furanoid linalool oxide (yield 30% and 5%, respectively) and *cis*- and *trans*-pyranoid linalool oxide (yield 14% and 1.5%, respectively)	*Aspergillus niger* (fungus)	Demyttenaere et al. (2001)	
	90% conversion to (*E*)-2,6-dimethyl-2,7-octadiene-1,6-diol	*Botrytis cinerea* (fungus)	Bock and Schreier (1986)	
	16% conversion to 8-hydroxy-linalool	*Nicotiana tabacum* cell suspension	Toshifumi et al. (1981)	
Linalyl acetate	40% transformation into linalool, geraniol, and α-terpineol	Suspension cultures of *Papaver bracteatum*	Hook et al. (1990)	Bergamot oil (30%)
	32% hydrolysis to linalool	*Geotrichum capitatum* (fungus, yeast)	van Dyk and Thomas (1998)	
Linoleic acid	Shifting of double bonds, via a hydroxylated derivative, to become conjugated linoleic acid	Species of *Lactobacillus*, *Bifidobacterium*, *Propionibacterium*, *Clostridium*, *Lactococcus*, *Pediococcus*, and other bacteria	Yang et al. (2017)	Cranberry seed oil (72%);
	31% conversion into δ-decalactone	*Yarrowia lipolytica* (fungus)	Kang et al. (2016)	hickory nut oil (32%)
	79% conversion to 13S-hydroxy-9(*Z*)-octadecenoic acid	*Escherichia coli* transformed with the *Lactobacillus acidophilus* linoleate hydratase	Park et al. (2015)	

(continued)

Table 14.5 (continued)

Substrate	Products	Transforming organism	Reference	Usual substrate % in potential plant source*
Myrcene	Myrcenoic acid, myrcenal, myrcen-8-ol, 4-methylhexanoic acid, 4-methylhexenoic acid	*Pseudomonas* sp. strain M1 (bacterium)	Soares-Castro et al. (2017)	Ledum oil (31% β-myrcene); orange oil (up to 18%); juniper berry oil (8%); frankincense (7%); cedarwood oil (5%)
	In 3 days, 90% conversion to 2,6-dimethyloctane and 7.7% to α-terpineol (7.7%). In 1.5 days 79.5% dihydrolinalool and 9.3% 2,6-dimethyloctane	*Pseudomonas aeruginosa* (bacterium)	Esmaeili and Hashemi (2011)	
	19 methylated, hydroxylated, epoxidated and dehydrogenated products at 1–10 $mg \cdot L^{-1}$ each, among which perillene	*Pleurotus ostreatus* (mushroom, fungus)	Krings et al. (2008)	
	Numerous hydroxylated, epoxidated, and carbonylated acyclic and monocyclic metabolites, but primarily myrcenol	*Ganoderma applanatura*, *Pleurotus flabellatus*, and *Pleurotus sajor-caju* (basidiomycete, fungi)	Esmaeili and Hashemi (2011)	
Oleic acid	58% transformation to 9-(nonanoyloxy) nonanoic acid	Hydratase from *Stenotrophomonas maltophilia*, alcohol dehydrogenase from *Micrococcus luteus*, and a monooxygenase from *Pseudomonas putida* KT2440, expressed in recombinant *Escherichia coli*	Koppireddi et al. (2016)	Moringa seed oil (70%); hickory nut oil (52%); cranberry
	72% conversion to *10S*-hydroxy- *8(E)*-octadecenoic acid	*Escherichia coli* cells expressing 10S-dioxygenase from *Nostoc punctiforme* PCC 73102	Kim et al. (2017)	seed oil (13%)
	32.1 $g \cdot L^{-1}$ citric acid	*Yarrowia lipolytica* (yeast, fungus)	Liu et al. (2016)	

	78% conversion to 10-ketostearic acid	Recombinant *Corynebacterium glutamicum* ATCC 13032 expressing oleate hydratase from *Stenotrophomonas maltophilia* and a secondary alcohol dehydrogenase from *Micrococcus luteus*	Lee et al. (2015)	
	94% to 10-hydroxystearic acid	*Lysinibacillus fusiformis* oleate hydratase	Kim et al. (2012)	
p-cymene	Oxidation of the methyl group to p-cumic alcohol	*Escherichia coli* carrying the p-cymene monooxygenase from *Pseudomonas putid*a	Nishio et al. (2001)	Eucalyptus oil (up to 27%)
p-Menthone	Reduction to menthol	*Chlorella vulgaris* (algae)	Ghasemi et al. (2010)	Geranium oil (7%)
	Reduction to menthol	*Isochrysis galbana*, *Porphyridium purpureum* (algae)	Hook et al. (2003)	
Thujopsene	55% conversion to a mixture consisting of 3-hydroxy-4-thujopsene (4%), mayurone (63%), and 3-epoxythujopsan-5-ol (33%)	Cultured cells of *Hibiscus cannabinus*	Chai et al. (2004)	Cedarwood oil (47%)
	Converted to mayurone (52%), 3β-hydroxy-4-thujopsene (16%), and 3β-epoxythujopsa-5β-ol (22%)	Cell suspension culture of *Caragana chamlagu*	Sakamaki et al. (2001)	
α-Cedrol	Converted to 12β-hydroxy cedrol, 10α-hydroxy cedrol, and 3α-hydroxy cedrol	*Neurospora crassa* (fungus)	Kiran et al. (2010)	Cedarwood oil (11%)
α-Pinene	65% epoxidation of pinene	*Aspergillus niger* lipase (fungal enzyme)	Tudorache et al. (2016)	Cedarwood bark oil (77%); juniper berry oil (51%); labdanum (up to 47%); tsuga oil (24%); curry leaf oil (19%); eucalyptus oil (up to 17%); nutmeg seed oil (13%); spruce oil (12%)
	Conversion to 0.25 $g \cdot L^{-1}$ α-terpineol	*Polyporus brumalis* (fungus)	Lee et al. (2015a)	
	Verbenone as the major product and myrtenol, camphor, and isopinocarveol as minor products	*Stereum hirsutum* (fungus)	Lee et al. (2015b)	

(continued)

Table 14.5 (continued)

Substrate	Products	Transforming organism	Reference	Usual substrate % in potential plant source*
	722 and 176 mg·L^{-1} verbenol and verbenone, respectively	*Chrysosporium pannorum* (fungus)	Trytek et al. (2015)	
	55% conversion to α-terpineol and isoterpineol	Cell suspensions of *Absidia corulea* (fungus)	Siddhardha et al. (2012)	
α-Thujone	Hydroxylation by 22–39% to 4-hydroxy-thujone and by 60–77% to 7-hydroxy-a-thujone	*Absidia* species (fungi)	Gniłka et al. (2015)	Western red cedar oil (62%)
	60% conversion to 2-S-hydroxylated α-thujone	*Aspergillus* sp. (fungus)	Alaoui and Benjilali (1994)	
β-Caryophyllene	30 g·L^{-1} (−)-β-caryophyllene oxide	*Nemania aenea* SF 10099–1 (basidiomycete, fungus)	Oda et al. (2011)	Black pepper oil (47–51%)
γ-Terpinene	Produces p-mentha-1,4- dien-9-ol	*Stemphylium botryosum* (mold, fungus)	Krings et al. (2005)	Tea tree leaf oil (23%); mandarin oil (16%); cedarwood oil (10%); lemon oil (9%); bergamot oil (7%); curry leaf oil (4–7%); tangerine oil (5%)

producing hydroxylated, epoxidated, or ketonized derivatives of the substrate or even derivatives carrying a carboxyl group. They can also be catabolic hydrolyzing or demethylation reactions. Rarely, such as in the cases of photosynthesizing biotransformers, can there be reductions. The products are very often minor components of the essential oil of which the biotransformed compound is a major ingredient. Of the products, of significant commercial interest are the perfume compounds decalactone, lilac aldehyde, lilac alcohol, phenylethanol, and caryophyllene oxide; the antimicrobials anisic acid (Saha et al. 2013), benzyl alcohol, (Gershanik et al. 1982), and verbenone (Santoyo et al. 2005); as well as the anticancer agent carveol (Crowell et al. 1992).

14.7 Conclusions

The usefulness and utilization of tree, shrub, and vine essential oils in biotechnological applications have only received limited exploration. In vitro cultured calli or cell suspensions produce greatly reduced amounts of essential oil components, even in the cases of genetically transformed tissues, carrying genes for terpenoid synthesis. The exception is the synthesis of alkaloids. This is an observation generally applicable to herbaceous species as well. The problem has to be tackled from a novel approach, since the availability of the necessary biosynthetic enzymes does not seem to be the limiting factor. Production of commercially useful compounds by the biotransformation of individual essential oil components by bacteria, fungi, or isolated enzymes is much more promising. The yields very often exceed the threshold for a commercially useful application of 1 g of product/L of culture medium. And the results are even more impressive in the case of microorganisms carrying transgenes used for the biosynthesis of essential oil ingredients.

References

Ab Rahman SFBT (2009) Analysis of agarwood oil composition by thin layer chromatography. Dissertation, Universiti Malaysia Pahang

Abraham W-R, Ernst L, Arfmann H-A (1990a) Rearranged caryophyllenes by biotransformation with *Chaetonium cochliodes*. Phytochemistry 29(3):757–763

Abraham W-R, Ernst L, Stumpf B (1990b) Biotransformation of caryophyllene by *Diplodia gossypina*. Phytochemistry 29(1):115–120

Adebola O, Ekundayo OO, Koenig WA (2005) Essential oil composition of *Lawsonia inermis* L. leaves from Nigeria. J Essent Oil Res 17:403–404

Adeboye PT, Olsson L, Bettiga M (2016) A coniferyl aldehyde dehydrogenase gene from *Pseudomonas* sp. strain HR199 enhances the conversion of coniferyl aldehyde by *Saccharomyces cerevisiae*. Bioresour Technol 212:11–19

Aguilar JA, Zavala AN, Diaz-Perez C, Cervantes C, Diaz-Perez AL, Campos-Garcia J (2006) The atu and liu clusters are involved in the catabolic pathways for acyclic monoterpenes and leucine in *Pseudomonas aeruginosa*. Appl Environ Microbiol 72(3):2070–2079

Ahmad N, Abbasi BH, ur Rahman I, Faza H (2013) *Piper nigrum*: micropropagation, antioxidative enzyme activities, and chromatographic fingerprint analysis for quality control. Appl Biochem Biotechnol 169:2004–2015

Al Harrasi A, Al Saidi S (2008) Phytochemical analysis of the essential oil from botanically certified oleogum resin of *Boswellia sacra* (Omani Luban). Molecules 13:2181–2189

Alaoui MI, Benjilali B (1994) Biotransformation of terpenic compounds by fungi. II-Metabolism of α-(−)-thujone. Nat Prod Lett 4:263–266

Ali MB, Singh N, Shohael AM, Hahn E-J, Paek K-J (2006) Phenolics metabolism and lignin synthesis in root suspension cultures of *Panax ginseng* in response to copper stress. Plant Sci 171(1):147–154

Allan E, Eeswara J, Jarvis A, Mordue A, Morgan E, Stuchbury T (2002) Induction of hairy root cultures of *Azadirachta indica* A. Juss. and their production of azadirachtin and other important insect bioactive metabolites. Plant Cell Rep 21:374–379

Aly U, Hanafy M (2008) Geranium oil production in suspension cultures of *Pelargonium graveolens* L. Med Aromat Plant Sci Biotechnol 2:24–28

Ambid C, Moisseeff M, Fallot J (1982) Biogenesis of monoterpenes bioconversion of citral by a cell suspension culture of Muscat grapes. Plant Cell Rep 1:91–93

Ammar AH, Bouajila J, Lebrihi A, Mathieu F, Romdhane M, Zagrouba F (2012) Chemical composition and *in vitro* antimicrobial and antioxidant activities of *Citrus aurantium* l. flowers essential oil (Neroli oil). Pak J Biol Sci 15:1034–1040

An J-U, Joo Y-C, Oh D-K (2013) New biotransformation process for production of the fragrant compound γ-dodecalactone from 10-hydroxystearate by permeabilized *Waltomyces lipofer* cells. Appl Environ Microbiol 79(8):2636–2641

Anderson JW, House CM (1979) Polarographic study of dicarboxylic-acid-dependent export of reducing equivalents from illuminated chloroplasts. Plant Physiol 64:1064–1069

Andrianoelisoa HS, Chantal Menut C, de Chatelperron PC, Saracco J, Ramanoelina P, Danthu P (2006) Intraspecific chemical variability and highlighting of chemotypes of leaf essential oils from *Ravensara aromatica* Sonnerat, a tree endemic to Madagascar. Flavour Frag J 21:833–838

Anterola AM, Jeon JH, Davin LB, Lewis NG (2002) Transcriptional control of monolignol biosynthesis in *Pinus taeda*: factors affecting monolignol ratios and carbon allocation in phenylpropanoid metabolism. J Biol Chem 277(21):18272–18280

Ashengroph M (2017a) *Salinivibrio costicola* GL6, a novel isolated strain for biotransformation of caffeine to theobromine under hypersaline conditions. Curr Microbiol 74:34–41

Ashengroph M (2017b) A novel strain of *Aureobasidium sp.* TeO12 for theophylline production from caffeine. 3 Biotech 7:176. https://doi.org/10.1007/s13205-017-0793-7

Atal CK, Kapur BM (1982) Cultivation and utilization of medical plants. Regional Research Laboratory, New Dehli

Azar PA, Nekoei M, Larijani K, Bahraminasab S (2011) Chemical composition of the essential oils of *Citrus sinensis* cv.*Valencia* and a quantitative structure–retention relationshipstudy for the prediction of retention indices by multiple linear regression. J Serb Chem Soc 76:1627–1637

Bakkali AT, Jaziri M, Foriers A, Vander Heyden Y, Vanhaelen M, Homes J (1997) Lawsone accumulation in normal and transformed cultures of henna, *Lawsonia inermis*. Plant Cell Tissue Organ Cult 51:83–87

Banthorpe DV, Barrow SE (1983) Monoterpene biosynthesis in extracts from cultures of *Rosa damascena*. Phytochemistry 22:2127–2728

Banthorpe DV, Branch SA, Njar VCO, Osborne MG, Watson DG (1986) Ability of plant callus cultures to synthesize and accumulate lower terpenoids. Phytochemistry 25(3):629–636

Başer KHC, Demirci B (2007) Studies on *Betula* essential oils. Arkivoc vii:335–348

Baumann TW, Rohrig L (1989) Formation and intracellular accumulation of caffeine and chlorogenic acid in suspension cultures of *Coffea arabica*. Phytochemistry 28:2667–2669

Bhagdeo M (2004) Determination of extracTables from cranberry seeds using supercritical CO_2. Dissertation, Virginia Polytechnic Institute and State University

Bhuiyan MNI, Begum J, Sardar PK, Rahman MS (2009) Constituents of peel and leaf essential oils of *Citrus Medica* L. J Sci Res 1:387–392

Bier MCJ, Medeiros ABP, Soccol CR (2017) Biotransformation of limonene by an endophytic fungus using synthetic and orange residue-based media. Fungal Biol 121:137–144

Bock G, Schreier P (1986) Biotransformation of linalool by *Botrytis cinerea*. J Food Sci 51(3):659–662

Bormann S, Etschmann MMW, Mirata M-A, Schrader J (2012) Integrated bioprocess for the stereospecific production of linalool oxides from linalool with *Corynespora cassiicola* DSM 62475. J Ind Microbiol Biotechnol 39:1761–1769

Boughendjioua H, Boughendjioua Z (2017) Chemical composition and biological activity of essential oil of mandarin (*Citrus reticulata)* cultivated in Algeria. Int J Pharm Sci Rev Res 44:179–184

Brokl M, Fauconnier M-L, Benini C, Lognay G, du Jardin P, Focant J-F (2013) Improvement of ylang-ylang essential oil characterization by GC×GC-TOFMS. Molecules 18:1783–1797

Bruneriel P, Benda I, Bock G, Schreier P (1987) Bioconversion of citronellol by *Botrytis cinerea*. Appl Microbiol Biotechnol 27:6–10

Buggeln RG, Bal AK (1977) Effects of auxins and chemically related non-auxins on photosynthesis and chloroplast ultrastructure in *Alaria esculenta* (Laminariales). Can J Bot 55(15):2098–2105

Carson CF, Hammer KA, Riley TV (2006) *Melaleuca alternifolia* (tea tree) oil: a review of antimicrobial and other medicinal properties. Clin Microbiol Rev 19:50–62

Chai W, Hayashida Y, Sakamaki H, Horiuchi A (2004) The biocatalytic oxidation of thujopsene by plant cultured-cells. J Mol Catal B Enzym 27:55–60

Chalchat JC, Chabard JL, Gorunovic MS, Djermanovic V, Bulatovic V (1995) Chemical composition of *Eucalyptus globules* oils from the Montenegro coast and east coast of Spain. J Essent Oil Res 7:147–152

Chen XQ, Zhang Y, Zu YG, Fu Y, Wang W (2011) Composition and biological activities of the essential oil from *Schisandra chinensis* obtained by solvent-free microwave extraction. LWT Food Sci Tecchnol 44:2047–2052

Chen Y, Zhou C, Ge Z, Liu Y, Liu Y, Feng W, Li S, Chen G, Wei T (2013) Composition and potential anticancer activities of essential oils obtained from myrrh and frankincense. Oncol Lett 6:1140–1146

Chow CK (2007) Fatty acids in foods and their health implications. CRC Press, Boca Raton\ London\New York

Combet E, Henderson J, Eastwood DC, Burton KS (2006) Eight-carbon volatiles in mushrooms and fungi: properties, analysis, and biosynthesis. Mycoscience 47(6):317–326

Crovadore J, Schalk M, Lefort F (2012) Selection and mass production of *Santalum album L. calli* for induction of sesquiterpenes. Biotechnol Biotechnol Eq 26:2870–2874

Crowell PL, Kennan WS, Haag JD, Ahmad S, Vedejs E, Gould MN (1992) Chemoprevention of mammary carcinogenesis by hydroxylated derivatives of d-limonene. Carcinogenesis 13(7):1261–1264

Cuevas-Glory L, Sauri-Duch E, Pino JA (2009) Volatile constituents of peel and leaf oils from Calamondin. J Essent Oil Bear Pl 12:656–660

da Fonseca AM, Marques DD, Lemos TLG, Aguiar GR, Bizarra AMC (2014) Fatty chemical composition and antioxidant activities of coconut oils (*Cocos nucifera* L). J Med Plant Res 8:1081–1085

de Alwis R, Fujita K, Ashitani T, Kuroda K (2009) Induced monoterpene and lignin production in mechanically stressed and fungal elicited cultured *Cupressus lusitanica* cells. Plant Biotechnol Rep 3:57–65

de Jesus HCR, Jeller AH, Debonsi HM, Alvesb PB, Porto ALM (2017) Multiple monohydroxylation products from rac-camphor by marine fungus *Botryosphaeria* sp. isolated from marine alga *Bostrychia radicans*. J Braz Chem Soc 28(3):498–504

de Souza RA, Ribeiro JB, Teixeira BG, Ferreira JLP, de A. Silva JR, do Amaral-Ferreira A, de Souza ROMA, Amaral ACF (2015) Hydroxylation of 1,8-cineole by *Mucor ramannianus* and *Aspergillus niger*. Braz J Microbiol 46(1):261–264

del Rio JA, Ortuño A, Puig DC, Iborra JL, Sabater F (1991) Accumulation of the sesquiterpenes nootkatone and valencene by callus cultures of *Citrus paradisi, Citrus limonia* and *Citrus aurantium*. Plant Cell Rep 10:410–413

Demyttenaere JCR, Adams A, Vanoverschelde J, De Kimpe N (2001) Biotransformation of (*S*)-(+)-linalool by *Aspergillus niger*: an investigation of the culture conditions. J Agric Food Chem 49(12):5895–5901

Demyttenaere JCR, Vanoverschelde J, De Kimpe N (2004) Biotransformation of (R)-(+)- and (S)-(−)-citronellol by *Aspergillus* sp. and *Penicillium* sp., and the use of solid-phase microextraction for screening. J Chromatogr A 1027:137–146

Dobreva A, Velcheva A, Bardarov V, Bardarov K (2013) Chemical composition of different genotypes oil-bearing roses. Bulg J Agric Sci 19:1213–1218

Dvořaková M, Valterová I, Vaněk T (2011) Biocatalytic conversion of turpentine – a wood processing waste – into oxygenated monoterpenes. Biocatal Biotransformation 29(5):204–211

El Hawary SS, El Tantawy ME, Rabeh MA, Badr WK (2013) Chemical composition and biological activities of essential oils of *Azadirachta indica* A. Juss. Int J Appl Res Nat Prod 6:33–42

Esmaeili A, Hashemi E (2011) Biotransformation of myrcene by *Pseudomonas aeruginosa*. Chem Cent J 5:26

Esmaeili A, Rohany S, Safaiyan S (2012) Biotransformation of citral by free and immobilized *Saccharomyces cerevisiae*. Chem Nat Compd 48(2):322–324

Esmaeili A, Tavassoli A (2010) Microbial transformation of citral by *Penicillium* sp. Acta Biochim Pol 57(3):265–268

Estevez JM, Cantero A, Reindl A, Reichler S, Leon P (2001) 1-Deoxy-D-xylulose-5-phosphate synthase, a limiting enzyme for plastidic isoprenoid biosynthesis in plants. J Biol Chem 276:22901–22909

