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Chapter

# Essential Oils: Partnering with Antibiotics

*Mariam Aljaafari, Maryam Sultan Alhosani, Aisha Abushelaibi, Kok-Song Lai and Swee-Hua Erin Lim*

## Abstract

Essential oils (EO) are volatile, non-lipid-based oils produced as a plant defense mechanism. Studies from our group have validated the potential usefulness of EOs to synergistically and additively work with antibiotics. In this book chapter, we aim to outline some background on the EOs and their uses and applications, to discuss the different mechanisms of action in partnering with antibiotics, and, finally, to explore their potential use against multidrug-resistant bacteria. Applications of EO in therapy will enable the revival of previously sidelined antibiotics and enhance the development of new drug regimens to better mitigate what may be the biggest health challenge by year 2050.

**Keywords:** lavender oil, cinnamon bark oil, peppermint oil, multidrug-resistant bacteria, synergistic interaction, antimicrobial

## 1. Introduction

Essential oil (EO) is a concentrated mixture of organic compounds. EOs are produced by plants as a form of defense in addition to being an attractant to insects for dispersion of pollens and seeds [1, 2]. These oils are formed by the glandular trichomes and specialized secretory structure like secretory hairs, ducts, cavities, and glands; they then diffuse to the surface organs of plant such as leaves and flowers [3, 4]. The process of EOs formation involves three pathways which are the methyl-D-erythritol-4-phosphate (MEP), mevalonate, and malonic acid pathways [5]. The MEP and mevalonate pathways contribute in the biosynthesis of isoprenoids, whereas the malonic acid pathway will form the phenolic compounds [6, 7].

EOs have been used for many years for different purposes, such as to preserve raw and processed food because it can inhibit the growth of microorganisms like bacteria, viruses, and fungi [1, 8, 9]. Besides food, EO was also utilized in the area of perfumery for many years especially for ancient civilizations of India, Greece, Egypt, and Rome [10, 11].

In addition, EOs also serve as an alternative medicine that is important for local populations to treat severe burns to accelerate healing [11] and also for diseases such as leishmaniasis, schistosomiasis, and malaria [12, 13]. To date, approximately 10% of all EOs have been analyzed and commercially used as an insect repellent, attributed by its low toxicity to mammalian cells and the environment [10, 14]. However, certain EOs may cause toxicity or allergies which results in health and safety problems. Hence, national and international organizations have set standards to control the use of EOs [15].

| Part | Name of essential oil | References |
|---|---|---|
| Flowers | Lavender, jasmine | [18] |
| Leaves | Mint, lemongrass | [19, 20] |
| Wood | Sandal, cedarwood | [21, 22] |
| Roots | Sassafras, valerian | [23, 24] |
| Seeds | Fennel, nutmeg | [25, 26] |
| Rhizomes | Ginger, orris | [27, 28] |
| Fruits | Orange, juniper | [18, 29] |

**Table 1.**
*EOs extracted from plant parts.*

EOs can be found in various plants species, in particular those that belong to the Coniferae, Myrtaceae, Rutaceae, Labiatae, Umbelliferae, Alliaceae, and Zingiberaceae families [16, 17]. EOs are derived from different plant parts, such as flowers, leaves, wood, roots, seeds, rhizomes, and fruits [18]. See **Table 1** for examples of EOs found in each of the plant parts.

## 2. Classification of essential oils

In general, EOs can be classified based on their chemical composition, aroma created by the oil, evaporation speed, taxonomy or the families they belong to, their therapeutic uses, consistency, their origin, and the alphabetical order [16, 30]. Classification based on consistency, for example, can be divided into essences, balms, and resins [16, 31]. See **Table 2** for definition and examples of each.

