Antimicrobial activity of essential oil from Indonesian medicinal plants against food-borne pathogens

by Titik Budiati

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Abstract. The aim of the study was to evaluate antimicrobial activity of essential oil from local plants or plants cultivated in Indonesia against three food-borne pathogens, *Salmonella* Thypimurium, *Bacillus cereus* and *Staphylococcus aureus*. A total 6 essential oil extracted from ginger (*Zingiber officinale*), turmeric (*Curcuma longa*), candlenut (*Aleurites moluccanus*), lemongrass (*Cymbopogon citratus*), clove bud (*Syzygium aromaticum*), and galangal (*Alpinia galanga*). By using disc diffusion assay, the highest antimicrobial activity against *S.* Tyhimurium, *B. cereus* and *S. aureus* was shown by clove bud oil (3.67 $\pm$ 0.58 mm), lemongrass oil (3.58 $\pm$ 0.14 mm) and clove bud oil (5.25 $\pm$ 0.75 mm), respectively. By using Minimum Inhibitory Concentration (MIC), the concentration of essential oil to inhibit the growth of *S.* Tyhimurium, *B. cereus* and *S. aureus* was found on clove bud oil for 0.39 %, lemongrass oil for 1.56 % and clove bud oil for 0.78 %, respectively. Out of the essential oil tested, clove bud oil and lemongrass oil showed promising antibacterial activity against *S.* Thypimurium, *B. cereus* and *S. aureus.*

1. Introduction

In developed and developing countries, food-borne diseases were considered by pathogenic bacteria as the primary causes. Assuring food safety, the addition of chemical agents has been used in food industries. However, it becomes a concern due to the adverse effect on human health. The consumers' preference tends to choose the food products with the fewer chemicals and more natural preservatives. Essential oils were potential alternatives to assure food safety by inhibiting food-borne pathogenic bacteria in food. Essential oils has been studied to show antimicrobial activity on pathogenic bacteria [1]. The structure, functional groups, and composition of the essential oils play an important role in determining their antimicrobial activity [2; 3; 4]. However, the data regarding the antimicrobial activity of essential oils from Indonesian medicinal plants, such as ginger oil (*Zingiber officinale*), turmeric oil (*Curcuma longa*), candlenut oil (*Aleurites moluccanus*), lemongrass oil (*Cymbopogon citratus*), clove bud oil (*Syzygium aromaticum*), and galangal oil (*Alpinia galanga*) to against food-borne pathogens is still limited. Food-borne pathogens continue to be a major public health problem worldwide. These also contribute to negative economic impacts because of the cost of surveillance investigation, prevention and the treatment of illness [5]. The pathogenic bacteria which has been known to cause public health problem were *Salmonella* Thypimurium, *Bacillus cereus and Staphylococcus aureus*

S. Thypimurium is Gram-negative bacteria, rod-shape that causes salmonellosis. These pathogenic bacteria caused enteric fever and acute gastroenteritis within incubation period of 6-72 hours [6]. *Salmonella* is the most frequently isolated as foodborne pathogen, and is predominantly found in eggs, dairy products and poultry [5]. Other food-borne pathogen is by *B. cereus* which has resistant endospores as a cause of intoxication [7]. The spore may be present in raw and cooked foods that can survive in high temperature during food processing [8]. *B. cereus* is Gram-positive bacteria causing an emetic or diarrheal syndrome [6]. Other food-borne disease is Staphylococcal food-borne disease (SFD). SFD is one of the most common food-borne diseases worldwide which caused by the contamination of *S. aureus* enterotoxins in food [9]. *S. aureus* is Gram-positive bacteria that does not form spores but can cause the contamination during food preparation and processing [9]. This pathogenic bacteria can grow in the range of 7° to 48.5 °C, pH of 4.2 to 9.3 and sodium chloride concentration up to 15% NaCl [9]. The present study was conducted to evaluate antimicrobial activity of essential oil from local plants or plants cultivated in Indonesia against three food-borne pathogens, *S.* Thypimurium, *B. cereus* and *S. aureus*.