Febres V, Latanya Fisher L, Khalaf A, Moore GA (2011) Citrus transformation: challenges and prospects. In: Alvarez M (ed) Genetic transformation. InTech, Rijeka

Ferrara MA, Almeida DS, Siani AC, Lucchetti L, Lacerda PSB, Freitas A, Tappin MRR, Bon EPS (2013) Bioconversion of R-(+)-limonene to perillic acid by the yeast *Yarrowia lipolytica*. Braz J Microbiol 44(4):1075–1080

Feussner I, Wasternack C (2002) The lipoxygenase pathway. Annu Rev Plant Biol 53:275–297

Fidelis CHV, Augusto F, Sampaio PTB, Krainovic PM, Barata LES (2012) Chemical characterization of rosewood (*Aniba rosaeodora* Ducke) leaf essential oil by comprehensive two-dimensional gas chromatography coupled with quadrupole mass spectrometry. J Essent Oil Res 24:245–251

Figueiredo AD, Barroso JG, Pedro LG, Scheffer JJC (2006) Potentialities of hairy root cultures for *in vitro* essential oil production. In: Teixeira da Silva JA (ed) Floriculture, ornamental and plant biotechnology: advances and topical issues, vol II. Global Science Books, Isleworth

Filová A (2014) Production of secondary metabolites in plant tissue cultures. Res J Agric Sci 46(1):236–245

Forster-Fromme K, Hoschle B, Mack C, Bott M, Armbruster W, Jendrossek D (2006) Identification of genes and proteins necessary for catabolism of acyclic terpenes and leucine/isovalerate in *Pseudomonas aeruginosa*. Appl Environ Microbiol 72(7):4819–4828

Gao S, Lin J, Liu X, Deng Z, Li Y, Sun X, Tang K (2006) Molecular cloning, characterization and functional analysis of a 2C-methyl-D-erythritol 2, 4-cyclodiphosphate synthase gene from *Ginkgo biloba*. J Biochem Mol Biol 39(5):502–510

García C, Rodríguez P, Días E, Heinzen H, Menéndez P (2009) Biooxidation of 1,8-cineole by *Aspergillus terreus*. J Mol Catal B Enzym 59:173–176

Garneau F-X, Collin G, Gagnon H, Pichette A (2012) Chemical composition of the hydrosol and the essential oil of three different species of the Pinaceae family: *Picea glauca* (Moench) Voss., *Picea mariana* (Mill.) B.S.P., and *Abies balsamea* (L.) mill. J Essent Oil Bear Pl 15:227–236

Gauthier L, Bonnin-Verdal M-N, Marchegay G, Pinson-Gadais L, Ducos C, Richard-Forget F, Atanasova-Penichon V (2016) Fungal biotransformation of chlorogenic and caffeic acids by *Fusarium graminearum*: new insights in the contribution of phenolic acids to resistance to deoxynivalenol accumulation in cereals. Int J Food Microbiol 221:61–68

Georgiev MI, Pavlov AI, Bley T (2007) Hairy root type plant *in vitro* systems as sources of bioactive substances. Appl Microbiol Biotechnol 74(6):1175–1185

Gershanik J, Boecler B, Ensley H, McCloskey S, George W (1982) The gasping syndrome and benzyl alcohol poisoning. N Engl J Med 307:1384–1388

Ghasemi Y, Mohagheghzadeh A, Ostovan Z, Moshavash M, Rasoul-Amini S, Morowvat MHH (2010) Biotransformation of some monoterpenoid ketones by *Chlorella vulgaris* MCCS 012. Chem Nat Compd 46(5):734–737

Ghazali HM, Abdulkarim SM (2011) *Moringa oleifera* seed oil: composition, nutritional aspects and health attributes. In: Preedy VR, Watson RR, Patel VB (eds) Nuts and seeds in health and disease. Academic Press, Amsterdam\Boston\Heidelberg\London\New York\Oxford\San Diego\San Francisco\Singapore\Sidney\Tokyo

Gniłka R, Majchrzak W, Wawrzenczyk C (2015) Microbial transformations of ()-a- and (+)-b-thujone by fungi of *Absidia* species. Phytochem Lett 13:41–46

Gök A, Kirbaşlar SI, Kirbaşlar FG (2015) Comparison of lemon oil composition after using different extraction methods. J Essent Oil Res 27:17–22

Goodwin TW, Mercer EI (1972) Introduction to plant biochemistry. Pergamon Press, Oxford, NY

Gounaris Y (2010) Biotechnology for the production of essential oils, flavours and volatile isolates. A review. Flavour Fragr J 25(5):367–386

Gretšušnikova T, Järvan K, Orav A, Koel M (2010) Comparative analysis of the composition of the essential oil from the shoots, leaves and stems the *wild Ledum palustre* L. from Estonia. Procedia Chem 2:168–173

Groeneveld M, van Beek HL, Duetz WA, Fraaije MW (2016) Identification of a novel oxygenase capable of regiospecific hydroxylation of D-limonene into (+)-trans-carveol. Tetrahedron 72:7263–7267

Guenther E (1982) The essential oils: individual essential oils of the plant families. Robert Krieger Publishing Company, Florida

Guo S, Geng Z, Zhang W, Liang J, Wang C, Deng Z, Du S (2016) The chemical composition of essential oils from *Cinnamomum camphora* and their insecticidal activity against the stored product pests. Int J Mol Sci 17:1836–1845

Hajati RJ, Payamnoor V, Bezdi KG, Chashmi NA (2016) Optimization of callus induction and cell suspension culture of *Betula pendula* Roth for improved production of betulin, betulinic acid, and antioxidant activity. *In Vitro Cell Dev Biol Plant* 52:400–407

Haluk J-P, Roussel-Bousta C (2003) Biosynthesis of tropolones from callus and suspension-cultured cells initiated from young seedlings of *Thuja plicata* Donn. Ann For Sci 60:271–276

Hamada H, Tanaka T, Furuya T, Takahatab H, Nemotob H (2001) Hydroxylation of benzylic and allylic sites by plant cultured suspension cells. Tetrahedron Lett 42:909–911

Heyen U, Harder J (1998) Cometabolic isoterpinolene formation from isolimonene by denitrifying *Alcaligenes defragrans*. FEMS Microbiol Lett 169:67–71

Höferl M, Stoilova I, Schmidt E, Wanner J, Jirovetz L, Trifonova D, Krastev L, Krastanov A (2014) Chemical composition and antioxidant properties of Juniper berry (*Juniperus communis* L.) essential oil. Action of the essential oil on the antioxidant protection of *Saccharomyces cerevisiae* model organism. Antioxidants 3:81–98

Hook I, Lecky R, McKenna B, Sheridan H (1990) Biotransformation of linalyl acetate by suspension cultures of *Papaver bracteatum*. Phytochemistry 29(7):2143–2144

Hook IL, Ryan S, Sheridan H (2003) Biotransformation of aliphatic and aromatic ketones, including several monoterpenoid ketones and their derivatives by five species of marine microalgae. Phytochemistry 63:31–36

Hopper DJ, Cottrell L (2003) Alkylphenol biotransformations catalyzed by 4-ethylphenol methylenehydroxylase. Appl Environ Microbiol 69(6):3650–3652

Hoschle B, Gnau V, Jendrossek D (2005) Methylcrotonyl-CoA and geranyl-CoA carboxylases are involved in leucine/isovalerate utilization (Liu) and acyclic terpene utilization (Atu), and are encoded by liuB/liuD and atuC/atuF, in *Pseudomonas aeruginosa*. Microbiology 151(Pt11):3649–3656

Indira-Iyer R, Jayaraman G, Ramesh A (2009) *In vitro* responses and production of phytochemicals of potential medicinal value in nutmeg, *Myristica fragrans* Houtt. Indian J Sci Technol 2:65–70

Itose R, Sakai K (1997) Improved culture conditions for the production of β thujaplicin by suspension cellcultures of *Cupressus lusitanica*. Plant Biotechnol 14:163–168

Jayaraman S, Mohamed R (2015) Crude extract of Trichoderma elicits agarwood substances in cell suspension culture of the tropical tree, *Aquilaria malaccensis* Lam. Turk J Agric For 39:163–173

Jeyakani Santhosh M, Rajalakshmi M (2016) Total polyphenol content present in tissue culture of *Melaleuca alternifolia*. World J Pharm Pharm Sci 5:1163–1169

Julsing MK, Rijpkema M, Woerdenbang HJ, Quax WJ, Kayser O (2007) Functional analysis of genes involved in the biosynthesis of isoprene in *Bacillus subtilis*. Appl Genet Mol Biotech 75(6):1377–1384

Kang W-R, Seo M-J, An J-U, Shin K-C, Oh D-K (2016) Production of δ-decalactone from linoleic acid via 13-hydroxy-9(Z)-octadecenoic acid intermediate by one-pot reaction using linoleate 13-hydratase and whole *Yarrowia lipolytica* cells. Biotechnol Lett 38:817–823

Karyagina TB, Gaevskaya OA, Gukasova EA, Timchenko TV, Bairamashvili DI (2007) The effect of phenylalanine on biosynthesis of protoberberine alkaloids in the cell culture of low meadow-rue. Russ J Plant Physiol 54(2):267–272

Kašparová M, Martin J, Tumová L, Spilková J (2017) Production of podophyllotoxin by plant tissue cultures of Juniperus virginiana. Nat Prod Commun 12:101–103

Kılıç Ö, Kocak A (2014) Volatile constituents of *Juniperus communis* L., *Taxus canadensis* Marshall. and *Tsuga canadensis* (L.) Carr. from Canada. J Agric Sci Technol B 4:135–140

Kim B-N, Joo Y-C, Kim Y-S, Kim K-R, Oh D-K (2012) Production of 10-hydroxystearic acid from oleic acid and olive oil hydrolyzate by an oleate hydratase from *Lysinibacillus fusiformis*. Appl Microbiol Biotechnol 95:929–937

Kim M-J, Seo M-J, Shin K-C, Oh D-K (2017) Production of 10S-hydroxy-8(E)-octadecenoic acid from oleic acid by whole recombinant *Escherichia coli* cells expressing 10S-dioxygenase from *Nostoc punctiforme* PCC 73102 with the aid of a chaperone. Biotechnol Lett 39:133–139

Kiran I, Durceylan Z, Kirimer N, KHC B, Noma Y, Demirci F (2010) Biotransformation of α-cedrol and caryophyllene oxide by the fungus *Neurospora crassa*. Nat Prod Commun 5(4):515–518

Kivrak Ş, Göktürk T, Kivrak İ (2017) Assessment of volatile oil composition, phenolics and antioxidant activity of bay (*Laurus nobilis*) leaf and usage in cosmetic applications. Int J Sec Metabolite 4:148–161

Kohda H, Kohjyouma M, Namera A, Tsuchiya H, Urimoto M, Ozaki M (1997) Production of anethole in callus of *Illicium verum* Hooker filius. Nat Med 51:519–522

Kongduang D, De-Eknamkul W, Sitthithaworn W, Wungsintaweekul J (2014) Terpenoid content and transcription profile analysis in callus and suspension cultures of *Croton stellatopilosus*. J Plant Biochem Biotechnol 23(1):61–68

Koppireddi S, Seo J-H, Jeon E-Y, Chowdhury PS, Jang H-Y, Park J-B, Kwon Y-K (2016) Combined biocatalytic and chemical transformations of oleic acid to ω-hydroxynonanoic acid and α,ω-nonanedioic acid. Adv Synth Catal 358(19):3084–3092

Kriaa D (2012) Secondary metabolites production from liquid tissue culture of *Citrus aurantium*. Dissertation, University of Sassari

Krings U, Brauer B, Kaspera R, Berger RG (2005) Biotransformation of g-terpinene using *Stemphylium botryosum* (Wallroth) yields p-mentha-1,4-dien-9-ol, a novel odorous monoterpenol. Biocatal Biotransformation 23(6):457–463

Krings U, Hapetta D, Berger RG (2008) Bioconversion of β-myrcene to perillene by *Pleurotus ostreatus*. Biocatal Biotransformation 26(4):288–295

Kusuma HS, Mahfud M (2016) The Extraction of essential oil from sandalwood (*Santalum album*) by microwave air-hydrodistillation method. J Mater Environ Sci 7:1597–1606

Lee B, Lee S, Kim H, Jeong K, Park J, Lee E, Lee J (2015) Biotransformation of oleic acid into 10-ketostearic acid by recombinant *Corynebacterium glutamicum*-based biocatalyst. Biotechnol Lett 37:1101–1106

Lee S-Y, Kim S-H, Hong C-Y, Kim H-Y, Ryu S-H, Choi I-G (2015b) Biotransformation of (−)-α-pinene by whole cells of white rot fungi, *Ceriporia* sp. ZLY-2010 and *Stereum hirsutum*. Mycobiology 43(3):297–302

Lee S-Y, Kim S-H, Hong C-Y, Park S-Y, Choi I-G (2015a) Biotransformation of (−)-α-pinene and geraniol to α-terpineol and p-menthane-3,8-diol by the white rot fungus, *Polyporus brumalis*. J Microbiol 53(7):462–467

Li Y-Q, Kong D-X, Wu H (2013) Analysis and evaluation of essential oil components of cinnamon barks using GC–MS and FTIR spectroscopy. Ind Crop Prod 41:269–278
Lindain AF, Reglos RA, de Guzman CC, Cedo MLO (2008) Tissue culture and essential oil production from callus cultures of Ilang-Ilang [*Cananga odorata* (Lamk) Hook.f. & Thomson]. Philipp Agric Sci 91:251–260
Liu X, Xu J, Xia J, Lv J, Wu Z, Deng Y (2016) Improved production of citric acid by *Yarrowia lipolytica* using oleic acid as the oxygen-vector and co-substrate. Eng Life Sci 16:424–431
Ma L, Liu X, Liang J, Zhang Z (2011) Biotransformations of cinnamaldehyde, cinnamic acid and acetophenone with *Mucor*. World J Microbiol Biotechnol 27:2133–2137
Ma T-W, Lai Y, Yang F-C (2014) Enhanced production of triterpenoid in submerged cultures of Antrodia cinnamomea with the addition of citrus peel extract. Bioprocess Biosyst Eng 37:2251–2261
Ma Y, Wang X, Xueling Nie X, Zhang Z, Yang Z, Nie C, Tang H (2016) Microbial degradation of chlorogenic acid by a *Sphingomonas* sp. strain. Appl Biochem Biotechnol 179:1381–1392
Mallavarapu GR, Ramesh S, Syamasundar KV, Chandrasekhara RS (1999) Composition of Indian curry leaf oil. J Essent Oil Res 11:176–178
Mariotti JP, Tomi F, Casanova J, Costa J, Bernardini AF (1997) Composition of the essential oil of *Cistus ladaniferus* L. cultivated in Corsica (France). Flavour Frag J 12:147–151
Maróstica MR Jr, Pastore GM (2006) Biotransformation of citronellol in rose-oxide using cassava wastewater as a medium. Ciencia Tecnol Alime 26(3):690–696
Mathew R, Sankari PD (2014) Comparison of major secondary metabolites quantified in elicited cell cultures, non-elicited cell cultures, callus cultures and field grown plants of *Ocimum*. Int J Pharm Pharm Sci 6(2):102–106
Mathur M, Kamal R (2012) Studies on trigonelline from *Moringa oleifera* and its *in vitro* regulation by feeding precursor in cell cultures. Braz J Pharmacog 22:994–1001
Mi J, Schewe H, Buchhaupt M, Holtmann D, Schrader J (2016) Efficient hydroxylation of 1,8-cineole with monoterpenoid resistant recombinant *Pseudomonas putida* GS1. World J Microbiol Biotechnol 32:112
Mirata M-A, Wiist M, Mosandl A, Sell D, Schrader J (2006) Lilac aldehydes and lilac alcohols as metabolic byproducts of fungal linalool biotransformation. In: Bredie WLP, Petersen MA (eds) Flavour science: recent advances and trends. Elsevier, Netherlands
Misra BB, Dey S (2013) Culture of East Indian sandalwood tree somatic embryos in air-lift bioreactors for production of santalols, phenolics and arabinogalactan proteins. AoB Plants 5:plt025. https://doi.org/10.1093/aobpla/plt025
Miyazawa M, Miyasato Y (2001) Biotransformation of (+)- and (−)-bornyl acetate using the plant parasitic fungus Glomerella cingulataas a biocatalyst. J Chem Technol Biotechnol 76:220–224
Miyazawa M, Takahashi T, Sakata K, Horibe I (2008) Biotransformation of three aromadendrane-type sesquiterpenoids by *Aspergillus wentii*. J Chem Technol Biotechnol 83:1006–1011
Miyazawa M, Uemura T, Kameoka H (1995) Biotrnaformation of sesquiterpenoids, (+)-aromadendrene and (−)-alloaromadendrene by *Glomerella cingulata*. Phytochemistry 40(3):793–796
Molina G, Bution ML, Bicas JL, Dolder MAH, Pastore GM (2015) Comparative study of the bioconversion process using R-(+)- and S-(−)-limonene as substrates for *Fusarium oxysporum* 152B. Food Chem 174:606–613
Möller R, Ball RD, Henderson AR, Modze G, Find J (2006) Effect of light and activated charcoal on tracheary element differentiation in callus cultures of *Pinus Radiata* D. Don. Plant Cell Tiss Org Cult 85(2):161–171
Monzotea L, Hillb GM, Cuellarc A, Scullc R, Setzerb WN (2012) Chemical Composition and antiproliferative properties of *Bursera graveolens* essential oil. Nat Prod Commun 7:1531–1534
Mordue AJ, Nisbet AJ (2000) Azadirachtin from the neem tree *Azadirachta indica*: its action against insects. An Soc Entomol Brasil 29(4):615–632
Mousavi ES, Dehghanzadeh H, Abdali A (2014) Chemical composition and essential oils of *Pelargonium graveolens* (Geraniaceae) by gas chromatography – mass spectrometry (GC/MS). Bull Env Pharmacol Life Sci 3:182–184
Mulas M, Melis RAM (2011) Essential oil composition of myrtle (*Myrtus communis*) leaves. J Herbs Spices Med Plants 17:21–34

Müller A, Hauer B, Rosche B (2006) Enzymatic reduction of the α,β-unsaturated carbon bond in citral. J Mol Catal B Enzym 38:126–130

Nakahashi H, Miyazawa M (2011) Biotransformation of (−)-camphor by *Salmonella typhimurium* OY1002/2A6 expressing human CYP2A and NADPH-P450 reductase. J Oleo Sci 60(10):545–548

Nanjundaiah S, Mutturi S, Bhatt P (2017) Modeling of caffeine degradation kinetics during cultivation of *Fusarium solani* using sucrose as co-substrate. Biochem Eng J 125:73–80

Nano GM, Binello A, Cravotto G, Bianco MA (2005) Biotransformation of (−)-bornyl acetate using submerged cultures of *Collybia velutipes, Trametes hirsuta* and *Ganoderma applanatum*. J Chem Technol Biotechnol 80:657–661

Niedz RP, Moshonas MG, Peterson B, Shapiro JP, Shaw PE (1997) Analysis of sweet orange (*Citrus sinensis* (L.) Osbeck) callus cultures for volatile compounds by gas chromatography with mass selective detector. Plant Cell Tissue Organ Cult 51:181–185

Nikolić M, Marković T, Mojović M, Pejind B, Savić A, Perić T, Marković D, Stević T, Soković M (2013) Chemical composition and biological activity of *Gaultheria procumbens* L. essential oil. Ind Crop Prod 49:561–567

Nishio T, Patel A, Wang Y, Lau PCK (2001) Biotransformations catalyzed by cloned p-cymene monooxygenase from *Pseudomonas putida* F1. Appl Microbiol Biotechnol 55:321–325

Njoroge SM, Mungai HN, Koaze H, NTL P, Sawamura M (2006) Volatile constituents of mandarin (*Citrus reticulata Blanco*) peel oil from Burundi. J Essent Oil Res 18:659–662

Njoroge SM, Koaze H, Karanja PN, Sawamura M (2005) Volatile constituents of redblush grapefruit (*Citrus paradisi*) and pummelo (*Citrus grandis*) peel essential oils from Kenya. J Agric Food Chem 53:9790–9794

Nogaim QA, Al-Duais M, Al-Warafi A, Al-Erianee H, Al-Sayadi M (2013) The chemical composition of Yemeni green coffee. J Food Chem Nutr 1:42–48

Noma Y, Okajima Y, Takahashi H, Asakawa Y (1991) Biotransformation of aromatic aldehydes and related compounds by *Euglena gracilis Z*. Phytochemistry 30(9):2969–2972

Nowak R (2005) Chemical composition of hips essential oils of some *Rosa* L. species. Z Naturforsch 60c:369–378

Oda S, Fujinuma K, Inoue A, Ohashi S (2011) Synthesis of (−)-β-caryophyllene oxide via regio- and stereoselective endocyclic epoxidation of β-caryophyllene with *Nemania aenea* SF 10099-1 in a liquid–liquid interface bioreactor (L–L IBR). J Biosci Bioeng 112(6):561–565

Oda S, Kato A, Matsudomi M, Ohta H (1996) Enantioselective oxidation of racemic citronellol with an interface bioreactor. Biosci Biotech Biochem 60(1):83–87