Furthermore, there are three classifications of EOs based on their origin which are natural, artificial, and synthetic [16]. The natural EOs are taken from the plant without physical or chemical modifications, while the artificial oils are obtained by

| Based on consistency | Definition | Examples |
|---|---|---|
| Essences | Volatile liquid at room temperature [16] | Lavender, jasmine, geranium, rose [32, 33] |
| Balsams | Thick very volatile natural extract from tree or bush [16] | Copaiba balsam, Peruvian balsam, Canada balsam, Tolu balsam, Cabreuva balsam, Bangui balsam [16, 34] |
| Resins | Solid or semisolid products that comprise of derivates and abietic acid [16] | Patchouli, sandalwood, frankincense [33] |

**Table 2.**
*Classification of EOs based on consistencies.*

| Types of essential oil | Disadvantages | Advantages |
|---|---|---|
| Natural | Expensive, need a lot of natural sources to create, can cause burns if not diluted [15, 35, 36] | Great smell, helpful for physical and mental health [36] |
| Synthetic | No therapeutic properties, damaging the skin and respiratory system [36] | Cheap, commonly used as fragrance and taste enhancers, long lasting [15, 36] |

**Table 3.**
*Classification of EOs based on their origin.*

enriching the essence with extra components (can be one or more). The synthetic EOs, however, are obtained by combining many chemical substances together [16]. See **Table 3** for comparison between natural and synthetic EOs.

## 3. Essential oil extraction

Five thousand years ago, the ancient civilizations have already incorporated the use of machines for EO extraction [11]. However, there has been an expansion of the different extraction methods today. One of the important methods is the hydro-distillation which is divided into water distillation, water-steam distillation, and steam distillation [37, 38]. Hydro-distillation method involves hydro-diffusion, hydrolysis, and decomposition by heat [18]. In addition, steam distillation is performed by using the Clevenger system to extract oil from both fresh and dried plants, and it takes about 3 h [1, 11]. Another method is the expression method which utilizes the machines to compress the EO out of the plant [9, 11]. Additionally, solvent extraction and ultrasonic extraction methods are also routinely used [17].

Throughout the distillation process, water is separated by gravity, and at the end it leaves the volatile liquid behind; this liquid is the EO [16, 39]. EOs that are extracted by the use of chemical solvents cannot be called true EOs according to the National Cancer Institute, because they can cause changes in the clarity, scent, and fragrance of the oil [40]. The four criteria that affect the amount of essential oils produced are (1) time of distillation, (2) temperature, (3) pressure, and (4) plant quality.

### 3.1 Hydro-distillation

Hydro-distillation is the most commonly used method of extraction of EOs in which the plant is boiled in water [41, 42]. This method takes 1 h of distillation for fresh samples and 1 h and 15 min for dried samples. In the hydro-distillation method, a round-bottomed flask is used to place the plant material in with distilled water; if the plant material is dry, 1000 ml of distilled water should be used for 75 g of plant material, and if it is fresh material, 400 ml of distilled water should be used with 200 g of plant material; if the sample of plant is smaller, however, they can adjust the amount of water using this ratio: 13.3 ml of distilled water for each gram of dry plant. For water distillation, the modified Clevenger trap should be used to extract EO, and at the end the volume of the oil should be determined, and the EO should be analyzed immediately [43–45]. An advanced distillation method which is the microwave-assisted hydro-distillation can be used to shorten extraction time [46, 47].

### 3.2 Steam distillation

Steam distillation is the traditional method of extraction of EOs from plants [37]. The fundamental principle of steam distillation is that the mixture is allowed to be distilled at a temperature that is lower than the boiling point of the component; EO substances have a high boiling point that can reach 200°C; however, these substances will be volatile when steam or boiling water is present which is in 100°C; then the hot gas mixture will be condensed to form oil if it passes through a cooling system [48]. In steam distillation, the steam is first passed into a flask that contains the plant material; after that the condensate at the bottom of the flask should be collected which will be the water and oil; then the extract is condensated three times with ethyl ether to ensure that the essential oil is fully extracted; then the moisture

should be removed by adding sodium sulfate to the ethyl ether, followed by rotary evaporation to remove ethyl ether; and finally the volume of the EO is determined [43]. The advantages of this method of extraction are that it is rapid and can be controlled by the operator and it gives an acceptable quality than EOs extracted with other methods [48].

### 3.3 Solvent extraction

Solvent extraction method or liquid-liquid method is done by separating compounds based on their part solubility [49]. The basic principle of the solvent extraction method is that between two immiscible solvents, the solute distributes itself in a fixed ratio, whereby one is usually water and the other is an organic solvent [50]. In this method, the plant material will be grinded in a mortar that contains anhydrous hexane $Na_2SO_4$, followed by four rounds of extraction with hexane to obtain the yellow extract, then this is followed by adding a sufficient amount of Norite A charcoal for all extracts to remove the yellow color after low-speed centrifugation, and eventually the solution will be concentrated under air stream at room temperature [37, 43, 49]. A newer method of solvent extraction, called the microwave-assisted simultaneous distillation-solvent extraction (MW-SDE), is faster and simpler and uses fewer solvents to determine volatile compounds than conventional methods [51].