2. Material and Methods

2.1 Essential oil

A total 6 Indonesian medicinal plants extracted from ginger (*Zingiber officinale*), turmeric (*Curcuma longa*), candlenut (*Aleurites moluccanus*), lemongrass (*Cymbopogon citratus*), clove bud (*Syzygium aromaticum*), and galangal (*Alpinia galanga*). The pure essential oils were purchased from Nusaroma (Indonesia).

2.2 *The strains of microorganisms*

Antimicrobial activity of the essential oil was investigated in 3 pathogenic bacteria *strains: S. enterica* serovar Typhimurium ATCC 14028, *B. cereus* ATCC 14579, *S. aureus ATCC 25923. S.* Typhimurium was cultured on Xylose Lysine Deoxycholate (XLD) agar (MerckKGaA, Damstads, Germany) for 24-48 hours at 37°C. Well colonies were appeared as red colonies with black centers and the agar will turn red. The colonies were purified by streaking onto tryptic soya agar (TSA) (MerckKGaA, Damstads, Germany). *Bacillus cereus* was cultivated on Brilliance *B. cereus* (BBC) agar (Oxoid, Baringstoke, Hampshire, United Kingdom) for 24 hours at 37°C. The colonies were appeared as blue colonies and were purified onto TSA bu streaking method. *S. aureus* was streaking on Manitol Salt Agar (MSA) (Himedia, India) and incubated at 37°C for 24-48 hours was appeared as yellow colonies. The colonies were purified onto TSA by using streaking methods.

2.3 Disc diffusion assay

Antimicrobial activity was evaluated using disc diffusion assay as Bauer [10]. Cultures were grown overnight in Triptic Soy Broth (TSB, Merck KGaA, Darmstadt, Germany) and incubated at 37 °C. The overnight cultures were diluted to a turbidity of 0.5 on McFarland scale. The cultures were streaked on Mueller Hinton Agar (Oxoid, Baringstoke, Hampshire, United Kingdom) plates using a cotton swab. After 30 min, 20 µL essential oil was dropped on the disc that has been placed on the plates and were incubated at 37 °C for 18–24 h. After the incubation period, the inhibition zones was measured in 4 different directions and recorded as milimeter.

2.4 Minimum Inhibitory Concentration (MIC)

Cultures were grown overnight in Triptic Soy Broth (TSB, Merck KGaA, Darmstadt, Germany) and incubated at 37 °C. The overnight cultures were diluted to a turbidity of 0.5 on McFarland scale. The cultures were dropped onto different concentration of essential oil. The first concentration was measured in 5 mL of essential oil. In the remaining concentration was added 2,5 mL Mueller Hinton

Broth (Oxoid, Baringstoke, Hampshire, United Kingdom) and was reduced gradually for 50%. Finally, each tube was added 20 µL of the bacterial suspension (10^6 CFU/mL). The tubes were then incubated for 24 hours at a temperature of 37 °C. The aliquot was dropped on Mueller Hinton Agar and incubated for 24 hours at 37 °C. The lowest concentration at which colonies grows occurred was taken as the MIC value (%). MIC values shown are mean values of three measurements. All experiments were performed in three replicates.

2.5 *Statistical Analysis*

The differences in MIC values of *S.* Typhimurium, *B. cereus*, *S. aureus* between different essential oils were determined by using one-way ANOVA (SPSS version 13.0) at a significance level of $P < 0.05$.

3. Results and Discussions

Minimum Inhibitory Concentration (MIC) of *S.* Typhimurium, *B. cereus* and *S. aureus* on six different essential oils was shown in table 1. By using disc diffusion assay, the three highest antimicrobial activity against *S.* Typhimurium was shown by clove leaf oil, lemongrass oil and ginger oil for 3.67 ± 0.58 mm, 2.92 ± 0.14 mm and 2.29 ± 0.36 mm, respectively. Statistical analysis showed significant difference ($P > 0.05$) between MIC value to inhibits the growth of each pathogenic bacteria.