Ogita S, Uefuji H, Morimoto M, Sano H (2004) Application of RNAi to confirm theobromine as the major intermediate for caffeine biosynthesis in coffee plants with potential for construction of decaffeinated varieties. Plant Mol Biol 54:931–941

Ogita S, Uefuji H, Yamaguchi Y, Koizumi N, Sano H (2003) RNA interference: producing decaffeinated coffee plants. Nature 423:823

Ogunwande IA, Olawore NO, Adeleke KA, Ekundayo O (2003) Chemical composition of essential oil of *Myristica Fragrans* Houtt (Nutmeg) from Nigeria. J Essent Oil Res 6:21–26

Ohara K, Matsunaga E, Nanto K, Yamamoto K, Sasaki K, Ebinuma H, Yazaki K (2010) Monoterpene engineering in a woody plant *Eucalyptus camaldulensis* using a limonene synthase cDNA. Plant Biotechnol J 8:28–37

Olivoto T, Maicon Nardino M, Carvalho IR, Follmann DN, Szareski VJ, Ferrari M, de Pelegrin AJ, de Souza VQ (2017) Plant secondary metabolites and its dynamical systems of induction in response to environmental factors: A review. Afr J Agric Res 12(2):71–84

Onken J, Berger RG (1999) Biotransformation of citronellol by the basidiomycete *Cystoderma carcharias* in an aerated-membrane bioreactor. Appl Microbiol Biotechnol 51:158–163

Park J-Y, Lee S-H, Kim K-R, Park J-B, Oh D-K (2015) Production of 13S-hydroxy-9(Z)-octadecenoic acid from linoleic acid by whole recombinant cells expressing linoleate 13-hydratase from *Lactobacillus acidophilus*. J Biotechnol 208:1–10

Park YJ, Baek HH, Bang O-K, Chang HC (2004) Conversion of citron (*Citrus junos*) peel oil by *Enterobacter agglomerans*. J Microbiol Biotechnol 14(6):1275–1279

Pawlowicz P, Piatkovski K, Siewinski A (1988) Enantiospecific hydrolysis of acetates of racemic monoterpenic alcohols by *Spirodela oligophiza*. Phytochemistry 27(9):2809–2811

Pawlowicz P, Stewinski A (1987) Enantioselective hydrolysis of esters and the oxidation of aromatic-aliphatic alcohols obtained therefrom by *Spirodela oligophiza*. Phytochemistry 26(4):1001–1004

Piantini U, Schrader J, Wawrzun A, Wüst M (2011) A biocatalytic route towards rose oxide using chloroperoxidase. Food Chem 129:1025–1029

Pierre-Leandri C, Fernandez X, Lizzani-Cuvelier L, Loiseau A-M, Fellous R, Garnero J, Oli CA (2003) Chemical composition of Cypress essential oils: volatile constituents of leaf oils from seven cultivated *Cupressus* species. J Essent Oil Res 15:242–247

Pimentel MR, Molina G, Bertucci TCP, Pastore GM (2012) Biotransformation of citronellol in rose oxide by *Pseudomonas* Spp. Chem Eng Trans 27:295–300

Proteau PJ (2004) 1-Deoxy-D-xylulose 5-phosphate reductoisomerase: an overview. Bioorg Chem 32(6):483–493

Rady MR, Youssef AA (1999) Comparison of essential oils and fats from *in vitro* cultures and field collected material of *Laurus nobilis*. J Agric Sci Mansoura Uni Egypt 24:3401–3412

Raimondi S, Anighoro A, Quartieri A, Amaretti A, Tomas-Barber FA, Rastelli G, Rossi M (2015) Role of bifidobacteria in the hydrolysis of chlorogenic acid. Microbiology 4(1):41–52

Rasheed-Uz-Zafar, Kausar A (2013) Biotransformation of limonene by freely suspended and immobilised cells of *Nigella sativa*. Int J Pharm Pharm Sci 5(suppl.1):23–26

Régimbal J-M, Collin G (1994) Essential oil analysis of balsam fir *Abies balsamea* (L.) Mill. J Essent Oil Res 6:229–238

Reila G, Bergerh RG (1996) Accumulation of chlorophyll and essential oils in photomixotrophic cell cultures of *Citrus* sp. Z Naturforsch 51c:657–666

Rmili R, Ramdani M, Ghazi Z, Saidi N, El Mahi B (2014) Composition comparison of essential oils extracted by hydrodistillation and microwave-assisted hydrodistillation from *Piper nigrum* L. J Mater Environ Sci 5:1560–1567

Rodrigo VB, Diego AA, MGP C, HGG J, LDR D (2010) Biotransformation of trans-cinnamaldehyde by the filamentous fungus *Aspergillus* sp. Vitae 17(3):281–290

Rodríguez A, San Andrés V, Cervera M, Redondo A, Alquézar B, Shimada T, Gadea J, Rodrigo MJ, Zacarías L, Palou L, López MM, Castañera P, Peña L (2011) Terpene down-regulation in orange reveals the role of fruit aromas in mediating interactions with insect herbivores and pathogens. Plant Physiol 156:793–802

Rodríguez P, Sierra W, Rodríguez S, Menéndez P (2006) Biotransformation of 1,8-cineole, the main product of *Eucalyptus* oils. Electron J Biotechnol 9(3):232–236

Rohdich F, Eisenreich W, Wungsintaweekul J, Hecht S, Schuhr CA, Bacher A (2001) Biosynthesis of terpenoids. 2C-Methyl-D-erythritol 2,4-cyclodiphosphate synthase (IspF) from *Plasmodium falciparum*. Eur J Biochem 268:3190–3197

Rohdich F, Wungsintaweeku J, Eisenreich W, Richter G, Schuhr CA, Hecht S, Zenk MH, Bacher A (2000) Biosynthesis of terpenoids: 4-Diphosphocytidyl-2-*C*-methyl-D-erythritol kinase from tomato. Proc Natl Acad Sci U S A 97:6451–8256

Rohdich F, Zepeck F, Adam P, Hecht S, Laupitz J, Grawert T, Amslinger S, Eisenreich W, Arigoni D (2003) The deoxyxylulose phosphate pathway of isoprenoid biosynthesis: studies on the mechanisms of the reactions catalyzed by IspG and IspH protein. Proc Natl Acad Sci U S A 100(4):1586–1591

Ryu J, Seo J, Lee Y, Lim Y, Ahn J-H, Hur H-G (2005) Identification of syn- and anti-anethole-2,3-epoxides in the metabolism of trans-anethole by the newly isolated bacterium *Pseudomonas putida* JYR-1. J Agric Food Chem 53:5954–5958

Sabra SMM, Al-Masoudi LMR, Hasan HAEE, Al-Gehani SAH, Abu-Harbah AAO (2015) The importance of the chemical composition of henna tree leaves (*Lawsonia inermis*) and its ability to eliminate *Tinea pedis*, with reference to the extent of usage and storage in the Saudi society, Taif, KSA. J Pharm Biol Sc 10(4):23–29

Saha R, Ghosh A, Saha B (2013) Kinetics of micellar catalysis on oxidation of *p*-anisaldehyde to *p*-anisic acid in aqueous medium at room temperature. Chem Eng Sci 99(9):23–27

Sakamaki H, Kitanaka S, Chai W, Hayashida Y, Takagi Y, Horiuchi A (2001) Biotransformation of Thujopsene by *Caragana chamlagu*. J Nat Prod 64(5):630–631
Sano H, Kusano T, Koizumi N (2002) Theobromine synthase polypeptide of coffee plant and the gene encoding said polypeptide. Patent CA 2363064 A1
Santoyo S, Cavero S, Jaime L, Ibañez E, Señoráns FJ, Reglero G (2005) Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. essential oil obtained via supercritical fluid extraction. J Food Prot 68(4):790–795
Sawamura M, Onishi Y, Ikemoto J, Tu NTM, Phi NTL (2006) Characteristic odour components of bergamot (*Citrus bergamia Risso*) essential oil. Flavour Fragr J 21:609–615
Saxena G, Banerjee S, ur-Rahman L, Verma PC, Mallavarapu GR, Kumar S (2007) Rose-scented geranium (*Pelargonium sp*.) generated by *Agrobacterium rhizogenes* mediated Ri-insertion for improved essential oil quality. Plant Cell Tissue Organ Cult 90:215–223
Scragg AH (1995) The problems associated with high biomass levels in plant cell suspensions. Plant Cell Tissue Organ Cult 43(2):163–170
Seidel V, Windhovel J, Eaton G, Alfermann AW, Arroo RRJ, Medarde M, Petersen M, Wooley JG (2002) Biosynthesis of podophyllotoxin in *Linum album* cell cultures. Planta 215(6):1031–1039
Sethi ML, Rao GS, Chowdhury BK, Morton JF, Kapadia GJ (1976) Identification of volatile constituents of *Sassafras albidum* root oil. Phytochemistry 15:1773–1775
Shams-Ardakani M, Ghannadi A, Badr P, Mohagheghzadeh A (2005) Biotransformation of terpenes and related compounds by suspension culture of *Glycyrrhiza glabra* L. (Papilionaceae). Flavour Fragr J 20:141–144
Shanker AK, Shanker C (2016) Abiotic and biotic stress in plants - Recent advances and future perspectives. InTeck. https://doi.org/10.5772/60477
Shen P, Song Z, Zhang Z, Zeng H, Tang X, Jiang C, Li J, Wu B (2014) Screening of Burkholderia sp. WGB31 producing anisic acid from anethole and optimization of fermentation conditions. J Basic Microbiol 54:1251–1257
Shestibratov K, Lebedev V, Podrezov A, Salmova M (2011) Transgenic aspen and birch trees for Russian plantation forests. BMC Proc 5(Suppl 7):P124
Si CY, Han X, Zhan Z, Tian S, Cui Q, Wang Y (2012) Chemical composition of essential oils of *Litsea cubeba* harvested from its distribution areas in China. Molecules 17:7057–7066
Siddhardha B, Kumar MV, Murty USN, Ramanjaneyulu GS, Prabhakar S (2012) Biotransformation of a-pinene to terpineol by eesting cell suspension of *Absidia corulea*. Indian J Microbiol 52(2):292–294
Soares-Castro P, Montenegro-Silva P, Heipieper HJ, Santosa PM (2017) Functional Characterization of a 28-kilobase catabolic island from *Pseudomonas* sp. strain M1 involved in biotransformation of β-myrcene and related plant-derived volatiles. Appl Environ Microbiol 83(9):e03112–e03116
Soler E, Feron G, Clastre M, Dargent R, Gleizes M, Ambid C (1992) Evidence for a geranyldiphosphate synthase located within the plastids of *Vitis vinifera* L. cultivated *in vitro*. Planta 187(2):171–175
Spadaro F, Costa R, Circosta C, Occhiuto F (2012) Volatile composition and biological activity of key lime *Citrus aurantifolia* essential oil. Nat Prod Commun 7:1523–1526
Srivastava AK, Prakash G (2010) Use of bioreactor plant cell cultivation as a novel technique for mass production of bio-pesticide. As Pac J Mol Biol Biotechnol 18:131–133
Srivastava S, Srivastava AK (2007) Hairy root culture for mass-production of high-value secondary metabolites. Crit Rev Biotechnol 27(1):29–43
Stewart CD, Jones CD, Setzer WN (2014) Essential oil compositions of *Juniperus virginiana* and *Pinus virginiana*, two important trees in Cherokee traditional medicine. Am J Essent Oil Nat Prod 2:17–24
Stiles J, Moisyadi I, Neupane KR (2000) Purified proteins, recombinant DNA sequences and processes for producing caffeine free beverages. USA Patent 6:075,184
Su G-J, Liu X-M, Li W-G, Chen M, Zhou Y-H, Guo C, Li S-H (2011) Optimization of biotransformation conditions for transformation of trans-anethole to anisic acid by *Pseudomonas sp*. BT-13. J Chem Eng of Chinese Univ 25(4):643–649

Suga T, Hirata T, Izumi S (1986) Enantioselectivity in the hydrolysis of bicyclic monoterpene acetates with the cultured cells of *Nicotiana tabacum*. Phytochemistry 25(12):2791–2792

Swift KAD (1997) Flavours and fragnances. Woodhead Publishing Limited, Cambridge

Szopa A, Ekiert H (2016) The importance of applied light quality on the production of lignans and phenolic acids in *Schisandra chinensis* (Turcz.) Baill. cultures *in vitro*. Plant Cell Tissue Organ Cult 127:115–121

Szopa BA, Kokotkiewicz A, Marzec-Wróblewska U, Bucinski A, Luczkiewicz M, Halina Ekiert H (2016) Accumulation of dibenzocyclooctadiene lignans in agar cultures and in stationary and agitated liquid cultures of *Schisandra chinensis* (Turcz.). Appl Microbiol Biotechnol 100:3965–3977

Tai Y-N, Xu M, Ren J-N, Dong M, Yang Z-Y, Pan S-Y, Fan G (2016) Optimisation of α-terpineol production by limonene biotransformation using *Penicillium digitatum* DSM 62840. J Sci Food Agric 96(3):954–961

Toshifumi H, Tadashi A, Takashi I, Takayuki S (1981) The Biotransformation of foreign substrates by tissue cultures. I. The hydroxylation of linalool and its related compounds with the suspension cells of *Nicotiana tabacum*. Bull Chem Soc Jpn 54(11):3527–3529

Trytek M, Jędrzejewski K, Fiedurek J (2015) Bioconversion of αpinene by a novel coldadapted fungus *Chrysosporium pannorum*. J Ind Microbiol Biotechnol 42:181–188

Tsiri D, Graikou K, Pobłocka-Olech L, Krauze-Baranowska M, Spyropoulos C, Chinou I (2009) Chemosystematic value of the essential oil composition of *Thuja* species cultivated in Poland—Antimicrobial activity. Molecules 14(11):4707–4715

Tudorache M, Gheorghe A, Viana AS, Parvulescu VI (2016) Biocatalytic epoxidation of α-pinene to oxy-derivatives over cross-linked lipase aggregates. J Mol Catal B Enz 134:9–15

Unterweger B, Bulach DM, Scoble J, Midgley DJ, Greenfield P, Lyras D, Johanesen P, Dumsday GJ (2016) CYP101J2, CYP101J3, and CYP101J4, 1,8-cineole hydroxylating cytochrome P450 monooxygenases from *Sphingobium yanoikuyae* strain B2. Appl Environ Microbiol 82:6507–6517

Valluri J (2009) Bioreactor production of secondary metabolites from cell cultures of periwinkle and sandalwood. In: Jain SM, Saxena PK (eds) Protocols for *in vitro* cultures and secondary metabolite analysis of aromatic and medicinal plants. Methods in molecular biology (methods and protocols), vol 547. Humana Press, Totowa, NJ

van Dyk MS, Thomas E (1998) Hydrolysis of linalyl acetate and a-terpinyl acetate by yeasts. Biotechnol Lett 20(4):417–420

Velasco-Bucheli R, Mesa A, Gil J, García C, Durango D (2015) Transformation of trans-anethole using the plant pathogenic fungus *Colletotrichum acutatum* as biocatalyst. Rev Mex Ing Quim 14(3):653–666

Villanueva MA, Torres RC, Başer KHC, Özek T, Kürkçüoğlu M (1993) The composition of Manila Elemi oil. Flavour Frag J 8:35–37

Volfová A, Chvojka L, Friedrich A (1978) The effect of kinetin and auxin on the chloroplast structure and chlorophyll content in wheat coleoptiles. Biol Plantarum 20(6):440–445

Wadenbäck J (2006) Lignin studies of transgenic Norway spruce. Dissertation, Univeristy of Uppsala

Waller GR, MacVean CD, Suzuki T (1983) Production of caffeine and related enzyme activities in callus cultures of *Coffea arabica* L. Plant Cell Rep 2:109–112

We FH, Chen FL, Tan XM (2015) Gas chromatographic-mass spectrometric analysis of essential oil of *Jasminum officinale* L var Grandiflorum flower. Trop J Pharm Res 14:149–152

Wei LL, Hua RM, Li MY, Huang YZ, Li SG, He YJ, Shen ZH (2014) Chemical composition and biological activity of star anise *Illicium verum* extracts against maize weevil, *Sitophilus zeamais* adults. J Insect Sci 14(80)

Weisshaar B, Jenkins GI (1998) Phenylpropanoid biosynthesis and its regulation. Curr Opin Plant Biol 1(3):251–257

Wiklof CA, Korus RA, Crawford DL, Pometto III AL (1984) Enzymatic conversion of aromatic aldehydes. In: Sixth Symposium on Biotechnology for Fuels and Chemicals, vol 14. Gatlinburg, TN, USA, pp. 419–423

Willrodt C, Halan B, Karthaus L, Rehdorf J, Julsing MK, Buehler K, Schmid A (2017) Continuous multistep synthesis of perillic acid from limonene by catalytic biofilms under segmented flow. Biotechnol Bioeng 114(2):281–290

Winkel-Shirley B (2001) Flavonoid Biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. Plant Physiol 126:485–493

Wolfertz M, Sharkey TD, Boland W, Kuhnemann F (2004) Rapid Regulation of the methylerythritol 4-phosphate pathway during isoprene synthesis. Plant Physiol 135(4):1939–1945

Yang B, Gao H, Stantond C, Rosse RP, Zhang H, Chen YQ, Chen H, Chen W (2017) Bacterial conjugated linoleic acid production and their applications. Prog Lipid Res 68:26–36

Zafra M, García-Peregrín E (1976) Seasonal variations in the composition of *Pinus halepensis* and *Pinus sylvestris*twigs and needles essential oil. J Agric Sci 86:1–6

Zeng X-F, Zhao D-G (2016) Expression of IPT in Asakura-sanshoo (*Zanthoxylum piperitum* (L.) DC. f. inerme Makino) alters tree architecture, delays leaf senescence, and changes leaf essential oil composition. Plant Mol Biol Rep 34:649–658

Zhang W, Wei R, Chen S, Jiang J, Li H, Huang H, Yang G, Wang S, Wei H, Liu G (2015) Functional characterization of *CCR* in birch (*Betula platyphylla* × *Betula pendula*) through overexpression and suppression analysis. Physiol Plantarum 154:283–296

Zheng Y, Xu X, Zou X (2016) Biotransformation of caffeine in oolong tea by *Paecilomyces gunnii*. Int Biodeterior Biodegradation 114:141–144

Zhou Y-Q, Li T-C, Cheng Y-P (2017) Production of schisandrin a and schisandrin b from callus and suspension cell cultures of schisandra chinensis. Nat Prod Commun 12(6):937–940

Zito SW, Srivastava V, Adebayo-Olojo E (1991) Incorporation of [1-^{14} *C*]-isopentenyl pyrophosphate into monoterpenes by a cell-free homogenate prepared from callus cultures of *Chrysanthemum cinerariaefolium*. Planta Med 57(5):425–427

Zubo YO, Yamburenko MV, Kusnetsov VV, Börner T (2011) Methyl jasmonate, gibberellic acid, and auxin affect transcription and transcript accumulation of chloroplast genes in barley. J Plant Physiol 168:1335–1344

Zucca P, Littarru M, Rescigno A, Sanjust E (2014) Cofactor recycling for selective enzymatic biotransformation of cinnamaldehyde to cinnamyl alcohol. Biosci Biotechnol Biochem 73(5):1224–1226

Chapter 15
Eremothecium Oil Biotechnology as a Novel Technology for the Modern Essential Oil Production

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15.1 Introduction

Essential oils are more significant products of plants, with sources ranging from fungi and algae to flowering plants. There are around 3000 varieties of plants existing in the world, which can be used as a source of essential oil. However, nowadays, only about 200 of them are used for essential oil production; the majority is plants of higher taxa. The quality of essential oil depends considerably on ecological factors such as location, where volatile-oil-bearing plants are cultivated. In addition, plantation cultivation is seasonal. Production using biotechnology is free from these disadvantages. However, the biotechnology of essential oil production using cultures of isolated cells and tissues is not as effective as the biotechnology based on the microbial synthesis.

Certain bacteria, yeasts, actinomycetes, fungi, and algae can synthesize essential oils and aromatic substances de novo and also bioconvert less valuable substances (fatty acids, alcohols, alkanes, etc.) into essential oils. These organisms are of particular interest as nontraditional sources in connection with the rapid development of modern industrial biotechnology. In nature, there are around 100,000 known species of microorganisms, but only a few hundred species synthesize products or provide reactions which are beneficial to mankind (Быков et al. 2003).

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15.1.1 Biotechnological Raw Material as a Nontrivial Source of Fragrant Substances

Specific features of oil-bearing plants and their complex and various essential oil compositions depend not only on their taxonomic status but also on the region they are cultivated, harvest time and method, drying procedure, and storage conditions. New species suitable for the production of essential oils were ascertained using comprehensive research methodologies, which are as follows:

- Search for new organisms using screening studies
- Study of essential oil accumulation dynamics in ontogeny
- Cultivation of introduced species and recommended development for rational use of raw materials
- Biotechnological study on production of biomass and bioactive substances
- Complex application of producers and raw materials using resource-saving technologies
- Development of test parameters required for reference documentation
- Improvement of applied methods

During introduction, new phytotechnologies and biotechnologies are developed and realized in industries at specialized enterprises (Pandal 2006). To date, search for new sources of essential oils and more raw materials is made possible using microorganisms and in vitro cultures. The latter permits manufacturers to achieve new forms with beneficial features, high phytomass yield, micropropagation, and plant sanitation (Шпичка and Семенова 2016a).