## 4. Composition of essential oils

### 4.1 Physical properties of EOs

EOs are volatile and become liquid at room temperature; they might be colorless or slightly yellow in color when extracted. Moreover EOs are lower in density than water, except for sassafras and clove essences [16]. EOs can be either liposoluble or soluble in alcohol and organic solvents, but they are only slightly soluble in water [4, 16, 32].

### 4.2. Chemical properties of EOs

Plants metabolites are divided into primary and secondary metabolites. The primary metabolites include proteins, DNA, and compounds that are important for cellular function. Secondary metabolites are produced by plants as a response of stress to deter herbivores or animals that would feed on them [52, 53]. Of the secondary metabolites, plant terpenes are the most numerous and diverse natural products of plant secondary metabolites which can be found in EOs [53]. They are found in monoterpene and diterpene oils and may be aliphatic, cyclic, or aromatic depending on the functional group [16]. According to the functional group, they can be alcohols, esters, ethers, hydrocarbon, and aldehydes [16].

The composition varies due to the place of origin, harvesting moment, extraction method, planting time, mineral fertilization, and climate [5, 16, 54]. For example, in warm places there will be more EOs than the cold or hot areas [16]. The concentration of EOs is extremely high due to the extraction methods used [23]. The simplest unit of EOs is the isoprene units that are composed of five carbons which can be assembled to form terpenes [16, 52]. EOs are composed of hydrocarbon molecules. Terpenes, for example, are hydrocarbon molecules that comprise of 10, 15, 20, and 30 carbon atoms and are made out of five-carbon isoprene units [55, 56].

EOs' main components are divided into terpenoid and non-terpenoid groups present in different concentrations [4]. The non-terpenoid group contains

short-chain aliphatic, aromatic, nitrogenated, and sulfated substances [16, 57]. The terpenoid group contains a different composition of hydrocarbon terpenes, terpenoids, and sesquiterpenes which is responsible for the special aroma [5, 58]. In general, the non-terpenoid group is less important than the terpenoid in terms of applications [53].

## 5. Use of essential oils against multidrug-resistant bacteria

Antibiotics are effective drugs that play an important role in treating infections and decreasing morbidity and mortality rates [59, 60]. In general, antibiotics kill multidrug-resistant (MDR) bacteria through various mechanisms. Examples include the β-lactam antibiotics that inhibit the bacterial cell wall synthesis, fluoroquinolones that inhibit DNA synthesis, tetracycline which is an inhibitor of protein synthesis, sulfonamides as a metabolic pathway or folic acid synthesis inhibitor, and polymyxin B which interferes with cell membrane integrity [60–63]. Antibiotic resistance develops naturally but is accelerated when the antibiotics are misused in both human and animals; the bacteria will evolve and develop resistance toward antibiotics, preventing the antibiotic from killing the bacteria [59, 64]. The bacteria subsequently become resistant by many mechanisms depending on the selective pressure incurred by the antibiotic used; for example, if the penicillin is used, the bacteria will become resistant to it by producing enzymes that will act against the antibiotic which is in this situation penicillinase enzyme [39]. For instance, a study conducted in 173 hospitals in Europe showed that high antibiotic consumption hospitals have a higher number of methicillin-resistant *Staphylococcus aureus* (MRSA) [65].

Antibiotic resistance in microorganisms is increasing at a worrisome rate [66]. Hence, over the years, researchers are exploring possible alternative sources that will be helpful to mitigate MDR bacteria. Of all the potential sources, EO was identified as one of the good alternative sources, because of their effectiveness in folk medicine [67]. Bacteria can be divided into two main types: the gram-positive and the gram-negative. The gram-positive have a thicker peptidoglycan layer than the gram-negative bacteria [68]. Besides that, the gram-negative bacteria also have an outer membrane that is absent in the gram-positive bacteria (**Figure 1**).

