Chapter 3

Antibacterial Properties of Essential Oil in Some Indonesian Herbs

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.78033

Abstract

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

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

1. Introduction

Essential oil has been used since ancient times as perfumery in ritual ceremony and folk medicine by native countries. Together with the time passed, essential oil utilizing has been developed not only in folk medicine but also in food industry as flavoring, cosmetics as fragrance, and additives [1]. These oil have many biological activities especially rich in mono and sesquiterpene which are known as antimicrobial. Indonesia has around 28,000 plant species and was predicted that more than 7000 species have potency as medicinal plants. Unfortunately just less than 300 species were used in pharmaceutical industry, whereas the rest still need the evidence [2]. One of the important compounds in Indonesian herbs is essential oil, a volatile oil from the plants composed of many phenolic compounds and responsible for strong antibacterial effect.

Essential oil is aromatic oily liquid obtained from different parts of the plants (root, bark, leaf, bud, flower, fruit, and seeds). This oil name bears the name of the plant species from which it is derived. This oil has a sharp smell that is produced as secondary metabolite and variable mixtures of terpenoid, monoterpene (C5), sesquiterpene (C15), and diterpene (C20). Another functional groups present in the molecule formed another molecules such as aldehydes, ketones, acids, lactones, etc. The amount of components varies from approximately 10–100, although usually the main part of the oil is composed of only a few components [3].

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

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

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

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

1.1. Antibacterial activity of essential oil

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

http://dx.doi.org/10.5772/intechopen.78033

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

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

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

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

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

2. Experiment

2.1. Material and methods

Five essential oils were used which are as follows: Lime oil (*Citrus aurantifolia*), Lemon oil (*Citrus limon* (L) Burm), Eugenia oil (*Eugenia uniflora*), Foeniculi oil (*Foeniculum vulgare* Mill), and Spilanthes oil (*S. paniculata*). Microorganisms were obtained from the Laboratory of Microbiology Faculty of Biology Universitas Kristen Satya Wacana. The strains used for the study were *Staphylococcus aureus* FNCC 0047, Bacillus subtilis ATCC 6051, *B. cereus* FNCC 0057, *Escherichia coli* IFO0091, Pseudomonas cepacia FNCC 0063, and *P. aeruginosa* FNCC 0063, respectively.

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

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

2.1.1. Isolation of essential oil

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

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

2.1.2. Direct bioautographic test

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

2.1.3. Determination of antibacterial activity

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

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

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

2.1.4. Data analysis

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

2.2. Result and discussion

2.2.1. Essential oil

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

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

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

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

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

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

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

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

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

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

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

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

2.2.1.2. Eugenia uniflora L. (Surinam cherry)

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

(4.16%), β–elemen (4.08%), and caryophyllene (2.97%). According to the report of [42], five dominant compounds of 16 compounds in the essential oil of *E. uniflora*: caryophyllene (8.812%), spathulenol (7.712%), isolongifolene (6.621%), viridiflorol (5781%), and alloaromadendrene (5.568%). Ref. [43] also found that the yield of EuEO was 0.3%, 32 components were identified in this oil by GC–MS, constituting 92.65% of the total mixture. EuEO was shown to be rich in oxygenated sesquiterpenes (62.55%) and sesquiterpene hydrocarbons (29.37%). Curzerene was the major constituent (47.3%), followed by *γ*-elemene (14.25%) and trans-*β*-elemenone (10.4%), (E) caryophyllene (4.33%), and atractylone (2.38%). Spathulenol and viridiflorol are also found in this oil but only in small amount less than 0.2%. Different with [44] indicated atractylone (26.78%) and curzerene (17.96%) as major constituents of *E. uniflora* essential oil. The main constituent of essential oil of *E. uniflora* may vary but the dominant classes are sesquiterpenes. *E. uniflora* has known antihypertensive [45], antitumor [46], and antinociceptive properties [47], and it shows good performance against microorganism. The important issue is that this essential oil is also used in industrial perfumery [48].

2.2.1.3. Foeniculum vulgare Mill. (fennel)

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

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

2.2.1.4. Spilanthes paniculata wall (toothache plant)

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

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

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

2.2.2. Antimicrobial activity

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

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

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

2.2.2.1. Lime and lemon essential oil

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

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

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

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

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

2.2.2.2. Eugenia uniflora L (Surinam cherry) essential oil

The essential oil was collected from leaf; the oil has yellowish white color, unidentified odor, and the aroma is quite difficult to express it. This oil also shows good antibacterial activity because in low concentration give medium to strong level MIC in 200–600 μg. The profile of essential oil composed of **spathulenol (12.03%),** dodecanol (11.78%), dodekanal (4.16%), β–elemen (4.08%), and *caryophyllene* (2.97%). The main constituent of this essential oil has big variation; there are no specific compounds in high amount. Antibacterial activity of the essential oil of *E. uniflora* showed the medium level at 200 μg and strong level at 300 μg against gram positive bacteria, whereas 300 μg for medium level and 600 μg for strong level against gram negative bacteria. Five major components of this oil have key role to show the antibacterial activity. Spathulenol, caryophyllene, alpha/beta pinene, humulene, and eugenol contributed to antibacterial activity of the essential oils [68–70]. It is also possible that the minor components might be involved in some type of synergism with the other active compounds [71]. Essential oil of several species of *Eugenia* also demonstrated antibacterial activity. Beta-Caryophyllene, spathulenol, 5-hydroxy calamenene, Bisabolene, caryophyllene, Farnesol, Selinene, Germacrene, and elemene β–elemen are important compounds in Eugenia oil [72].

2.2.2.3. Foeniculum vulgare Mill (fennel) essential oil

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

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

2.2.2.4. Spilanthes paniculata wall (toothache plant) essential oil

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

Genus *Spilanthes* is one of the oil-rich genera belonging to the family Asteraceae, although only a few species have been explored for their essential oils [79]. The composition of the essential oil is very variable, suggesting the existence of a high number of chemotypes. From the flower heads of *S. acmella*, volatile constituents were characterized [80]. In the same plant, the presence of a mixture of C22 to C35 hydrocarbons was also reported [77, 81]. Seven components from the essential oil have been identified, including the sesquiterpene caryophyllene oxide, caryophyllene, limonene, and myrcene as significantly dominating compounds of the essential oil from the inflorescences of *S. calva* DC [82].

2.2.3. Discussion

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

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

3. Conclusions

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

Conflict of interest

The authors declare that there is no conflict of interest.

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