Intensification of phyto- and biotechnologies for bioactive substance production needs the fundamental and applied studies in the field of life sciences, including genomics, proteomics, metabolomics, and nanobiotechnology. This also includes the development of novel life system and product technologies aimed at increasing the time and quality of life and supporting reproductive and labor potential in all countries. Industrial introduction of new scientific achievements ensures technological, economic, and social development (Родов et al. 1987; Семенова and Богданов 2000). Nowadays, together with the increase in production capacities and expansion of cultured plant variety, we should take measures to improve the raw material quality, including ecological cleanness, high yield achievement, support of biological balance, nature protection, rational use of raw materials, and so on. The raw material base expansion for oil-bearing plants is possible due to their cultivation under controlled conditions (Dudareva et al. 2013). Recently, such technologies as industrial hydroponics, biotechnology, and greenhouse farming have become common.

For a long time, plants were the only source of volatile fragrant substances. However, issues related to their cultivation and shortage in raw materials limit the development of perfume, cosmetic, pharmaceutical, and food industries. Moreover,

the quality of these essential oils may vary under extreme conditions. Therefore, the search for new sources of volatile fragrant substances has become crucial, and biotechnological approaches are of high interest (Krings, and Berger 1998; Bicas et al. 2010). Particularly, microorganisms, which can produce such substances de novo or convert less valuable substrates, are proven to be promising (Soetaert and Vandamme 2010). To date, several companies produce 4-decalactone with peach scent (BASF), macrocyclic musk components (Nippon Mining Co., Quest International), vanillin (Evolva), and so on (Janssens et al. 1992; Bomgardner 2012).

All studied bacteria species can be divided into four groups depending on differing scents of the synthesized substances. The first group is the rod bacteria which belong to phyla *Firmicutes* and *Actinobacteria* and produces pyrazines with nutty scent. The second group includes Gram-positive cocci and bacilli (*Firmicutes*, *Lactobacillales*), which can synthesize creamy-scented ketones, ethers, aldehyde, and simple acyloins. The third group consists of various microorganisms which produce alcohols and esters with fruity scent (Table 15.1). The fourth group consists of streptomyces synthesizing geosmin with earthy smell.

For fragrant compound industrial scaling, microorganisms from the second group are of particular interest because these substances define organoleptic properties of dairy products. Therefore, their extraction may decrease the end product quality. However, revealing the pathways and mechanisms of creamy-scented compound synthesis and the conditions influencing their cultivation is crucial for the dairy

Table 15.1 Bacteria and microalgae synthesizing fruity-floral fragrant substances

Species	Taxonomic status	Synthesized substances	Odor
Clostridium acetobutilicum	*Bacteria, Firmicutes, Clostridiales, Clostridiaceae*	Butyl butyrate	Fruity, pineapple
Erwinia carotovora (syn. Erwinia arrocepitae Pectobacterium carotovorum)	*Bacteria, Proteobacteria, Enterobacteriales, Enterobacteriaceae*	Aliphatic esters, 3-methylbutyl acetate, isobutyl acetate, metionol, metionol acetate, isobutanol, β-phenylethanol, tryptophol	Banana, sweet
Pseudomonas aeruginosa	*Bacteria, Proteobacteria, Pseudomonadales, Pseudomonadaceae*	2-Aminoacetophenone	Grape-like, jasmine-like, sweet
Pseudomonas fragi	*Bacteria, Proteobacteria, Pseudomonadales, Pseudomonadaceae*	Ethyl butyrate, ethyl isovalerate, ethyl-3-methyl butyrate, ethyl hexanoate, ethyl crotonate, ethyl-2-methyl hexanoate	Fruity, pineapple, strawberry
Scenedesmus incrassatulus	Plantae, Chlorophyta, Chlorophyceae, Scenedesmaceae	Isopropenyl acetate, phytol, ferruginol, benzyl cinnamate, butandiol	Floral-balsamic

Janssens et al. (1992); Шпичка and Семенова (2013b); Kambourova et al. (2003); our data

industry (Longo and Sanroman 2006). The fruity odor of bacteria from the third group usually consists of 2–5 components, which can be easily produced via chemical synthesis in high yields (Janssens et al. 1992).

Microalgae are a promising source to produce fragrant products. The comparative analysis of cyanobacteria and green and red algae, belonging to *Calothrix*, *Cylindrospermum*, *Anabaena*, *Nostoc*, *Spirulina*, *Chlorella*, and *Cyanidium*, showed that the quantity of the synthesized volatile fragrant compounds may reach up to 3 mg L^{-1} of cultural liquid (Погорельская et al. 1999; Семенова and Бугорский 1989). The biomasses from *Chlorella vulgaris*, *Spirulina platensis*, and others may be used to prepare ethanol oakmoss resinoid-like extracts for coloring and odor fixation in perfumes (Table 15.2) (Mitishev at al. 2016).

The most promising microorganisms for essential oil and fragrant compound production are fungi. They can accumulate interesting and bioactive metabolites in large quantities (Tables 15.2 and 15.3). Moreover, micromycetes can synthesize more complex fruity-scented compounds (lactones) than bacteria (Vandamme 2003). Despite their chemical synthesis, lactones produced by fungi are optically active and easily accessible. For instance, *Trichoderma viride* generates strong coconut odor during its cultivation on simple media. This odor is mostly caused by the 6-pentyl-2-pyrone synthesis, and its quantity reaches 170 mg L^{-1} of cultural liquid. To produce this compound via chemical synthesis, at least seven steps are required. Peach-like scent can be obtained using *Sporobolomyces odorus* cultures that synthesize 4-decalactone (Haeusler and Muench 1998; Hansen et al. 2009; Bicas et al. 2010).

Basidiomycetes and other fungi produce volatile fragrant substances with mushroom odor. It is caused by aliphatic eight-carbon compounds (e.g., 1-octen-ol, 1-octen-3on, 1-octen-3ol, 3-octanol), some pyrazines, and pyrroles. Hence, deep cultivation of these fungi allows obtaining natural mushroom flavorings for use in the food industry (Krings, and Berger 1998; Longo and Sanroman 2006).

Ceratocystis, *Trichoderma*, *Eremothecium*, *Pichia*, and *Saccharomyces* genera are of special interest for the extraction of essential oil (Christen 1995; Haeusler and Muench 1998; Gordente et al. 2012). Quantity of aroma-forming compounds, synthetized by fungi, may vary from hundreds of μg (*C. populina*) to hundreds of mg (*C. variospora, C. moniliformis, E. asbyi, T. viride*) —per liter of cultural liquid. Ascomycetes *Ceratocystis* sp. and *Eremothecium* sp. produce the highest amount of aroma-forming compounds during the minimal fermentation time. For instance, *C. variospora* can synthetize up to 1 g of essential oil per liter of cultural liquid on the fifth day of cultivation. Different basidiomycetes give the following yield: *Bjerkandera adusta*, 30 mg on 24th day; *Lepista irina*, 3–81 mg on the 28th day; and *Lentinus lepideus*, 100 mg in the 15th week.

Thus, microorganisms can synthetize a wide range of chemical compounds with a variety of scents: woody, fruity, creamy, earthy, and so on. That is why they are important alternative sources of essential oils and individual volatile fragrant compounds. In addition, further chemosystematic study of bioobjects, development of biotechnology, and its introduction in industry are required.

Table 15.2 Yeast and yeast-like fungi which synthesize volatile fragrant compounds with fruity-floral scent

Species	Taxonomic status	Synthesized substances	Odor
Geotrichum candidum (Staron)	*Fungi, Ascomycota, Saccharomycetes, Endomycetaceae*	Ethyl isobutyrate, ethyl-2-methyl butyrate, ethyl-3-methyl butyrate	Fruity
Geotrichum candidum	*Fungi, Ascomycota, Saccharomycetes, Endomycetaceae*	Ethyl acetate, 3-methyl butanol, 3-methylbutyl acetate, β-phenylethanol, β-phenylethyl acetate	Melon
Geotrichum penicillatum (syn. Trichosporon penicillatum)	*Fungi, Ascomycota, Saccharomycetes, Endomycetaceae*	Ethyl esters, ethyl-2-methyl butyrate, ethyl-3-methyl butyrate, ethyl isobutyrate, ethyl butyrate	Fruity
Dipodascus magnusii	*Fungi, Ascomycota, Saccharomycetes, Dipodascaceae*	Higher alcohols and esters	Apple, fruity
Hansenula anomala (syn. Pichia anomala)	*Fungi, Ascomycota, Saccharomycetes, Saccharomycetaceae*	Ethyl acetate, isobutyl acetate, 3-methylbutyl acetate, phenylethyl acetate, phenylethanol	Fruity-floral
Hansenula mrakii	*Fungi, Ascomycota, Saccharomycetes, Saccharomycetaceae*	2-Methylbutyl acetate, 3-methylbutyl acetate, isobutyl acetate	Fruity, banana
Saccharomyces fermentati	*Fungi, Ascomycota, Saccharomycetes, Saccharomycetaceae*	Linalool, nerolidol, trans-farnesol	Floral
Saccharomyces rosei	*Fungi, Ascomycota, Saccharomycetes, Saccharomycetaceae*	β-Myrcene, limonene, linalool, α-terpineol, farnesol	Floral, fruity-floral
Zygosaccharomyces rouxii	*Fungi, Ascomycota, Saccharomycetes, Saccharomycetaceae*	4-Hydroxy-2(or 5)-ethyl-5(or 2)-3(2H)-furanone, furaneol	Intensive sweet
Schizosaccharomyces pombe	*Fungi, Ascomycota, Schizosaccharomycetes, Schizosaccharomycetaceae*	Vanillin	Vanilla
Sporobolomyces odorus (syn. Sporidiobolus salmonicolor)	*Fungi, Basidiomycota, Microbotryomycetes, Sporidiobolaceae*	4-Decanolide, 5-decanolide, cis-7-decen-5-olide, cis-6-dodecen-4-one	Intensive peach
Sporobolomyces roseus	*Fungi, Basidiomycota, Microbotryomycetes, Sporidiobolaceae*	4-Decalactone	Peach

Krings and Berger (1998); Longo and Sanroman (2006); Bicas et al. (2010); Шпичка and Семенова (2013b); Mitishev et al. (2016); Alchihab et al. (2010); Carrau et al. (2005); our data

Table 15.3 Mycelial fungi which synthesize volatile fragrant compounds with fruity-floral scent

Species	Taxonomic status	Synthesized substances	Odor
Bjerkandera adusta (syn. Polyporus adustus)	*Fungi, Basidiomycota, Agaricomycetes, Polyporaceae*	4-Methoxybenzaldehyde, 3,4-dimethoxybenzaldehyde, 3,4-dimethoxybenzyl alcohols, 4-decanolide	Sweet, odorous, vanilla-like
Lentinus lepideus	*Fungi, Basidiomycota, Agaricomycetes, Polyporaceae*	Methyl cinnamate, cinnamic acid, sesquiterpenes with cadinane structure, cadinol, muurolol, cubenols, farnesol, drimenol, sesquiterpene esters with murolan structure, terrestrol	Fruity, odorous, anisic, cedar
Polyporus durus	*Fungi, Basidiomycota, Agaricomycetes, Polyporaceae*	4-Butanolide, 4-pentanolide, 3-penten-4-olide, 4-hexanolide, 2-hexen-4-olide, 5-hexen-4-olide, 5-hexanolide, 2-hepten-4-olide, 4-heptanolide, 4-octanolide, 2-nonen-4-olide, 2-decen-4-olide, 4-decanolide, sesquiterpenes	Coconut, pineapple
Polyporus tuberaster	*Fungi, Basidiomycota, Agaricomycetes, Polyporaceae*	Methyl benzoate, ethyl benzoate, benzaldehyde	Fruity-floral with ylang-ylang notes
Pycnoporus cinnabarinus	*Fungi, Basidiomycota, Agaricomycetes, Polyporaceae*	Vanillin, methyl anthranilate	Vanilla
Trametes odorata (syn. Gloeophyllum odoratum; syn. Osmoporus odoratus)	*Fungi, Basidiomycota, Agaricomycetes, Polyporaceae*	Methylanisate, anisaldehyde, δ-cadinene	Anise-like
Wolfiporia cocos	*Fungi, Basidiomycota, Agaricomycetes, Polyporaceae*	Linalool	Lily of the valley
Gloeophyllum odoratum	*Fungi, Basidiomycota, Agaricomycetes, Gloeophyllaceae*	Drimenol, methyl-3-hydroxy-3,7-dimethyl-6-octenoate, 1-octen-3-ol	Pleasant, fruity
Ischnoderma benzoinum	*Fungi, Basidiomycota, Agaricomycetes Fomitopsidaceae*	Benzaldehyde, 4-methoxybenzaldehyde, β-phenylethanol	Almond, hawthorn flowers
Poria aurea (syn. Auriporia aurea)	*Fungi, Basidiomycota, Agaricomycetes Fomitopsidaceae*	2-Octen-4-olide	Sweet
Lentinellus cochleatus	*Fungi, Basidiomycota, Agaricomycetes Auriscalpiaceae*	Trans-nerolidol, fokienol, 6-phormyl-2,2-dimethylchromene	Anise-like

(continued)

Table 15.3 (continued)

Species	Taxonomic status	Synthesized substances	Odor
Lepista irina	*Fungi, Basidiomycota, Agaricomycetes Tricholomataceae*	(3S, 4S, 10R)-3,10-epoxy-11-oxobisabola-1,8-diene—lepistirone	Iris oil, orange flowers
Mycoacia uda	*Fungi, Basidiomycota, Agaricomycetes Meruliaceae*	p-Tolualdehyde, p-methyl acetophenone, p-methyl benzyl alcohol, p-tolyl-1-ethanol	Fruity
Phlebia radiata	*Fungi, Basidiomycota, Agaricomycetes Meruliaceae*	4-Decanolide	Fruity with peach note
Oospora suaveolens	*Fungi, Basidiomycota, Agaricomycetes Botryobasidiaceae*	Amino acid esters	Fruity
Pleurotus euosmus	*Fungi, Basidiomycota, Agaricomycetes Pleurotaceae*	Linalool, coumarin, cis- and trans-linalool oxides	Sweet, floral
Cystostereum muraii	*Fungi, Basidiomycota, Basidiomycetes, Cystostereaceae*	1-Octen-3-one, benzofuran terpenoids, bisabolan	Vanilla, coconut flakes
Tyromyces sambuceus	*Fungi, Basidiomycota, Basidiomycetes, Polyporaceae*	4-Decalactone (4-decanolide), other lactones	Peach, passion fruit, coconut
Aspergillus oryzae	*Fungi, Ascomycota, Eurotiomycetes, Trichocomaceae*	1-Octen-1-ol	Pineapple
Aspergillus terreus	*Fungi, Ascomycota, Eurotiomycetes, Trichocomaceae*	Ethyl acetate	Fruity
Trichothecium roseum	*Fungi, Ascomycota, Ascomycetes, Incertae sedis*	Nerol, linalool, citronellol, terpineol, nerolidol, linalyl acetate, citronellyl acetate, geranyl acetate, 1-octen-3-ol, 3-octanol, 1,5-octadien-3-ol, octan-1-ol, 2-octen-1-ol	Floral, mushroom
Ceratocystis coerulescens	*Fungi, Ascomycota, Sordariomycetes, Ophiostomataceae*	6-Methyl-5-hepten-2-on, 6-methyl-5-hepten-2-ol, nerolidol, citronellol, citronellyl acetate, 2,3-dihydrofarnesol, trans-farnesol, geraniol, geranyl acetate, nerol, linalool, α-terpineol, neryl acetate	Fruity
Ceratocystis fimbriata	*Fungi, Ascomycota, Sordariomycetes, Ophiostomataceae*	Linalool, citronellol, geraniol, α-terpineol	Sweet, fruity

(continued)

Table 15.3 (continued)

Species	Taxonomic status	Synthesized substances	Odor
Ceratocystis populina	*Fungi, Ascomycota, Sordariomycetes, Ophiostomataceae*	Bicyclic sesquiterpenes with 1,7-dimethyl-4-isopropyldecaline skeleton, δ-cadinol, δ-cadinene	Pleasant, fruity
Ceratocystis variospora	*Fungi, Ascomycota, Sordariomycetes, Ophiostomataceae*	Vitronellol, citronellyl acetate, geranial, neral, geraniol, linalool, geranyl acetate, nerol, α-terpineol	Odorous, geranium-like
Leptographium lundbergii	*Fungi, Ascomycota, Sordariomycetes, Ophiostomataceae*	Sesquiterpene alcohols with africanane skeleton (africanols): leptografiol, isoleptografiol, isoafricanol	Sweet, fruity
Fusarium pore	*Fungi, Ascomycota, Sordariomycetes, Nectriaceae*	τ-Lactones, τ-decalactone, (Z)-6-τ-dodecenolactone	Fruity, peach
Hypomyces odoratus	*Fungi, Ascomycota, Sordariomycetes, Hypocreaceae*	Sesquiterpene esters and alcohols, 1-octen-3-ol	Camphor-like
Trichoderma koningii	*Fungi, Ascomycota, Sordariomycetes, Hypocreaceae*	6-Pentyl-α-pyrone	Coconut
Trichoderma reesei	*Fungi, Ascomycota, Sordariomycetes, Hypocreaceae*	6-Pentyl-2-pyrone	Coconut
Trichoderma viride	*Fungi, Ascomycota, Sordariomycetes, Hypocreaceae*	6-Pentyl-2-pyrone, 6-(pent-1-enyl)-2-pyrone	Coconut
Cladosporium cladosporioides	*Fungi, Ascomycota, Dothideomycetes, Davidiellaceae*	Isobutyl alcohol, isobutyl acetate, 3-methyl-butanol, 3-methylbutyl acetate, β-phenylethanol, β-phenyl acetate	Fruity
Cladosporium suaveolens	*Fungi, Ascomycota, Dothideomycetes, Davidiellaceae*	γ-Decalactone, δ-dodecalactone	Coconut
Monilia fruticola	*Fungi, Ascomycota, Leotiomycetes, Sclerotiniaceae*	4-Octalactone, 4-decalactone	Peach

Etschmann et al. (2002); Medeiros et al. (2006); Шпичка and Семенова (2013b); our data

15.1.2 Screening of Bioobjects with Different Taxonomic Statuses Which Produce Rose-Scented Essential Oil

Oil-bearing rose raw material belongs to floral-grassy volatile-oil-bearing raw materials in industrial processing.

Rose essential oil (Bulgarian, Crimean, French, etc.) refers to the floral-scented essential oil according to the classification of perfumes. It is rather valuable and the most expensive in the world today. However, biotechnology for producing rose essential oil conforming to international standards has not yet been developed.

It was revealed that oil content in rose cell culture is significantly less than in intact petals. Furthermore, composition of extractable oil differs from phytogenous rose oil (Mulder-Krieger et al. 1988; Егорова and Ставцева 2006). During the period 1980–1990, microorganisms were used to extract natural fragrant substances for the first time. Among all the studied organisms, a group which produces rose-scented alcohols and esters was singled out Шпичка and Семенова 2015a (Tables 15.4 and 15.5).