Generally, the gram-positive bacteria are less resistant to EOs than gram-negative bacteria [69, 70]. In gram-positive bacteria, hydrophobic molecules are able to penetrate the cell and act on the cell wall and cytoplasm. This is exemplified by the phenolic compounds in EOs against gram-positive bacteria [66]. In the gram-negative bacteria, a thin layer of peptidoglycans is present with an outer membrane that contains LPS. LPS consists of lipid A, core LPS, and O-side chain, which makes the gram-negative bacteria more resistant to EOs than gram-positive bacteria [66, 71]. Small hydrophilic solutes will make use of the porin proteins in the gram-negative bacteria to pass through the outer membrane; it is this porin selectivity that also makes the gram-negative bacteria less susceptible to hydrophobic antibiotics [66, 72, 73].

EOs via their different components have different targets against microorganisms such as the membrane and the cytoplasm [8]. Scientists have also found that the solubility of EO in water allowed them to decipher how EOs penetrated the cell wall of microbes; in other words EOs, being soluble in the cell membrane phospholipid bilayer, diffuse through the membrane [74]. A study done using the EO of *Melaleuca alternifolia* (tea tree) against MDR gram-negative bacteria (e.g., *Escherichia coli* and carbapenem-resistant *K. pneumoniae*) and methicillin-resistant *S. aureus* (MRSA) showed that there is a bactericidal effect of tea tree EO on these microorganisms [75]. This indicated that the EO can be used to kill