Research conducted on objects promising for aroma product biotechnology revealed the differences in the biosynthetic activity and essential oil composition between species and strains (Шпичка and Семенова 2013a). To extract rose-scented essential oil, researchers were interested in the genera *Ceratocystis*, *Eremothecium*, *Pichia*, and *Saccharomyces*. Considerable part of volatile fragrant compounds, synthetized by them, are floral-scented, generally with rose scent. In most of the microorganisms, this odor is caused by β-phenylethanol. The latter is synthetized in enzymatic reactions of deamination, decarboxylation, and oxidation of L-phenylalanine (Бугорский et al. 1986). However, *Ceratocystis* sp., *Eremothecium*

Table 15.4 Mycelial fungi which synthesize volatile fragrant compounds with roselike scent

Species	Taxonomic status	Synthesized substances	Odor
Inocybe coridalina, I. pyrlodora, I. odorata	*Fungi, Basidiomycota, Agaricomycetes, Cortinariaceae*	Methyl cinnamate	Fruity, roselike
Mycena pura	*Fungi, Basidiomycota, Agaricomycetes, Tricholomataceae*	Citronellol	Rose
Aspergillus niger	*Fungi, Ascomycota, Eurotiomycetes, Trichocomaceae*	Methyl ketones, β-phenylethanol	Unpleasant, roselike
Penicillium decumbens	*Fungi, Ascomycota, Eurotiomycetes, Trichocomaceae*	Thujopsene, 3-octanone, nerolidol, 1-octen-3-ol, β-phenylethanol	Pine-like, roselike, apple-like, mushroomlike
Ceratocystis moniliformis	*Fungi, Ascomycota, Sordariomycetes Ophiostomataceae*	3-Methylbutyl acetate, geraniol, citronellol, nerol, linalool, α-terpineol, geranial, neral, citronellyl acetate, geranyl acetate	Banana, pear, rose, peach
Ceratocystis virescens	*Fungi, Ascomycota, Sordariomycetes Ophiostomataceae*	Citronellol, geraniol, linalool, geranyl acetate, nerol, α-terpineol, geranial, neral, citronellyl acetate, neryl acetate	Fruity, roselike
Ascoidea hylecoeti	*Fungi, Ascomycota, Saccharomecetes, Ascoidaceae*	β-Phenylethanol, furan-2-carboxylic acid, citronellol, nerol, linalool, α-terpineol, citronellal, limonene, myrcene, citronellyl acetate	Fruity-floral, roselike

Janssens et al. (1992); Krings and Berger (1998); Шпичка and Семенова (2013b); our data

Table 15.5 Yeast and yeast-like fungi which synthesize volatile fragrant compounds with roselike scent

Species	Taxonomic status	Synthesized substances	Odor
Ambrosiozyma cicatricosa, A. monospora	*Fungi, Ascomycota, Saccharomycetes, Saccharomycopsidaceae*	Geraniol, citronellol, nerol, β-phenylethanol, α-terpineol, citral, linalool	Roselike
Eremothecium ashbyi, E. gossypii	*Fungi, Ascomycota, Saccharomycetes, Eremotheciaceae*	Geraniol, citronellol, nerol, β-phenylethanol, linalool, citral, farnesol	Roselike
Hansenula saturnus	*Fungi, Ascomycota, Saccharomycetes, Saccharomycetaceae*	Ethyl acetate, 3-methylbutanol, 3-methylbutyl acetate, β-phenylethanol, 2-phenylethyl acetate	Roselike
Kluyveromyces lactis	*Fungi, Ascomycota, Saccharomycetes, Saccharomycetaceae*	Citronellol, geraniol, linalool, β-phenylethanol, esters, isoamyl alcohol, acetoin, 2-phenyl acetate, isobutanol, isovaleric acid	Roselike, fruity, floral
Kluyveromyces marxianus	*Fungi, Ascomycota, Saccharomycetes, Saccharomycetaceae*	β-Phenylethanol	Roselike
Pichia farinosa	*Fungi, Ascomycota, Saccharomycetes, Saccharomycetaceae*	Ethyl acetate, 3-methylbutanol, 3-methylbutyl acetate, β-phenylethanol, 2-phenylethyl acetate	Roselike
Pichia fermentans	*Fungi, Ascomycota, Saccharomycetes, Saccharomycetaceae*	β-Phenylethanol	Roselike
Saccharomyces cerevisiae	*Fungi, Ascomycota, Saccharomycetes, Saccharomycetaceae*	β-Phenylethanol, 4-decanolide; linalool, geraniol, citronellol, α-terpineol; vanillin	Roselike, fruity-floral, vanilla
Saccharomyces vini	*Fungi, Ascomycota, Saccharomycetes, Saccharomycetaceae*	β-Phenylethanol	Roselike
Torulopsis utilis (syn. Candida utilis)	*Fungi, Ascomycota, Saccharomycetes, Saccharomycetaceae*	β-Phenylethanol, ethyl acetate	Roselike

Etschmann et al. (2002), Longo and Sanroman (2006), Mitrović et al. (2011), Шпичка and Семенова (2013b); our data

sp., and *Kluyveromyces* sp. can also synthesize terpene alcohols (geraniol, citronellol, nerol, linalool, farnesol), which are the main components of rose essential oil (Родов et al., 1987).

Monoterpene alcohols can be extracted during cultivation of these producers, so there are some specified pathways of its biosynthesis. These pathways are associated with the formation of isopentenyl diphosphate—precursor of terpenes and terpenoids (Klein-Marcuschamer et al. 2007; Schwab et al. 2008; Baydar and Baydar 2013; Langenheim 1994). Understanding the mechanisms underlying the isopentenyl diphosphate synthesis pathways and isomerization conditions of unstable

monoterpene alcohols is very important. It enables us to design the fermentation process to obtain the desired final product via altering the specific component composition. For example, affinity between *Saccharomyces* sp., *Kluyveromyces* sp., and *Eremothecium* sp. (Kurtzman 1995; Ajikumar et al. 2008) and their ability to synthetize terpene compounds allow assuming similar enzymatic systems. Results of the study of monoterpene alcohol synthesis for one of these genera can be used for study of the other, including the following: catalysis systems, regulation of formation and polymerization of isopentenyl diphosphate, and isomerization of geranyl diphosphate.

The quantity of aroma-forming compounds, synthetized by some fungi (e.g., *C. moniliformis, E. asbyi, E. gossypii)*, considerably exceeds 100 mg L^{-1} of cultural liquid. According to our research, *Ceratocystis*, *Eremothecium*, and *Kluyveromyces* genera are most promising for further studies (Семенова and Бугорский 1990; Semenova et al. 2011; Семенова and Шпичка 2012; Jianping 1993). They have high growth rates and produce the largest quantities of fragrant compounds. However, microorganisms of the *Ceratocystis* and *Eremothecium* genera produce more valuable essential oils. It has the most similar component composition to rose oil. Furthermore, it was revealed that *E. ashbyi* produce more than 180 mg of essential oil per liter of cultural liquid during the first two days of cultivation, and that is one of the highest rates. Comparable quantity of oil is contained in 500–600 g of rose flowers. In essential oil, which is produced by *E. ashbyi* and *E. gossypii*; geraniol, nerol, citronellol, and β-phenylethanol are the main aroma-forming compounds Бугорский and Semenova 1991. Although *E. gossypii* can synthetize higher amounts of these compounds, the quantity of monoterpene alcohols is similar to rose essential oil for this species (Table 15.6).

Thus, there are numerous bioobjects, which can serve as alternative sources of natural essential oil and volatile fragrant compounds with rose smell. That emphasizes their important role. Biogenic bioactive compounds are more similar to internal environment of human body than synthetic ones. They can easily take part in metabolic processes and rarely have any side effects. Many of these compounds are precursors of physiologically active substances (hormones, mediators, etc.).

Table 15.6 Composition of *Eremothecium* and rose essential oil, average value

Organism	Aroma product	Mass concentration, %				
		MTA[c]	Geraniol	Citronellol	Nerol	β-Phenylethanol
E. ashbyi	1[a]	61.9–77.6	65.5–80.9	6.0–11.4	1.8–3.4	21.7–37.5
	2[b]	78.0–84.9	43.3–64.2	2.6–5.1	0.5–3.5	9.8–12.7
E. gossypii	1	56.7–66.4	31.5–69.7	0.3–4.6	0.1–6.8	33.1–43.2
	2	52.8–61.9	35.0–52.4	1.2–2.8	0.2–2.7	37.3–46.3
Oil-bearing rose	1 (OST 10–60–87)	≥8.0	–	–	–	75.0–88.0
	2 (GOST 31,791–2012)	≥8.0	–	–	–	75.0–88.0

Note: [a]1 hydrodistillated essential oil, [b]2 extracted essential oil, [c]MTA monoterpene alcohols Шпичка and Семенова (2016a)

15.1.3 *Structural and Functional Aspects of* Eremothecium ashbyi *and* Eremothecium gossypii *Ontogenesis*

We studied ontogenesis of ascomycetes *E. ashbyi* and *E. gossypii* and morphological and cytological alterations that had occurred. The study showed that there were some structural features connected with vegetative growth and sexual and asexual reproduction (Fig. 15.1).

Vegetative reproduction is realized via separation and further growth, branching or septation of mycelium Шпичка and Семенова 2016b; Bezrukova et al. 2016. During microscopy of solid media surface, several areas with dichotomous branching of hypha fragment were discovered (Fig. 15.2).

Research showed that asexual reproduction occurs via terminal and lateral formation of spindle-shaped or oblong conidia (Fig. 15.3). Conidium formation was detected for both the species. L. R. Batra (1973) supposed that *E. ashbyi* is not

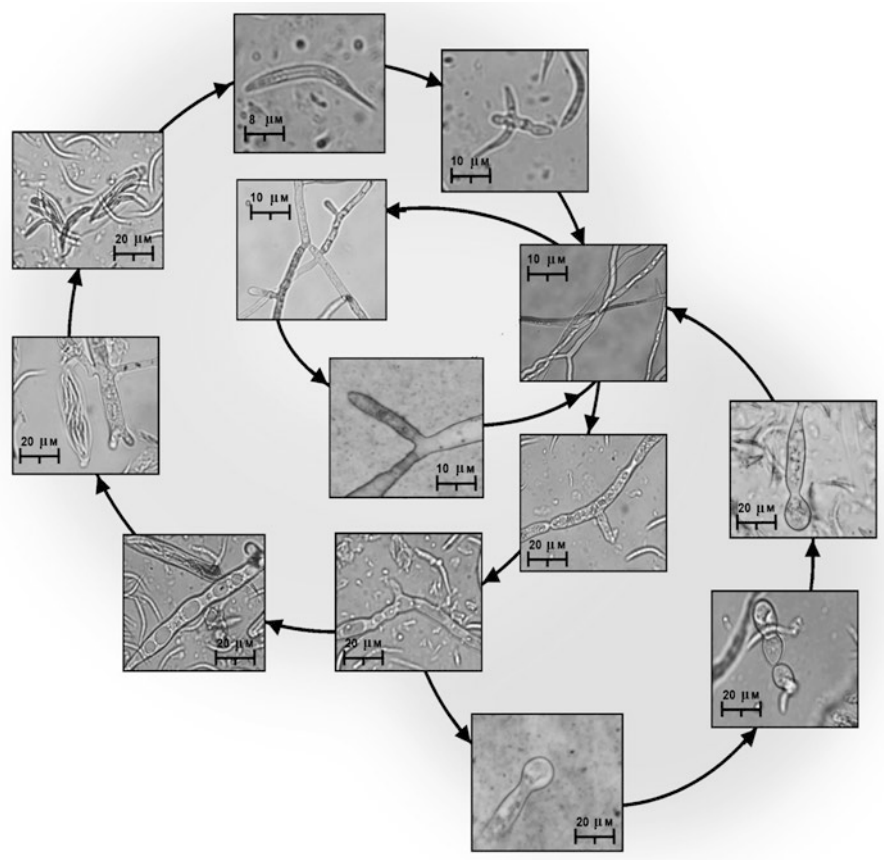

Fig. 15.1 Life, sexual reproduction; internal circle, asexual reproduction (conidium formation); left circle, asexual reproduction (budding) (Семенова et al. 2013)

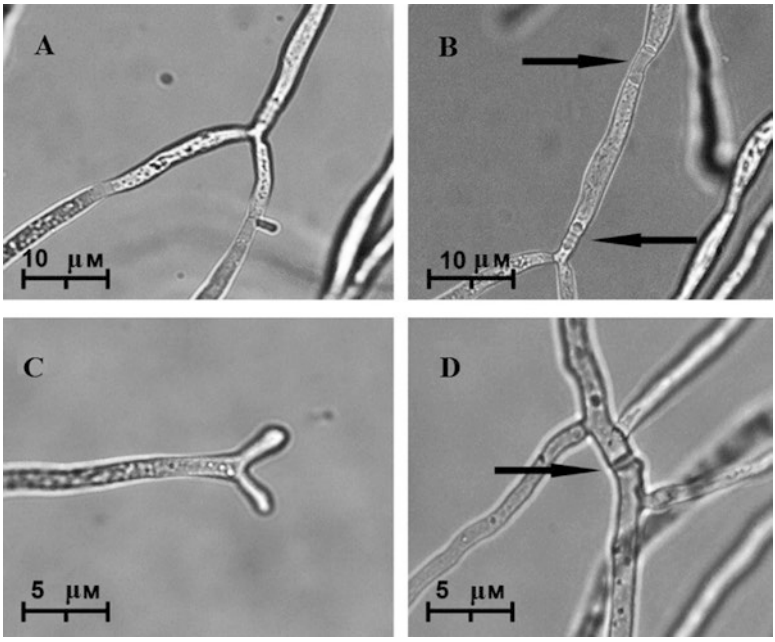

Fig. 15.2 Mycelium structure of *Eremothecium* sp.: dichotomous branching of hypha ((**a**), *E. ashbyi;* (**c**), *E. gossypii*), septation (arrow points at intercellular septum, (**b**), *E. ashbyi*; (**d**), *E. gossypii*) (Семенова et al. 2013)

capable of this process. However, this opinion was not approved or disproved in other scientific literature. Asexual reproduction of studied microorganisms can also occur via budding cells (Fig. 15.3) that can grow and form a separate mycelium. These cells can generate yeast phase of micromycete development in case we change the cultivation method. For example, it is possible if we reduce the supply of oxygen to the liquid medium and do not allow it to spread evenly. These conditions can be created if there is no shaking during cultivation or the shaking rates are significantly reduced. Thus, studied species tend to dimorph. This was not noted in previous cytomorphologic investigations. It also disproves the statement made by M. Nadal et al. (2008). They supposed these microorganisms to have only mycelial growth.

During sexual reproduction, special unicellular structures (meiosporangia) are formed. Sporangia of *E. ashbyi* are oblong (65.2–90.8 × 14.7–20.2 mkm), *E. gossypii*—clavate, cylindrical, or sigmoid (86.8–157.2 × 12.4–19.6 mkm) (Fig. 15.4). It was noted, that asci can be located singly or in groups and chains. A specified number of separate ascospores (Бугорский et al., 1986; Быков et al., 2003; Величко et al., 2015; Войткевич, 1999; Гуринович & Пучкова, 2005; Егорова & Ставцева, 2006; Жученко et al., 2015; Маркелова et al., 2014; Митишев et al., 2014; Митишев et al., 2017; Погорельская et al., 1999; Родов et al., 1987; Семенова, 2007) develop endogenously in sporangium. Spores grow after being

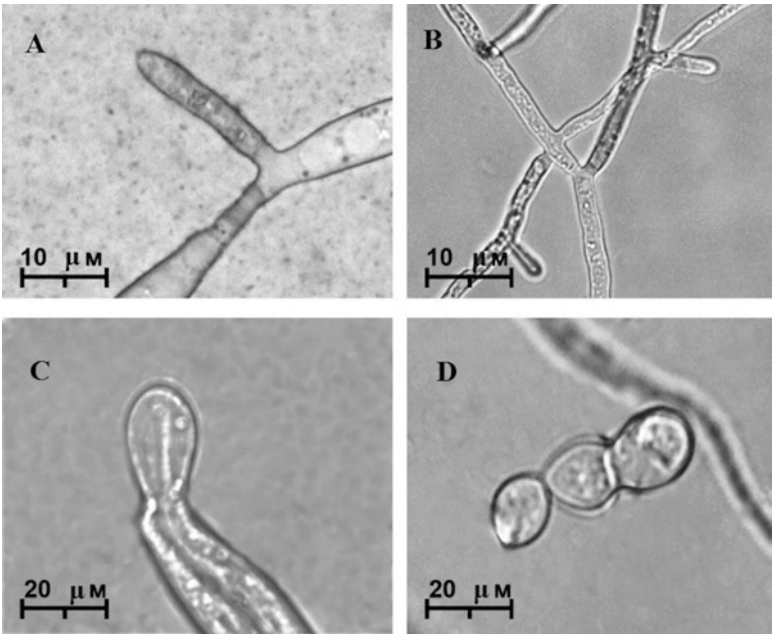

Fig. 15.3 Structures for asexual reproduction: spindle-shaped conidia, which are located laterally on hypha ((**a**), *E. ashbyi;* (**b**), *E. gossypii*); budding yeast-like cells (**c**, **d**) (Семенова et al. 2013)

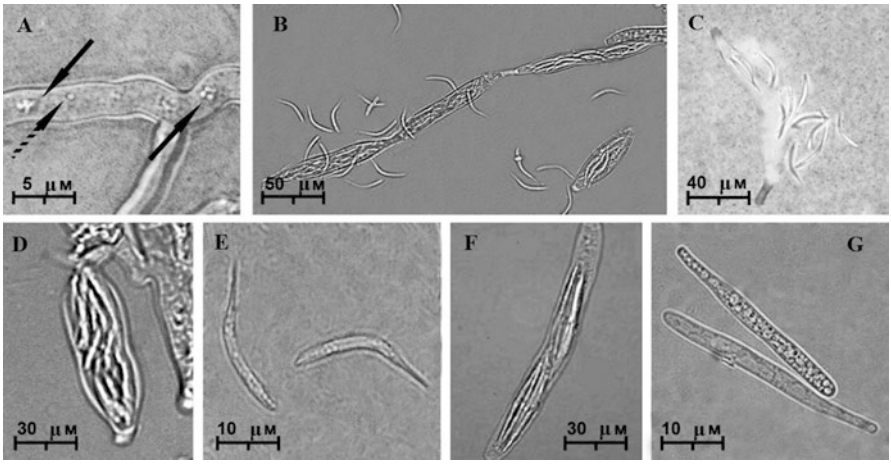

Fig. 15.4 Structures for sexual reproduction: (**a**) nuclei fusion in hypha of mycelium (pointed by arrows; single nucleus is pointed by dashed arrow); (**b**) chain formed by 3 asci and single ascus; (**c**) rupture of ascus and releasing of ascospores; (**d** and **e**) polysporous single sporangia and ascospores of *E. ashbyi*; (**f** and **g**) polysporous single sporangia and ascospores of *E. gossypii* (Семенова et al. 2013)

released via rupture of seed coat. They are species-specific: *E. ashbyi* has simple spores that are clavate and needle-shaped, straight, or bent with a narrow tip and granule-free (20.1–27.2 × 2.4–2.9 mkm). *E. gossypii* have spindle-shaped or needle-shaped ascospores, often with thin septum in the middle (17.5–45.8 × 1.3–4.6 mkm) (Fig. 15.4).

It should be noted that reproduction process is different for studied species of microorganisms (Figs. 15.5 and 15.6). In the life cycle of *E. ashbyi*, there is no spore

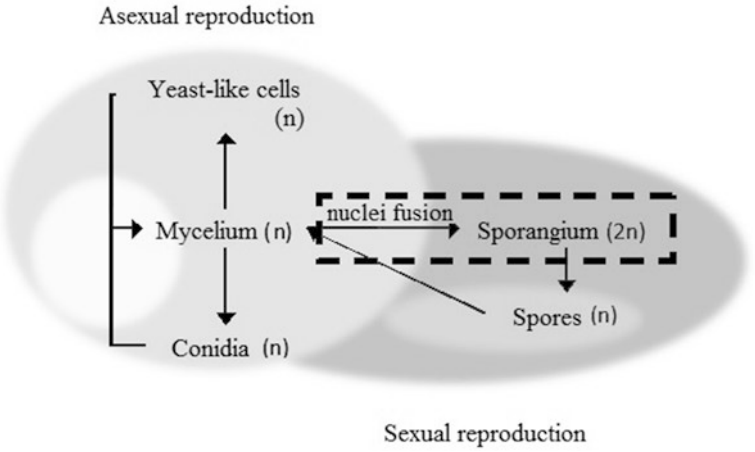

Fig. 15.5 Diagram of sexual and asexual reproduction of *E. ashbyi* (Семенова et al. 2013)

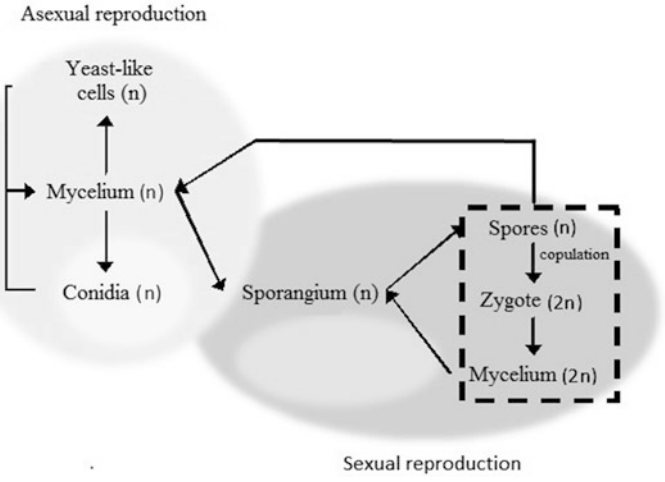

Fig. 15.6 Diagram of sexual and asexual reproduction of *E. gossypii* (Семенова et al. 2013)

fusion, and that is important. Ascospores of *E. ashbyi* ripen in intercalary-located sporangia and form new mycelium. During initial growth phase, there are several nuclei in hypha. But at a later time, while asci are forming, only one nucleus is found to be present. This phenomenon allows to assume that nuclei fuse during karyogamy (Fig. 15.5). L. R. Batra (1973) has a similar opinion; in his work, he has mentioned about the alteration in fungi cell.

Spores of *E. gossypii* conjugate after their release caused by the rupture of oblong clavate or sigmoid ascum. These spores form a zygote, which grow and form uninuclear diploid mycelium. After that, sporangia are formed terminally on hyphae of this mycelium. However, ascospores can also grow into a new multinuclear haploid mycelium with several septa without fusion (Fig. 15.6).

Different conditions of cultivation influence sexual and asexual reproduction significantly. For instance, addition of yeast extract to the glycose peptone medium allows increasing the number of ascospores from 0.2–0.5 to 6.0–7.0 mln ml^{-1}. Besides that, asexual reproduction was more typical for *E. gossypii* during conducted experiments.

The dynamics of an *E. ashbyi* biomass accumulation during the cultivation in the fluid medium is subordinated by known laws for periodic cultures. The growth goes exponentially till 36 h and reaches 2.0 g of dry biomass per liter of cultural liquid. Then the growth speed deceleration is observed which is typical for the stationary phase and the beginning of culture autolysis until the end of fermentation. There occurs a pH shift. The pH value reaches 5.5 in the period of active growth and increases until it reaches 6.2 in the stationary and the lysis phase. The synthesis and the accumulation of the riboflavin begin in the stationary growth phase and increase gradually during culture lysis until 30 μg g^{-1} of dry biomass is achieved (Fig. 15.7). The maximal accumulation of a basic monoterpene alcohol of the essential oil composition in fungus (geraniol) occurs in the period between 36 and 48 h of cultivation and compounds 25 μg g^{-1} of dry biomass. It correlates with periods of the maximal synthesis of intracellular neutral lipids. The growth dynamics of *E. gossypii* strains is subordinated by the same laws which are confirmed by Nieland's and Stahmann's research studies (2013). The productivity of *E. ashbyi* with regard to the essential oil by submerged cultivation on a soybean fermentation medium is 80.2–473.1 μg L^{-1}, while the productivity of *E. gossypii* reaches 565.5 μg L^{-1} of aromatic products.

E. ashbyi and *E. gossypii* are etiological agents of stigmatomycosis and, as it was noted earlier, are able to produce riboflavin and essential oils Шпичка et al. 2013. These industrially important properties necessitate the study of their biology. Formation and development of some cell structures correlate to the character and the level of biosynthetic activity (Fig. 15.8) Семенова et al. 2015. The beginning of the essential oil biosynthesis and the peak of its accumulation precede the active sporification (Figs. 15.7 and 15.8). Therefore, this clearly indicates the influence of essential oil on the processes of sexual reproduction, particularly, ascospore formation and maturing.

Thus, analysis of our data and scientific literature (Foerster et al. 2001; Семенова et al. 2011; Schiestl 2010; Nieland and Stahmann 2013; Wasserstorm et al. 2013; Semenova et al. 2016; Semenova et al. 2017a, b, c; Knyazkova et al. 2016) expands

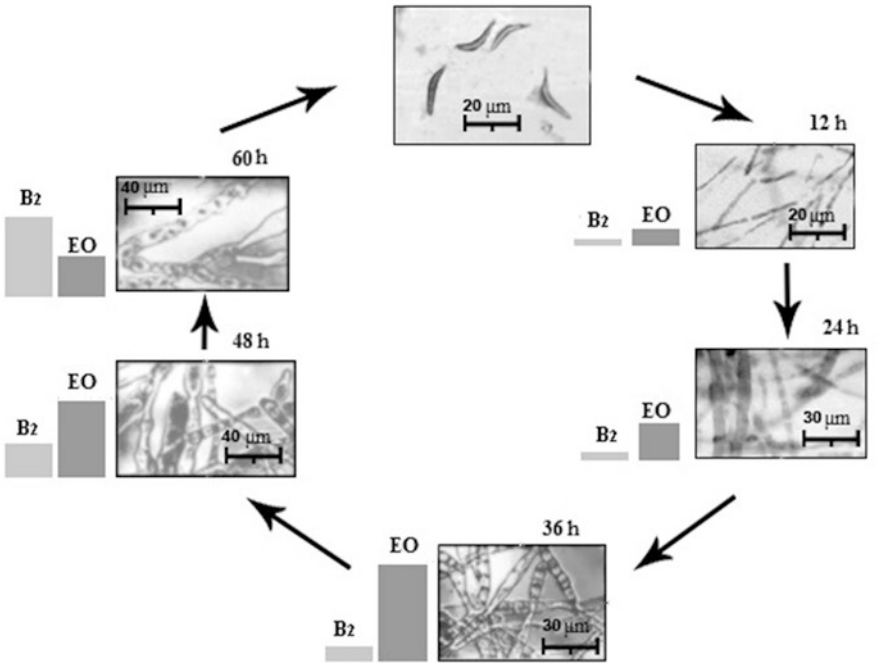

Fig. 15.7 Dynamics of essential oil and riboflavin accumulation during *E. ashbyi* ontogenesis: cultivation stages, starting from spore germination (ЭМ, essential oil; B_2, riboflavin) (Семенова et al. 2013)

and systematizes knowledge of *E. ashbyi* and *E. gossypii* growth and life cycles and structural and functional alterations connected with it. This analysis can be used in mycological and phytopathological studies of mentioned objects.

15.1.4 Aromatic and Monoterpene Alcohol Accumulation by Eremothecium ashbyi *Strains Differing in Riboflavinogenesis*

In our study, we compared different *E. ashbyi* strains: industrial VKPM F-340, yellow VKM F-3009, and white VKM F-124 (a mutant obtained by E. F. Semenova (Семенова et al. 2013). The color intensity of strain colonies differed depending on the riboflavinogenesis level (Revuelta et al. 2017).

We examined the dynamics of biomass accumulation during strain submerged cultivation and revealed no significant differences from the known regularities. The log phase of growth was observed up to 36 h and followed by a slowdown in growth rate (transition to stationary phase). Cell autolysis occurred at the end of fermentation (Fig. 15.9).

Overall, 60-h cultivation of strains VKPM F-340 and VKM F-3009 and a white mutant of strain VKM F-124 resulted in achieving minimum (1.06 g × L^{-1}), medium

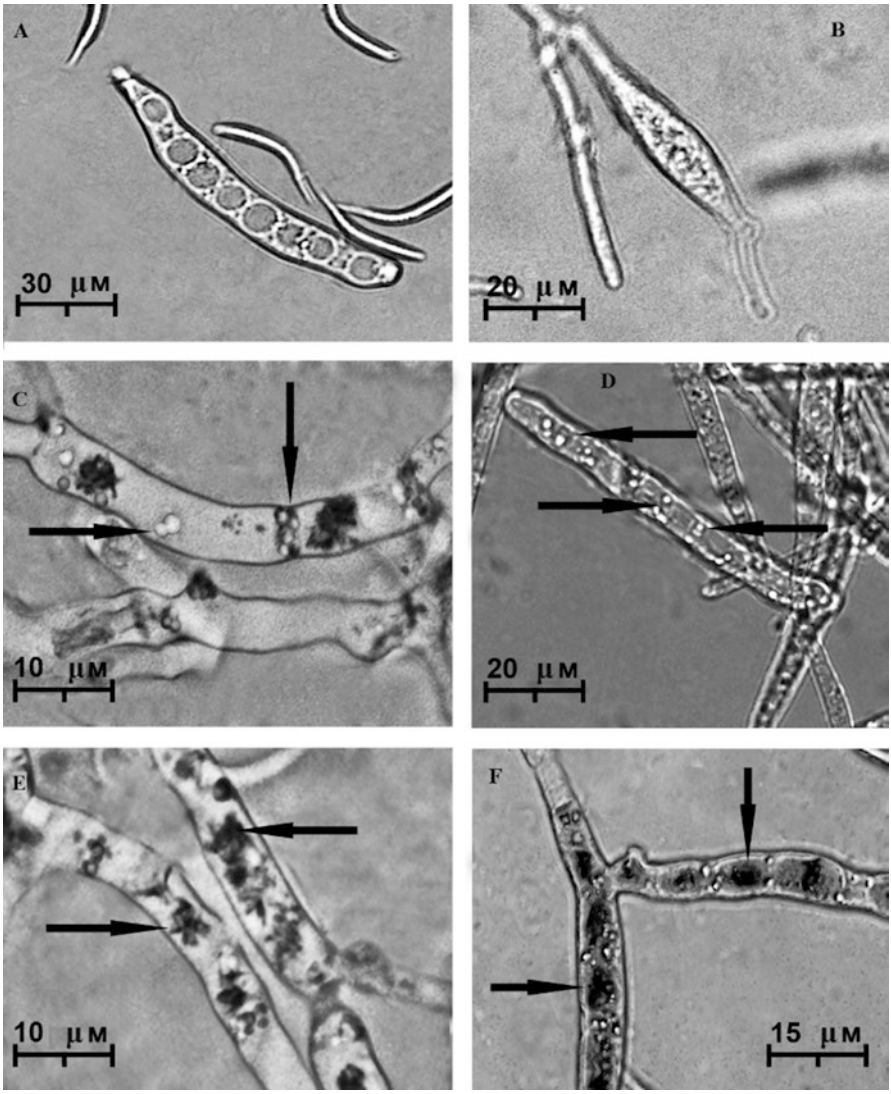

Fig. 15.8 Morphological alterations connected with biosynthetical activity: thickening and vacuolization of hypha (1, *E. ashbyi*; 2, *E. gossypii*); lipidic droplets within the mycelium (pointed with arrow, 3, *E. ashbyi*; 4, *E. gossypii*); crystalline inclusions of riboflavin within hypha vacuoles (pointed with arrow, 5, *E. ashbyi*; 6, *E. gossypii*) (Семенова et al. 2013)

(2.01 g × L^{-1}), and maximum (2.89 g × L^{-1}) values of biomass accumulation, respectively. The submerged microorganism cultivation led to medium acidification during log phase and alkalization during stationary and decline phases. After 60 h, strain VKM F-124 had higher levels of biomass accumulation and medium alkalization than other strains. The degree of medium alkalization might be caused

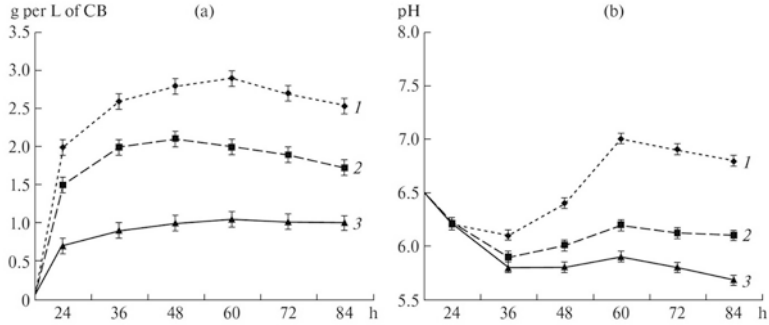

Fig. 15.9 (**a**) Dry biomass accumulation and (**b**) pH changes of CB during submerged cultivation of (1) VKM F-124, (2) VKM F-3009, and (3) VKPM F-340 strains of *E. ashbyi*

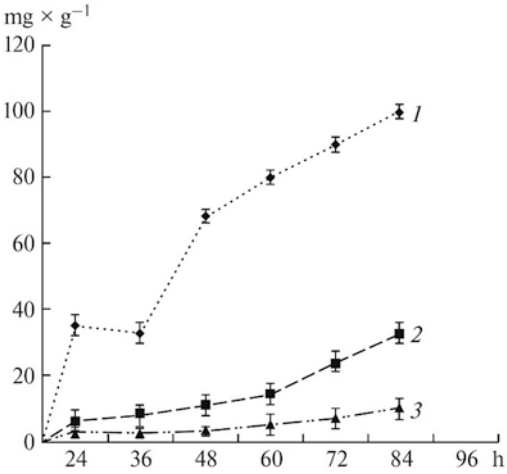

Fig. 15.10 Accumulation (mg × g^{-1}) of vitamin B_2 during cultivation of (1) VKPM F-340, (2) VKM F-3009, and (3) VKM F-124 strains of *E. ashbyi*

by the changes in acid-base balance of the accumulated biomass and the intensity of alkaline metabolite excretion.

The riboflavin synthesis and accumulation in culture medium began in the log phase and increased during stationary and decline phases (Fig. 15.10). The riboflavin synthesis by strain VKPM F-340 was the most intense. Strain VKM F-3009 and the white mutant of strain VKM F-124 had less intense riboflavin synthesis (three and ten times, respectively) than strain VKM F-340.

The intensity of accumulation of the main aroma-forming compounds (geraniol, citronellol, nerol, and 2-phenylethanol) was different among these strains Шпичка and Семенова 2014. The most intense accumulation of these compounds was observed in the stationary phase (Figs. 15.11 and 15.12).

Significant differences in geraniol synthesis were noticed at certain stages of culture development. The highest increase in geraniol accumulation by strain VKM

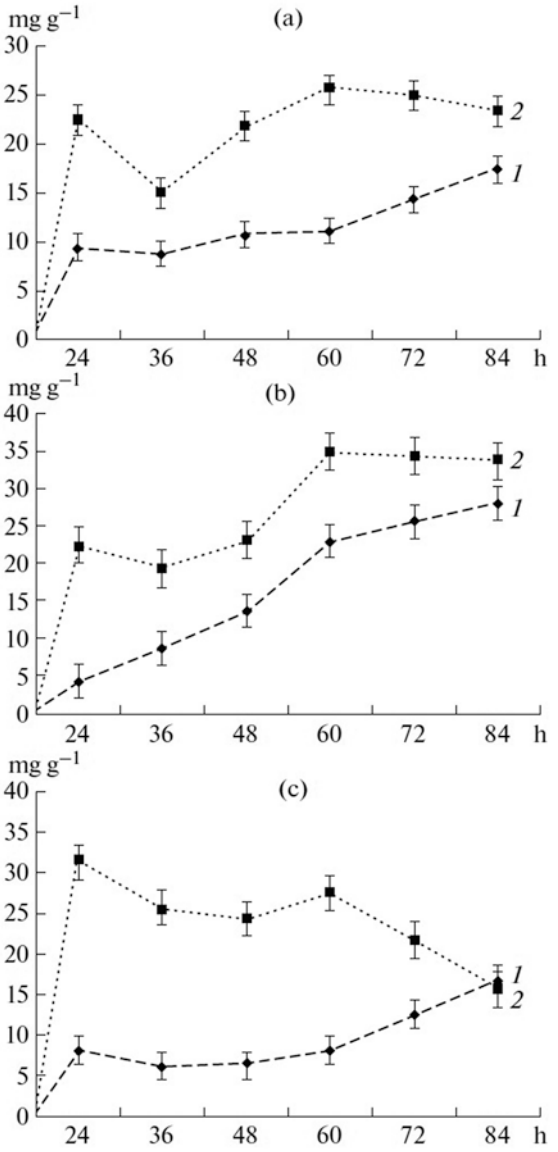

Fig. 15.11 Accumulation (mg × g^{-1}) of (1) geraniol and (2) β-phenylethanol during submerged cultivation of (**a**) VKM F-3009, (**b**) VKPM F-340, and (**c**) VKM F-124 strains of *E. ashbyi*

Fig. 3. Accumulation (mg × g^{-1}) of (*1*) geraniol and (*2*) β-phenylethanol during submerged cultivation of (a) VKM F-3009, (b) VKPM F-340, and (c) VKM F-124 strains of *E. ashbyi*.

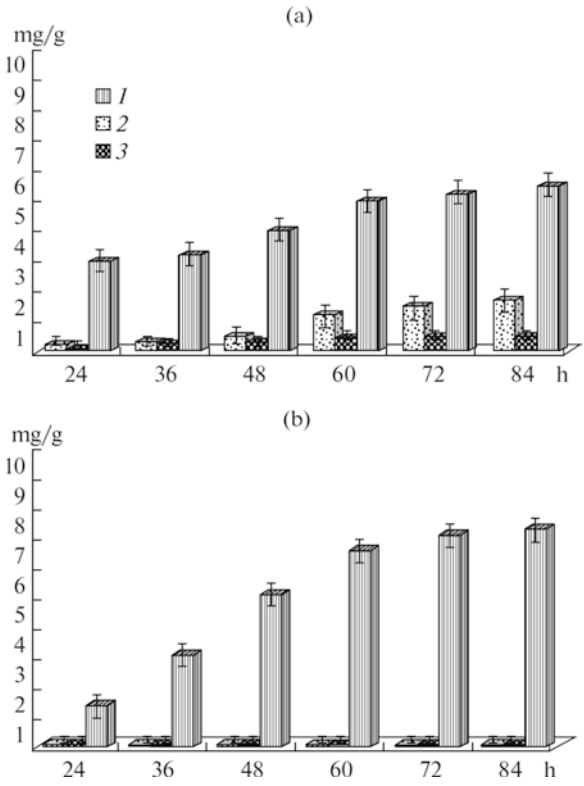

Fig. 15.12 Accumulation (mg × g^{-1}) of (**a**) citronellol and (**b**) nerol during submerged cultivation of (1) VKPM F-340, (2) VKM F-3009, and (3) VKM F-124 strains of *E. ashbyi*

F- 3009 was observed between 48 and 60 h, while for strain VKPM F-340 and VKM F-124 it was between 48 and 60 h. Nevertheless, the latter had the insignificant geraniol increase.

It was noted above that in 24 h, essential oil and riboflavin began to accumulate within mycelia and in medium, and lipid bodies formed in micromycete vegetative hyphae (Fig. 15.13). Among all strains, the increase in the essential oil synthesis efficiency (Figs. 15.11 and 15.12) was accompanied with the growth in spherosome quantities and sizes.

The marked vacuolization (after 36–48 h) and the beginning of sporogenesis (after 48–60 h) were observed during the period of highest accumulation of aroma-forming compounds and vitamin B_2 (Fig. 15.14).

Strain VKPM F-340, which possessed the highest intensity of essential oil synthesis, had more intense vacuolization (numerous small vacuoles) than other strains. The vacuolization of this strain began at earlier stages and finished at the later stages of growth than that of other strains. Strain VKPM F-340 possessed more intense protoplasm lipophilicity (osmophilicity in electron microscopy) than other strains (Fig. 15.15).

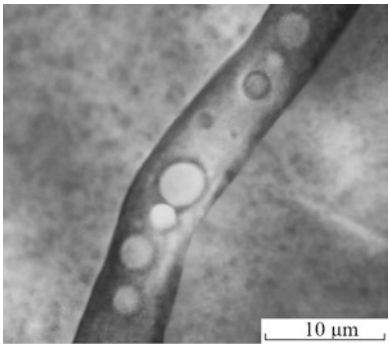

Fig. 15.13 Lipid droplets in a *E. ashbyi* hypha (Sudan III staining)

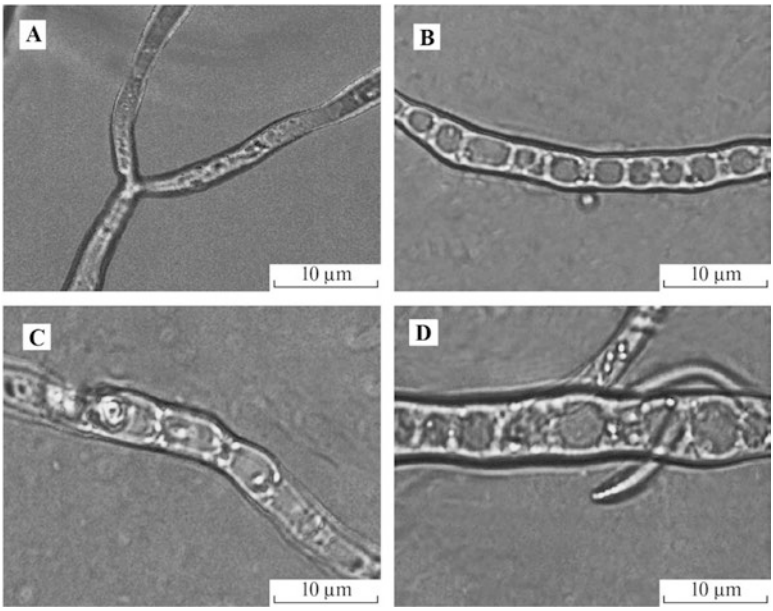

Fig. 15.14 Mycelium of *E. ashbyi* during (**a**) log phase, (**b**) a slowdown in the growth, (**c**) stationary phase, and (**d**) at the beginning of decline phase

Protoplasm lipid bodies virtually disappeared after the release of lipophilic metabolites (phenylethyl and monoterpene alcohols) into the medium at the end of the stationary phase. The excretion of aroma-forming compounds might be a way to control their synthesis using the mechanisms of overflow or excess metabolite excretion (Ledesma-Amaro et al. 2013, 2014).

The content analysis of data on the metabolic pathways of riboflavin and aroma-forming compound biosynthesis in eukaryotes (Takahashi et al. 2007; Marx et al. 2008; Misawa 2011) allowed us to propose a hypothetical model, which describes

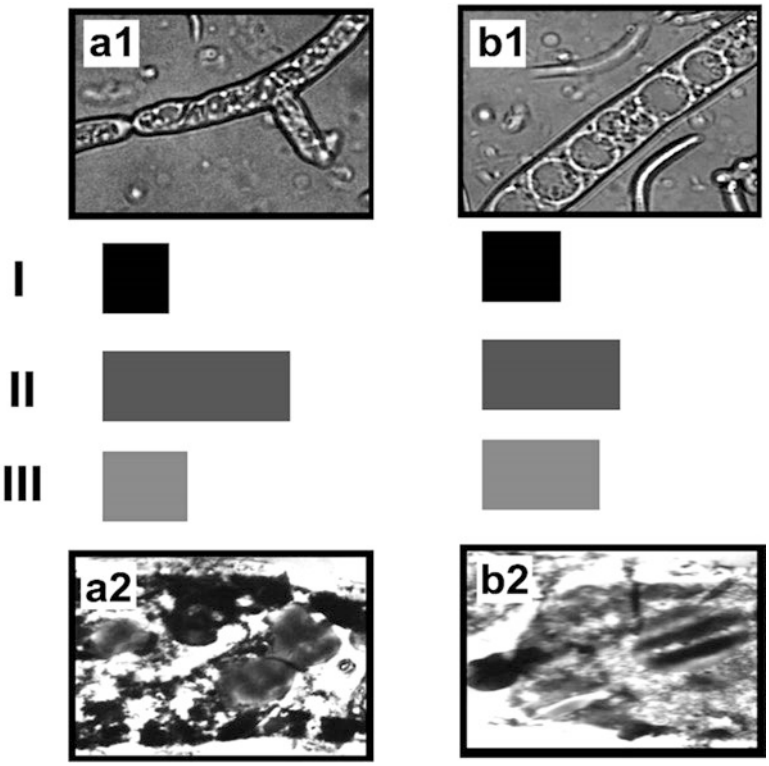

Fig. 15.15 Cytomorphological features of *Eremothecium* mycelium and alteration of the main biologically active substance accumulation: I, riboflavin; II, essential oil; III, geraniol; a1, a2 36 h; b1, b2 48 h of cultivation in liquid medium (magnification: a1, b1,– ×100; a2, ×18500; b2, ×20000) (Семенова et al. 2015)

the riboflavin and essential oil biogenesis in *E. ashbyi* and includes basic biochemical reactions for correlation determination among all reactions (Fig. 15.16).