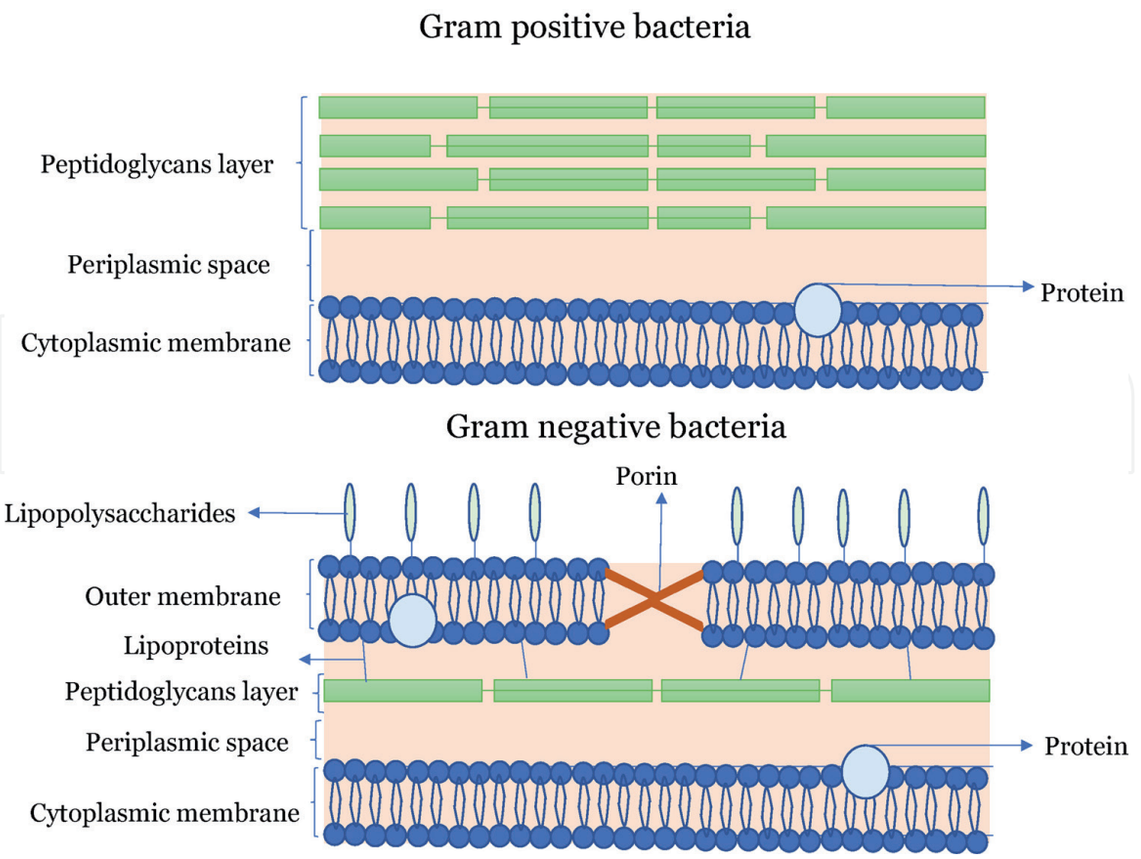


**Figure 1.**
*Schematic of different gram-positive (at the top) and gram-negative (at the bottom) cell walls.*

resistant bacteria [74]. Moreover, EO phenolic compounds' effect is concentration-dependent, whereby at low concentrations the phenolic compound will work with enzymes to produce energy, while at high concentration it will denature proteins [66, 76].

### 5.1 Determination of MIC of EOs for bacteria

Minimal inhibitory concentration (MIC) is the lowest concentration of a specific drug to inhibit the growth of microorganisms such as bacteria [1, 77]. After knowing that a particular EO has bactericidal, viricidal, and antiparasitic effects, the lowest concentration of EO to inhibit microbial growth should be measured [57, 78]. There are many assays to evaluate and screen for antimicrobial activity such as the disk diffusion test, microdilution (resazurin) or broth method, and agar dilution method [79, 80]. The agar disk diffusion test is commonly used to determine the antibacterial activity of the EO, but this method works only for EO with known components. This is because, for the EOs with unknown components, the antimicrobial effect may give rise to false or negative result caused by the unknown components [81]. Previously, in a study performed using the disk diffusion test to examine the antimicrobial activity of *Eucalyptus globulus* leaves, EO showed that there was a bacterial inhibitory effect on *E. coli* and *S. aureus* [82].

The commonly used alternative method to determine antimicrobial activity is the dilution method through a serial dilution of the EO in several tubes, and then determining the MIC after adding the test microorganism, turbidity is measured as a signal for growth [81]. In this method, the EO is first diluted; then it will be added to the medium that contains the broth culture, followed by incubation for 18 h in 37°C [69]. After the incubation period, the tube with the lowest concentration that showed no sign of growth is the MIC of the EO [69, 83]. However, this method

requires a large quantity of the plant extract [81]. A study using the redox dye resazurin for the new modified microdilution method has been carried out to determine the MIC for tea tree EO (*Melaleuca alternifolia*) against the gram-positive and gram-negative bacteria. The results showed that the resazurin method is accurate to determine the MIC and is higher in sensitivity than the results obtained from the agar dilution assay [80].

## 6. Mechanisms of actions with antibiotic

EOs' mechanism of action is poorly understood, but in general it depends on their chemical composition [8, 66, 84]. As antimicrobial resistance to antibiotics is increasing, scientists are currently exploring the ability of the plant extract to modify bacterial resistance against drugs [39]. The three main types of interactions that occur between the combination of antibiotic and EO are synergism, additivity, and antagonism [85]. Synergistic interaction is when the effect of the combined chemicals is greater than the effect of a chemical alone; additive interaction is when the sum of two chemicals is equal to the sum of chemical effect alone, while antagonism is when the whole effect of the two chemicals is less than the sum of effect of a single chemical alone [86]. In a study performed using the tea tree EO against the MDR bacteria, when a combination of tea tree EO with antibiotic (e.g., oxacillin) was tested on the bacteria, in particular the MRSA, a high synergistic index in the sub-inhibitory concentration was recorded [75]. This indicates that the EO can be used to overcome bacterial resistance to antibiotic. The synergism level increases when the combined effect is higher than the individual effect in the combination therapy [39].

Combination therapy is a new method that combines antibiotics and EO to kill resistant bacteria, via enhancement of the antimicrobial activity [39, 87]. Moreover, EOs have more components possessing different mechanisms of actions for many targets than antibiotics that have only one target. Combination therapy would be useful and able to provide a new treatment option for resistance bacteria [39].