According to this model, fatty acids, glucose, aspartate, asparagine, glycine, serine, and threonine are the main substrates for the riboflavin synthesis, whereas fatty acids, glucose, and leucine are substrates for the monoterpene alcohol synthesis (geraniol, nerol, and citronellol); phenylalanine participates in the aromatic alcohol synthesis (β-phenylethanol). Acetyl-CoA, which is formed during glucose and fatty acid catabolism, is a key compound in vitamin and monoterpene synthesis. This metabolic model enables us to understand the relationships among the processes of the synthesis of flavins and terpene and aromatic alcohols, their direction, cellular localization, and the regulation mechanisms of *E. ashbyi* productivity Бугорский et al. 1990.

Figures 15.10, 15.11, and 15.12 show that strain VKPM F-340 had the highest vitamin and aroma-forming compound productivity. However, our study of the dynamics of formation of these compounds revealed that in 60 h, the accumulation of monoterpenes had no changes and remained approximately the same, while that of riboflavin continued to increase. This might have been caused by the decreased

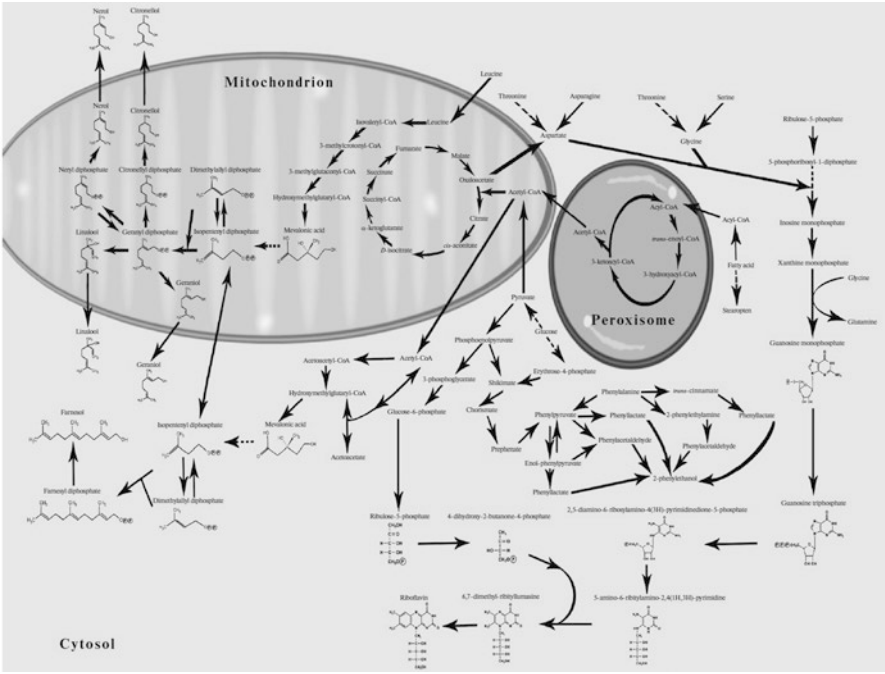

Fig. 15.16 Hypothetical metabolic model of riboflavin, phenylethanol, and terpene alcohol biosynthesis by *E. ashbyi* cells

medium glucose concentration, which led to cessation or reduction of the rate of monoterpene alcohol synthesis (geraniol, nerol, and citronellol) and affected the synthesis of riboflavin and β-phenylethanol to a lesser extent. The latter can be synthesized from amino acids (Cheng et al. 2011; Walther and Wendland 2012; Ravasio et al. 2014). β-Phenylethanol was detected in CB of all strains; this circumstance indirectly indicates the expression of active genes, for example, ARO8a, ARO8b, ARO10, or ARO80, which are responsible for aromatic amino acid catabolism (Семенова and Шпичка 2014).

Literature analysis (Takahashi et al. 2007; Nieland and Stahmann 2013; Ledesma-Amaro et al. 2013, 2014; Schindler and Schmid 1982) and our data suggested that the riboflavin and monoterpene biosyntheses might be coupled in regard to their dynamics and cellular localization (compartmentation), and their gene regulation may also be interconnected. The correlation analysis of the content of riboflavin, geraniol, citronellol, and nerol and their total content in CB confirmed this assumption with a confidence level of 95.0–99.9% (Table 15.7).

The Spearman's coefficients showed strong (VKM F-124) and very strong (VKM F-3009 and VKPM F-340) direct (positive), simple (between riboflavin and monoterpene alcohols), and multiple (between riboflavin and monoterpene alcohol

Table 15.7 Spearman's correlation coefficients between riboflavin and monoterpene alcohol syntheses by *E. ashbyi* strains

Compound	Correlation coefficients			Significance level range
	Producer			
	VKM F-124	VKM F-3009	VKPM F-340	
Geraniol	0.81	0.94	0.94	0.005–0.052
Citronellol	0.97	1.00	0.94	0.001–0.050
Nerol	–	1.00	0.94	0.005–0.050
Geraniol + citronellol	0.90	0.94	0.94	0.005–0.015
Geraniol + nerol	0.81	1.00	0.94	0.005–0.052
Citronellol + nerol	0.97	1.00	0.94	0.005–0.050
MTA[a] Phenylethanol/ (citronellol + nerol)	0.90 −0.90	1.00 −0.94	0.94 −0.94	0.005–0.050 0.000–0.005

Note. [a]MTA monoterpene alcohols, "–" means that the compound was not detected

combinations) correlations. Therefore, the high level of riboflavinogenesis can serve as a marker of high cell metabolic activity and an additional criterion for the selection of mutants with high levels of aroma-forming compound synthesis.

These results are of great importance for the development of methods for regulation of the biosynthesis of industrially important metabolites, such as riboflavin and phenyl ethyl and monoterpene alcohols. However, these data should be further evaluated using molecular biology techniques to determine possible methods of gene regulation of these processes.

15.2 In Vitro Study of Antimicrobial and Toxic Properties of *Eremothecium* Oil Depending on Its Component Composition

As it was noted, homothallic ascomycetes *E. ashbyi* and *E. gossypii* are able to produce rose-scented essential oil, whose composition was similar to that of natural rose essential oil (Tables 15.8 and 15.9). While previous studies have mainly focused on the study of fundamental problems, none have assessed the biological activity of this new pharmaceutical substance (*Eremothecium* oil). We, therefore, sought to reveal its antimicrobial and toxic effects depending on its component composition.

Eremothecium oil possesses the highest antibiotic activity against E. *coli*, *P. aeruginosa*, *Myxococcus* sp., *L. acidophilus*, *L. lactis* ssp. *lactis*, *S. maltophilia*, *A. baumannii*, *K. pneumoniae*, *S. aureus*, *B. subtilis*, *B. megatherium*, and *C. albicans* (Table 15.10 and Fig. 15.17 Бибарсова et al. 2018). We revealed the minimal

Table 15.8 Aroma-forming ability of *Eremothecium* strains

Strain	Mass concentration of main components, %				Mass concentration of stearoptenes, %	Biosynthetical activity, mg L^{-1}
	β-Phenyl-ethanol	MTA[a]				
		Geraniol	Citronellol	Nerol		
E. ashbyi						
ВКПМ F-36	0.8–8.0	21.8–64.8	1.8–9.2	1.1–2.6	30.0–38.4	80.3–173.8
ВКМ F-4566	6.8–24.0	34.6–64.8	9.2–27.0	2.6–11.9	–	122.9–293.1
ВКМ F-4565	0.6–20.4	43.8–68.4	5.6–9.4	1.4–2.8	0.0–46.8	172.0–362.7
ВКМ F-3009	11.2–23.1	64.7–75.6	5.8–12.3	1.9–21.2	–	156.7–473.1
ВКПМ F-340	10.2–19.0	79.0–88.3	1.7–2.1	–	–	143.2–178.5
ВКПМ F-1320	40.7–63.4	41.1–53.0	0.9–2.0	0.0–1.8	–	193.9–551.1
E. gossypii						
ВКМ F-2627	53.1–58.2	35.3–48.8	0.5–2.9	0.0–5.4	–	197.0–565.5
ВКМ F-3276	39.8–57.6	0.6–58.9	2.1–4.7	0.1–6.9	10.6–97.2	159.2–247.5
ВКМ F-3296	50.1–52.1	1.7–49.8	2.2–3.6	0.5–0.7	–	52.4–87.9
ВКПМ F-1321	46.8–56.2	36.3–46.8	1.5–3.9	1.0–5.4	–	251.3–595.1

Note. [a]MTA monoterpene alcohols, "–" means that the compound was not detected

inhibitory concentrations of distillated oil from *E. ashbyi* for *C. albicans, S. aureus*, and *E. coli*. These concentrations correspond with those of oil from rose petals (Семенова et al. 2014; Шпичка and Семенова 2015b). The study of test-culture growth kinetics showed that *Eremothecium* oil had bacteriostatic activity against *E. coli* and *S. aureus* (death rate higher than 85.0%) at a concentration of 7.8 μL mL^{-1}; however, it does not affect *C. albicans* (Fig. 15.17).

After 20 h, oil at a concentration of 860 μg mL^{-1} showed the highest toxicity (100%) on *P. caudatum* (Table 15.12). At the same time, the average lethal concentration among all oil samples was 210 μg mL^{-1} (Table 15.11). Oil at concentrations of 860–1720 μg mL^{-1} possessed an acute toxic effect (cell viability of *P. caudatum* decreased more than 50% than in control); concentrations from 210 to 430 μg mL^{-1} do not have acute and subacute toxicity. Effect intensity correlated with phenyl ethanol ($R = -0.9$; strong negative association), geraniol ($R = 0.6$; moderate positive association), nerol ($R = -0.55$; moderate negative association),

Table 15.9 Comparative analysis of aroma products synthetized by *Eremothecium* strains and *Rosa* species

Producer	Ratio			Efficiency of oil production process, mg per g of biomass/h
	β-Phenyl-ethanol/ MTA[a]	Geraniol/ citronellol	Geraniol/ erol	
E. ashbyi				
ВКМ F-3009	0.02–0.12	9.12–15.30	13,6–24.65	0.813–1.298
ВКМ F-4565	0.01–0.37	4.66–12.21	15.64–48.86	1.032–1.682
ВКМ F-4566	0.08–0.31	0.98–6.90	2.22–10.17	0.825–1.237
ВКПМ F-36	0.22–0.39	2.51–7.04	3.65–68.20	0.930–1.358
ВКПМ F-340	0.11–0.24	37.62–51.94	–	0.976–1.240
ВКПМ F-1320	0.79–1.41	20.50–58.89	0.00–29.45	1.347–2.334
E. gossypii				
ВКМ F-2627	1.12–1.27	13.92–77.33	6.96–34.72	1.514–1.915
ВКМ F-3276	0.79–1.29	7.76–13.21	4.79–26.42	0.627–2.198
ВКМ F-3296	0.01–16.82	1.53–8.31	0.00–39.73	0.326–0.759
ВКПМ F-1321	0.85–1.53	9.31–31.20	6.72–46.8	0.873–2.066
Essential oil-bearing *Rosa* species				
R. alba	0.05–0.13	0.26–1.20	1.43–3.20	0.002–0.003
R. gallica	2.33–3.00	2.00–2.40	1.00–4.20	0.004–0.006
R. damascena	0.04–0.08	0.13–1.12	0.55–7.67	0.001–0.005

Note. [a]MTA monoterpene alcohols, "–" means that the compound was not detected

Table 15.10 Biological activity of *Eremothecium* oil against *L. lactis* spp. *lactis* and *L. acidophilus*

Dilution of *Eremothecium* oil	Indicators of delay or absence of culture growth					
	L. lactis spp.*lactis*			*L. acidophilus*		
	Lim, mm	CV, %	$\bar{x} + S_x$, mm	Lim, mm	CV, %	$\bar{x} + S_x$, mm
I	10…20	26.9	16,0 ± 4.3	12...15	9.5	13.7 ± 1.3
II	5…9	45.5	7.5 ± 2.5	8...10	11.1	9,0 ± 1.0
III	5…7	16.7	6,0 ± 1,0	6…11	29.4	8.5 ± 2.5

linalool ($R = -0.74$; strong negative association), and total monoterpene alcohol ($R = 0.5$; moderate positive association) content (Table 15.13 and Fig. 15.18). We revealed the weak negative correlation between sample activity on *P. caudatum* and citronellol ($R = -0.19$) content.

The content of the aroma-forming compounds and their combination, which possessed additional inhibitory activity, causes the antimicrobial and toxic action of *Eremothecium* oil Markelova and Semenova 2017. These data correspond with those for essential oil from rose petals (Семенова 2007; Маркелова et al. 2014; Величко et al. 2015).

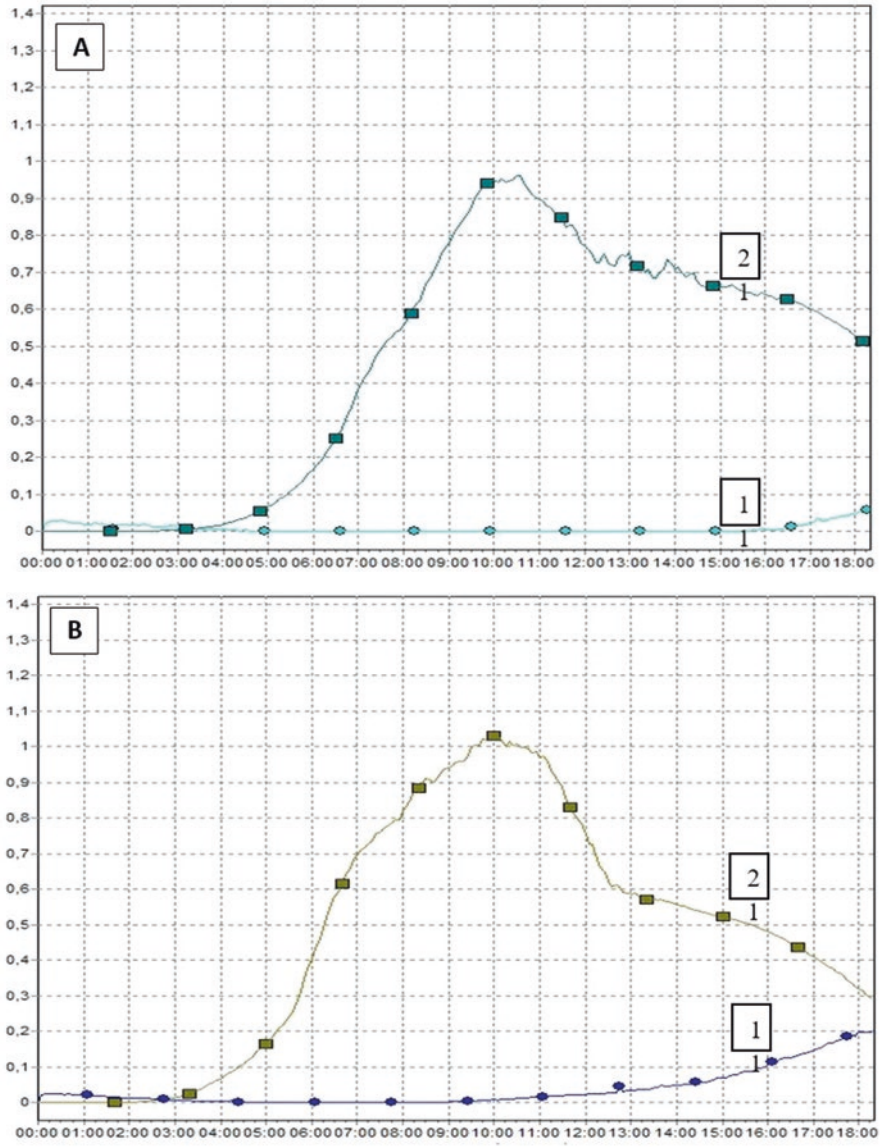

Fig. 15.17 Growth curves of test bacteria (1, in a medium containing *Eremothecium* oil in bacteriostatic concentration 2107, 81 mkg ml^{-1}; 2, in a medium without essential oil); (**a**) *S. aureus*; (**b**) *E. coli*; *x*-axis, measurement time (hours); *y*-axis, optical density increment (Янина et al. 2017)

Table 15.11 Component composition of *Eremothecium* oil samples

Sample	Phenylethanol, %	Monoterpene alcohols, %				
		Geraniol	Citronellol	Nerol	Linalool	Total amount
1	16.32	67.17	6.22	4.02	0.83	78.24
2	13.85	74.66	5.64	3.60	0.25	84.15
3	7.52	68.31	4.06	4.15	0.22	76.74
4	15.84	61.03	8.24	6.97	0.00	76.24
5	35.89	41.34	6.90	6.59	1.08	55.91
6	4.40	43.30	5.50	3.35	0.00	52.15
7	10.50	56.10	7.50	2.10	0.00	65.70

(Янина et al. 2017)

Table 15.12 *Eremothecium* oil influence on *P. caudatum*

Sample	LC_{100}, mkg ml^{-1}	EC_{50}, mkg ml^{-1}	LC_{50}, mkg ml^{-1}	LT_{50}, h	Lethality, % at the following concentration of oil, mkg ml^{-1}			
					1720	860	430	210
1	860	430	430	20	100±0	100±0	85±1.6	50±2.2
2	1720	430	860	20	100±0	72.5±0.8	48.8±1.3	42.5±1.3
3	860	210	210	20	100 ±0	97.5±1.3	73.8±0.9	47.5±1.3
4	430	210	210	20	100±0	97.5±2.1	92.5±0.6	47.5±1.3
5	860	210	430	20	97.3±0.5	91.3±1.3	78.8±0.9	51.3±2.5
6	860	860	210	20	100±0	100±0	90±0.8	82.5±2.4
7	1720	430	210	6	100±0	90±1.8	62.5±2.1	46.3±2.6

Note. LC_{100}, absolute lethal concentration; LC_{50}, median lethal concentration; LT_{50}, median lethal time; EC_{50}, half-maximal effective concentration
(Янина et al. 2017)

Table 15.13 Influence of individual compounds on *P. caudatum*

Compound	LC_{100}, mkg ml^{-1}	EC_{50}, mkg ml^{-1}	LC_{50}, mkg ml^{-1}	LT_{50}, h	Lethality, % at the following concentration, mkg ml^{-1}			
					1720	860	430	210
Phenylethanol	1720	430	860	24	99 ± 2	87.3±2.2	68±1.8	49.5±1
Geraniol	210	<210	<210	1	100±0	100±0	97.5±2.8	93.5±1.3
Citronellol	1720	430	210	24	100±0	88.8±1.5	68.3±2.4	50±0
Nerol	210	<210	<210	<0.016	100±0	98.3±2.1	92±2.2	87.8±0.5
Linalool	860	430	210	24	100±0	100±0	95±0.8	90±1.8

15.3 Conclusion

Essential oils are widely spread in the vegetable world, from fungi and algae to flowering plants. Earth shelters around 3000 plants that can be used a source of essential oil. But nowadays, a limited number of higher plants are used in industries. The quality of essential oil depends considerably on ecological factors such as

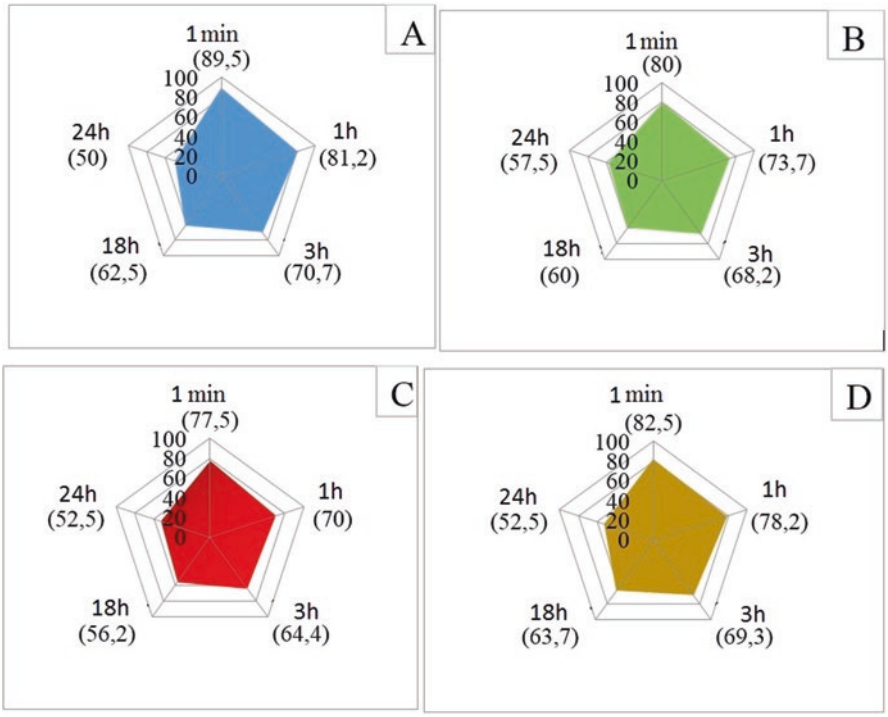

Fig. 15.18 *P. caudatum* viability dynamics in media containing *Eremothecium* oil of different origins with concentration 210 mkg ml^{-1} (samples (**a**) 1; (**b**) 2; (**c**) 3; (**d**) 4)

location, where volatile-oil-bearing plants are cultivated. In addition, plantation cultivation is characterized with seasonal prevalence. Biotechnological production suffers a few disadvantages. But the biotechnology of essential oil production in the culture of isolated cells and tissues is not competitive in comparison with the biotechnology based on the microbial synthesis.

It is well known that *E. ashbyi* and *E. gossypii* are etiological agents of stigmatomycosis and are able to produce riboflavin and essential oil, which are important for industry. These biological properties of the micromycetes are of potential interest for industrial production of different products. Research revealed the structural and functional features of vegetative, asexual, and sexual reproduction for the representatives of *Eremothecium* genus. There are a number of differences among the studied species. Under experimental conditions, the asexual reproduction was more common in *E. gossypii* than in *E. ashbyi.* This might be explained by the different impacts of the environment on the expression of certain genes which play an essential role in the regulation of sporulation. Formation and development of some cell structures correlate to the character and the level of biosynthetic activity. The process of the beginning of essential oil biosynthesis and the peak of its accumulation precede the active sporification, thus suggesting that essential oil can influence

processes of the sexual reproduction, particularly, ascospore formation and maturing. The analysis of received data and sources supplement and systematize knowledge about individual development cycles of *E. ashbyi* and *E. gossypii* and related changes in dynamics of culture growth. This new data may be used in mycological and phytopathological studies of mentioned objects.

The cultivation of oil-bearing rose plants could not meet the increasing demand of the industry. Therefore, the interest in fungal strains of *E. ashbyi* and *E. gossypii* is increasing. A study was conducted to determine the features of secretory structures of the *Rosa* and *Eremothecium* species. The investigation of aspects of biosynthesis, accumulation, and secretion of essential oils with a rose scent is crucial for the development of new ways to produce them and the characteristic of biological roles of *Rosa* and *Eremothecium* secondary metabolites.

Processes of accumulation of phenyl ethanol, geraniol, citronellol, and nerol were studied among *E. ashbyi* strains that showed different levels of riboflavin synthesis. There was a significant positive correlation between riboflavin and monoterpene alcohol biosynthesis (Spearman correlation coefficients = 0.81–1.00, $p \leq 0.05$). While accumulating the main secondary metabolites (vitamin B_2 and aroma-forming substances), the strains showed an increase in lipid drop quantity, and their vacuoles were filled with lipophilic compounds. This could be an indirect measure of riboflavinogenesis intensity and essential oil synthesis.

Thus, the comparative biotechnological characteristic of producers permits to recommend *E. ashbyi* and *E. gossypii* for the production of riboflavin (which is main end product for *E. ashbyi* and side additional product for *E. gossypii*) and essential oil (which is main end product for *E. gossypii* and side additional product for *E. ashbyi*).

References

Бибарсова АА, Семенова ЕФ, Преснякова ЕВ (2018) Влияние розового и эремотецевого масла на рост некоторых молочнокислых бактерий. Вопросы биологической, медицинской и фармацевтической химии 5. https://doi.org/10.29296/25877313-2018-05-04

Бугорский ПС, Семенова ЕФ, Родов ВС (1990) Влияние ионов водорода, калия и натрия на продуктивность гриба *Eremothecium ashbyi*. Микробиологический журнал 52(3):44–47

Бугорский ПС, Семенова ЕФ (1991) Душистые вещества мицелиального гриба *Ashbya gossypii*. Химия природных соединений 3:428

Бугорский ПС, Родов ВС, Носов АМ (1986) Состав эфирного масла мицелиального гриба *Eremothecium ashbyi*. Химия природных соединений 6:790–791

Быков ВА, Жученко АА, Рабинович АМ, Чечеткина НВ (2003) Средообразующие фитотехнологии 21 века. Нетрадиционные сельскохозяйственные, лекарственные и декоративные растения 1:74–87

Величко ВП, Семенова ЕФ, Стойко ТГ, Шпичка АИ, Моисеева ИЯ (2015) О токсичности розового и эремотецевого масла в отношении культуры *Paramecium caudatum.* Ученые записки Крымского федерального университета имени В.И. Вернадского. Серия «Биология, химия» 1(67):10–15

Войткевич СА (1999) Эфирные масла для парфюмерии и ароматерапии. Пищевая промышленность, Москва

Гуринович ЛК, Пучкова ТВ (2005) Эфирные масла: химия, технология, анализ и применение. Школа Косметических Химиков, Москва

Егорова НА, Ставцева ИВ (2006) Некоторые итоги и перспективы биотехнологических исследований эфиромасличных растений. Научные труды Института эфиромасличных и лекарственных растений 26:19–26

Жученко ЕВ, Семенова ЕФ, Маркелова НА, Шпичка АИ (2015) Влияние эфирных масел на микроорганизмы различной таксономической принадлежности в сравнении с современными антибиотиками. Сообщение 3. Действие масел лаванды, розового дерева, эвкалипта, пихты на некоторые грамотрицательные бактерии. Известия высших учебных заведений. Поволжский регион. Серия «Естественные науки» 1(9):30–41

Маркелова НА, Семенова ЕФ, Шпичка АИ, Жученко ЕВ (2014) Влияние эфирных масел на микроорганизмы различной таксономической принадлежности в сравнении с современными антибиотиками. Сообщение 1. Действие розового эфирного масла и антибиотических субстанций на некоторые грамотрицательные бактерии. Известия высших учебных заведений. Поволжский регион. Серия «Естественные науки» 3(7):39–48

Митишев АВ, Преснякова ЕВ, Семенова ЕФ, Гурина МА (2014) Сравнительный анализ штаммов продуцента и инновационного продукта как основных элементов биотехнологии резиноида хлореллы. Известия высших учебных заведений. Поволжский регион. Серия «Естественные науки» 4(8):19–29

Митишев АВ, Семенова ЕФ, Преснякова ЕВ (2017) К вопросу разработки технологии культивирования и переработки *Chlorella vulgaris* для получения резиноида. Вестник Воронежского университета. Серия «Химия. Биология. Фармация» 4:62–65

Погорельская АН, Кочетков ЕС, Бугорский ПС, Семенова ЕФ (1999) Биотехнологические методы в помощь создания рентабельного эфиромасличного производства с наиболее полной утилизацией отходов. Труды Института эфиромасличных и лекарственных растений 25:170–180

Родов ВС, Бугорский ПС, Семенова ЕФ (1987) Получение эфирного масла розового направления на основе биотехнологии. Труды ВНИИ эфиромасличных культур 18:13–15

Семенова ЕФ (2007) Биосинтетическая активность и антимикробные свойства *Eremothecium ashbyi* Guill. Известия вузов. Поволжский регион. Серия «Медицинские науки» 4:44–50

Семенова ЕФ, Богданов НИ (2000) Некоторые результаты биотехнологии ароматических продуктов. Инновационные технологии и продукты 4:9–13

Семенова ЕФ, Бугорский ПС (1989) Некоторые итоги поиска биотехнологически перспективных ароматообразующих культур. Труды ВНИИ эфиромасличных культур 20:14–16

Семенова ЕФ, Бугорский ПС (1990) К вопросу утилизации отходов эфиромасличного производства. Труды/ ВНИИ эфиромасличных культур 21:179–183

Семенова ЕФ, Преснякова ЕВ, Жужжалова ТП (2014) Репродуктивная биология видов и форм *Rosa* L. Изд-во ЦНТИ, Воронеж

Семенова ЕФ, Шпичка АИ, Моисеева ИЯ (2011) Культурально-морфологические и физиолого-биохимические свойства видов рода *Eremothecium*. Фундаментальные науки 6:210–214

Семенова ЕФ, Шпичка АИ, Преснякова ЕВ, Меженная НА (2015) Структурные основы маслонакопления в лепестках некоторых видов рода *Rosa* (Rosaceae) и мицелии аскомицетов *Eremothecium* (Eremotheciaceae). Растительные ресурсы 51(4):500–509

Семенова ЕФ, Шпичка АИ, Преснякова ЕВ (2013) Фармацевтическая микология видов рода *Eremothecium* (Биология, технология, практическое использование). Изд-во ПГУ, Пенза

Семенова ЕФ, Шпичка АИ (2014) О методических аспектах селекции продуцентов эфирного масла розового направления запаха. Вестник Воронежского госуниверситета. Серия «Химия. Биология. Фармация» 2:91–95

Семенова ЕФ, Шпичка АИ (2012) Фармбиотехнологическая характеристика *Eremothecium* – продуцента рибофлавина и эфирного масла. Разработка, исследование и маркетинг новой фармацевтической продукции 67:368–372

Шпичка АИ, Семенова ЕФ (2016a) Маркетинговый анализ разработки биотехнологии эремотецевого масла как инновационной технологии современного эфирномасличного производства. Научное обозрение Биологические науки 5:28–49

Шпичка АИ, Семенова ЕФ (2015a) О скрининге продуцентов комплекса биологически активных соединений. Таврический вестник аграрной науки 1(3):25–28

Шпичка АИ, Семенова ЕФ, Преснякова ЕВ (2013) Цитоморфологическое исследование представителей рода *Eremothecium*. Известия высших учебных заведений. Поволжский регион. Серия «Медицинские науки» 4:53–60

Шпичка АИ, Семенова ЕФ (2013a) Современное состояние и перспективы развития биотехнологии на основе эремотеция – продуцента рибофлавина и эфирного масла. Успехи современного естествознания 11:87–98

Шпичка АИ, Семенова ЕФ (2015b) Сравнительный анализ эфирных масел, синтезируемых в лепестках *Rosa* L.(*Rosaceae*) и мицелии *Eremothecium* Kurtzman (*Eremotheciaceae*). Вопросы биологической, медицинской и фармацевтической химии 7:42–44

Шпичка АИ, Семенова ЕФ (2013b) Сравнительная характеристика микроорганизмов, синтезирующих de novo летучие душистые вещества. Фундаментальные исследования 8(5):1113–1124

Шпичка АИ, Семенова ЕФ (2016b) Структурно-функциональные аспекты онтогенеза фитопатогенных грибов *Eremothecium ashbyi* и *Eremothecium gossypii*. Микология и фитопатология 50(1):53–61

Шпичка АИ, Семенова ЕФ (2014) Характеристика аромапродуктов *Eremothecium* и перспективы их использования. Курский научно-практический вестник «Человек и его здоровье» 2:103–106

Янина ЕА, Семенова ЕФ, Митишев АВ (2017) Биотестирование in vitro токсичности эремотецевого масла. Актуальные проблемы медицинской науки и образования 6:146–149

Ajikumar PK, Tyo K, Carlsen S, Mucha O, Phon TH, Stephanopoulos G (2008) Terpenoids: opportunities for biosynthesis of natural product drug using engineered microorganisms. Mol Pharm 5(2):167–190

Alchihab M, Destain J, Aguedo M, Thonart P (2010) Production d'aromes de type lactone par des levure. Biotechnol Agron Soc Environ 14(4):681–691

Batra LR (1973) *Nematosporaceae* (*Hemiascomycetidae*): taxonomy, pathogenicity, distribution, and vector relations. Tech Bull 1469:1–73

Baydar NG, Baydar H (2013) Phenolic compounds, antiradical activity and antioxidant capacity of oil-bearing rose (Rosa damascena Mill.) extracts. Ind Crop Prod 41:375–380

Bezrukova EI, Semenova EF, Velichko VP, Knyazkova AA, Shpichka AI (2016) The problem of optimization of seed material preparation *Eremothecium ashbyi*. Top Issues New Drugs Dev 1:337–338

Bicas JL, Silva JC, Dionisio AP, Pastore GM (2010) Biotechnological production of bioflavors and functional sugars. Cienc e Technol de Alimentos 30(1):7–18

Bomgardner MM (2012) The sweet smell of microbes. Chem Eng News 90(29):25–29

Carrau FM, Medina K, Boido E, Farina L, Caggero C, Dellacassa E, Versini G, Henschke PA (2005) De novo synthesis of monoterpenes by *Saccharomyces cerevisiae* wine yeasts. FEMS Microbiol Lett 243:107–115

Cheng X, Zhou J, Huang L, Li K (2011) Improved riboflavin production by *Eremothecium ashbyi* using glucose and yeast extract. Afr J Biotechnol 10(70):15777–15782

Christen P (1995) Producción de aromas porfermentación en medio solido. Tópicos de Investigacióny Posgrado IV 2:102–109

Dudareva N, Klempien A, Muhlemann JK, Kaplan I (2013) Biosynthesis, function and metabolic engineering of plant volatile organic compounds. New Phytol 198(1):16–32

Etschmann MMW, Bluemke W, Sell D, Schrader J (2002) Biotechnological production of 2-phenylethanol. Appl Microbiol Biotechnol 59:1–8

Foerster C, Revuelta JL, Kraemer R (2001) Carrier-mediated transport of riboflavin in *Ashbya gossypii*. Appl Microbiol Biotechnol 55:85–89

Gordente AG, Curtin CD, Varela C, Pretorius IS (2012) Flavor-active wine yeast. Appl Microbiol Biotechnol 66:1–18

Haeusler A, Muench T (1998) Microbial production of natural flavors. ASM News 63(10):551–559

Hansen EH, Lindberg Moller B, Kock GR, Buenner CM, Kristensen C, Jensen OR, Okkels FT, Olsen CE, Motawia MS, Hansen J (2009) De novo biosynthesis of vanillin in fission yeast and baker's yeast. Appl Environ Microbiol 75(9):2765–2774

Janssens L, De Pooter HL, Schamp NM, Vandamme EJ (1992) Production of flavours by microorganisms. Process Biochem 27:195–215

Jianping J (1993) Identification of volatile compounds produced *by Kluyveromyces lactis*. Biotechnol Tech 7(12):863–866

Kambourova R, Bankova V, Petkov G (2003) Volatile substances of the green alga *Scenedesmus incrassatulus*. Z Naturforsch 53:187–190

Klein-Marcuschamer D, Ajikamar PK, Stephanopoulos G (2007) Engineering microbial cell factories for biosynthesis of isoprenoids. Trends Biotechnol 25(9):417–424

Knyazkova AA, Semenova EF, Shpichka AI, Velichko VP, Bezrukova EI (2016) About the maintenance in the active state of high-productive variant of *Eremothecium ashbyi*. Top Issues New Drugs Dev 1:356–357

Krings U, Berger RG (1998) Biotechnological production of flavours and fragrances. App Microbiol Biotechnol 49:1–8

Kurtzman CP (1995) Relationships among the genera *Ashbya, Eremothecium, Holleya* and *Nematospora* determined from rDNA sequence divergence. J Ind Microbiol 14:523–530

Langenheim JH (1994) Higher plant terpenoids: a phytocentric overview of their ecological role. J Chem Ecol 20(6):1223–1280

Ledesma-Amaro R, Kerkhoven EJ, Revuelta JL, Nieland J (2013) Genome scale metabolic modeling of the riboflavin overproducer *Ashbya gossypii*. Biotechnol Bioeng 9999:1–10

Ledesma-Amaro R, Santos M, Jimenez A (2014) Strain design of *Ashbya gossypii* for single-cell oil production. Appl Environ Microbiol 80(4):1237–1244

Longo MA, Sanroman MA (2006) Production of food aroma compounds: microbial and enzymatic methodologies. Food Technol Biotechnol 44(3):335–353

Markelova NN, Semenova EF (2017) Sensitivity of nonfermentative gram-negative bacteria to essential oils of different origin. Microbiologie 86(5):610–617

Marx H, Mattanovich D, Sauer M (2008) Overexpression of the riboflavin biosynthetic pathway in *Pichia pastoris*. Microb Cell Factories 7:23

Medeiros ABP, Pandey A, Vandenberghe LPS, Pastore GM, Soccol CR (2006) Production and recovery of aroma compounds produced by solid-state fermentation using different absorbents. Food Technol Biotechnol 44(1):47–51

Misawa N (2011) Pathway engineering for functional isoprenoids. Curr Opin Biotechnol 22:1–7

Mitishev AV, Semenova EF, Velichko VP, Shpichka AI, Moiseeva IY (2016) Determination of the biological activity of chlorella resinoids in regard to *Paramecium caudatum*. Int Res J 4(46), 5:23–27

Mitrović T, Stamenković S, Cvetković V, Nikolić M, Tošić S, Stojičić D (2011) Lichens as a source of versatile bioactive compounds. Biologica Nyssana 2(1):1–6

Mulder-Krieger T, Verpoorte R, Baerheim A, Svendsen J, Scheffer JC (1988) Production of essential oils and flavours in plant cell and tissue cultures. A review. Plant Cell Tiss Org Cult 13:85–154

Nadal M, García-Pedrajas MD, Gold SE (2008) Dimorphism in fungal plant pathogens. FEMS Microbiol Lett 284:127–134

Nieland S, Stahmann K-P (2013) A developmental stage of hyphal cells shows riboflavin instead of sporulation in *Ashbya gossypii*. Appl Microbiol Biotechnol 97:10143–10153

Pandal N (2006) The global market for flavors and fragrances. BBC Research, London

Ravasio D, Wendland J, Walther A (2014) Major contribution of the Ehrlich pathway for the 2-phenylethanol/rose flavor production in *Ashbya gossypii*. FEMS Yeast Res 1:1–12

Revuelta JL, Ledesma-Amaro R, Lozano-Martinez P, Díaz-Fernández D, Buey RM, Jiménez A (2017) Bioproduction of riboflavin: a bright yellow history. J Ind Microbiol Biotechnol 44:659–665

Schindler J, Schmid RD (1982) Fragrance or aroma chemicals. Microbial synthesis and enzymatic transformation – a review. Process Biochem 17:2–8

Schiestl FP (2010) The evolution of floral scent and insect chemical communication. Ecol Lett 13(5):643–656

Schwab W, Davidovich-Rikanati R, Lewinsohn E (2008) Biosynthesis of plant-derived flavor compounds. Plant J 54:712–732

Semenova E, Presnyakova V, Goncharov D, Goncharov M, Presnyakova E, Presnyakov S, Moiseeva I, Kolesnikova S, Moiseev J (2017a) Spectrophotometric method for quantitative measuring essential oil in aromatic water and distillate with rose smell. J Phys: Conference Series 784 1:012053. https://doi.org/10.1088/1742-6596/784/1/012053. http://iopscience.iop.org/article/10.1088/1742-6596/784/1/012053/meta

Semenova EF, Rodov VS, Shpichka AI (2011) Influence of conditions of inoculating material preparation on accumulation of aroma building substances in culture of *Eremothecium ashby*i Guill. Int J Appl Fundam Res 6:87

Semenova EF, Shpichka AI (2012) Some pharmbiotechnological characteristics of *Eremothecium*, producer of riboflavin and essential oil. Int J Appl Fundam Res 1:170–172

Semenova EF, Shpichka AI, Moiseeva IY (2012a) About explanation of elaboration of essential Eremothecium oil biotechnology. Int J Exp Educ 3:35–36

Semenova EF, Shpichka AI, Moiseeva IY (2012b) About essential oils biotechnology on the base of microbial synthesis. Eur J Nat Hist 4:29–31

Semenova EF, Shpichka AI, Presnyakova EV, Mezhennaya NA (2016) Processes of essential oil accumulation in petals of *Rosa (Rosaceae*) and mycelium of *Eremothecium*. Bull State Nikit Botan Gard 118:25–33

Semenova EF, Shpichka AI, Presnyakova EV (2017b) Aromatic and monoterpene alcohol accumulation by *Eremothecium ashbyi* strains differing in riboflavinogenesis. Appl Biochem Microbiol 53(3):374–380

Semenova EF, Shpichka AI, Presniakova EV, Presniakova VS, Goncharov MA, Goncharov DA (2017c) Development of a novel biotechnological fragrant product, Eremothecium oil. Indian J Pharm Educ Res 51(3):136–138

Soetaert W, Vandamme EJ (2010) Industrial biotechnology: sustainable growth and economic success. Willey, Washington

Takahashi S, Yeo J, Greenhagen BT, McMullin T, Song L, Maurina-Brunker J, Rosson R, Noel JP (2007) Chappell Metabolic engineering of sesquiterpene metabolism in yeast. Biotechnol Bioeng 97(1):170–181

Vandamme EJ (2003) Bioflavours and fragrances via fungi and their enzymes. Fungal Divers 13:153–166

Walther A, Wendland J (2012) Yap1-dependent oxidative stress response provides a link to riboflavin production in *Ashbya gossypii*. Fungal Genet Biol 49(9):697–707

Wasserstorm L, Lengeler KB, Walther A, Wendland J (2013) Molecular determinants of sporulation in *Ashbya gossypii*. Genet 195:87–99

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