## 7. Application of essential oils in therapy

Daily, the human body comes into contact with EOs through various sources such as herbs, spices, orange, spearmint, lemongrass, etc., but only limited information about the amount of EO uptake is known [4, 88]. Effects of EO begin to appear after it penetrates the human body in several ways such as by ingestion, by absorbing the EO or diffusion, and by inhalation [4, 89]. EOs can be taken by inhalation through the lungs and distributed into the blood because of their volatility [90–92]. Moreover, consumption of EO by ingestion should be taken with care because EOs may cause probable toxicity [4]. EOs are used in folk medicine to treat many health problems and can also be used as food preservatives by giving antimicrobial, antioxidant, and anti-inflammation properties [93, 94].

Many studies investigated the efficiency of EOs in combination with antibiotics to combat bacterial resistance; EOs with its compounds and secondary metabolites have shown promising synergistic interaction as an indication that they would be helpful to treat and decrease bacterial resistance to antibiotics [39, 95]. The advantages that make the EOs preferable are that they will decrease adverse reactions, besides being comparatively more cost-effective, with more public acceptance due to traditional usage, and being renewable with better biodegradability properties [39, 96].

## 8. Synergistic activity of essential oil

The synergistic effects between the EOs and antibiotics against the MDR bacteria have been investigated [97]. The synergistic effect can be defined as the ability of EO components to act together with the antibiotic component to increase the activity of the EO against MDR bacteria [98]. This is important because it will help to reduce the use of antibiotics and decrease the rates of antibiotic resistance [97]. Some studies have been done to assess the combinatory activities of lavender, cinnamon bark, peppermint, and other EOs against bacteria, and the results show there is a synergistic effect [97]. Some of these EOs will be discussed in the following sections.

### 8.1 Lavender essential oil

The lavender EO is used in traditional medicine as well as in cosmetic products; this oil is believed to have sedative, anti-inflammatory, and antimicrobial effects [99]. Lavender EO shows a synergistic effect when combined with piperacillin antibiotic against beta-lactamase-producing *Escherichia coli* under study with fractional inhibitory concentration (FIC) index between 0.26 and 0.5 [97]. This finding shows that it's possible to use the lavender EO as an agent in modifying the antibiotic resistance [97]. Another study which aimed to compare the antimicrobial efficacy of four types of lavender oil on MSSA and MSRA shows that by direct contact the oil inhibits the growth of these microbes [100]. Fusidic acid is one of the compounds within this oil which gives it the antimicrobial activity, the mechanism of which is to cause bacterial cell damage by reducing synthesis of proteins [101].

### 8.2 Cinnamon bark essential oil

The cinnamon bark EO can be obtained from different parts of the tropical evergreen tree, which is important for human health and agriculture uses [102]. Previously, a study reported that a combination of cinnamon bark EO with piperacillin resulted in a synergistic relationship with FIC $\leq 0.5$, and this result indicates the possibility of using cinnamon bark EO as a resistance-modifying agent against MDR bacteria [97, 103]. Cinnamon bark oil contains cinnamaldehyde which is one of the compounds that inhibit the activity of amino acid decarboxylase; this compound with others within the oil gives this oil the ability to inhibit some pathogenic bacteria [104].

### 8.3 Peppermint essential oil

Peppermint EO is significant in inhibiting the microbial growth and increasing the shelf-life of food by preventing food spoilage [105]. Combination of piperacillin and peppermint EOs with FIC in the range 0.26–0.5 was found showing a synergistic effect that is absent in 31 other combination pairs that were studied, indicating a promising alternative to reduce the use of antibiotic and achieve the reverse beta-lactam antibiotic resistance [91]. The antibacterial activity for this oil is associated with menthol and ethyl acetate in high concentrations [106].

## 9. Future perspectives

Research about the reversal antibiotic resistance is important to preserve the healthy microbial ecosystem in the human host. It is imperative to understand the cause of antimicrobial resistance and to find solutions to alleviate the present

situation. As discussed above, combination therapy between EOs and antibiotic provides a promising alternative to mitigate MDR bacteria, possibly by disrupting the bacterial cell wall. Although EOs have been proven to be useful for mitigating MDR bacteria spread, there is still much to be done in terms of the combination stability, selectivity, definite mechanism of action, chemical nature, availability of these products in human body, optimal dose, and adverse reactions as a treatment. These gaps need to be taken into consideration before applying EOs for clinical usage. In addition, there is also a need for animal study and human trials in the future, if one intends to employ EOs as a therapeutic option in medical settings.

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## Conflict of interest

The authors declare they have no competing interests.

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