Table 1. Inhibition zone (IZ) and Minimum Inhibitory Concentration (MIC) of food-borne pathogenic bacteria *S.* Typhimurium, *B. cereus* and *S. aureus* on six different essential oils

Food-borne pathogen bacteria	Ginger oil		Turmeric oil		Candlenut oil		Lemongrass oil		Clove bud oil		Galangal oil	
	IZ (mm)	MIC (%)	IZ (mm)	MIC (%)	IZ (mm)	MIC (%)	IZ (mm)	MIC (%)	IZ (mm)	MIC (%)	IZ (mm)	MIC (%)
Salmonella Typhimurium	2.29 ± 0.36	6.25 ± 0^b	1.75 ± 0	12.5 ± 0^c	No inhibiton	No inhibiton	2.92 ± 0.14	6.25 ± 0^b	3.67 ± 0.58	0.39 ± 0^a	1.17 ± 0	6.25 ± 0^b
B. cereus	1.92 ± 1.23	6.25 ± 0^d	1.63 ± 0.57	12.33 ± $0{,}29^e$	No inhibiton	No inhibiton	3.58 ± 0.14	1.56 ± 0^c	3.25 ± 0.9	0.78 ± 0^b	0.75 ± 0.43	0.39 ± 0^a
S. aureus	4.33 ± 0.58	1.55 ± 0.05^b	1.88 ± 0.29	3.13 ± 0^c	0.5 ± 0	23.33 ± 2.89^e	1.75 ± 1.56	6.25 ± 0^d	5.25 ± 0.75	0.78 ± 0^a	1.92 ± 0.38	0.39 ± 0^a

Note : a,b = different alphabet means significant different at P<0.05 in the same row of MIC value

By using MIC, the highest antimicrobial activity of essential to inhibit the growth of *S.* Typhimurium was found on clove bud oil for 0.39 ± 0%. It was followed by three essential oils (lemongrass oil, ginger oil and galangal oil) that showed the same MIC value (6.25 ± 0%). The result was similar with Dorman and Deans [2]. Dorman and Deans reported that clove oil composed by volatile oil compounds which can inhibits *Salmonella* Pullorum and *S. aureus*. These were mainly carvacrol, δ-3-carene, eugenol and cis/trans citral. Other compounds were identified as carvacrol methyl ester and geraniol. There was only borneol which did not has microbial activity on *S.* Pullorum and carvacrol methyl ester on *S. aureus* [2]. Other study has reported that the component with phenolic structures, such as carvacrol and thymol have synergetic antimicrobial activity against major foodborne pathogens such as *S.* Typhimurium by attacking the bacterial membrane [11] and disrupting the cytoplasmic membrane of Gram-negative bacteria [12]. By altering the fatty acid composition of the bacterial membrane, carvacol might promote the sub-lethal injury to the cells [12]. However Moon reported that eugenol was not enough to show bactericidal effect to *S.* Typhimurium

[11]. Other compounds were reported has antimicrobial effect on *Salmonella* sp. and *S. aureus*. These were geranyl acetate, cis-hex-3-en-1-ol, menthone, α-pinene, β-pinene, terpinen-4-ol, α-terpineole [2].

By using disc diffusion assay, the highest antimicrobial activity against *B. cereus* was shown by lemongrass oil for 3.58 ± 0.14 mm. This was followed by clove bud oil and ginger oil for 3.25 ± 0.9 mm and 1.92 ± 1.23 mm, respectively. It was also observed on the minimum concentration inhibitory data as shown in table 1. Clove bud oil and lemongrass oil showed has high antimicrobial activity for 0.78 ± 0% and 1.56 ± 0% , respectively. The antimicrobial activity of the compounds in clove bud oil and lemongrass oil showed relatively higher on Gram-positive bacteria than those of Gram-negative bacteria. These results were similar to other studies. Moon [11] and Burt [3] reported that Gram-positive bacteria are more susceptible to carvacrol and thymol than Gram-negative bacteria. However α-terpineole reported to be more effective to inhibit Gram-negative (*S.* Typhimurium) than those Gram-positive bacteria (*S. aureus* and *B. cereus*) [3]. These compounds were composed by hydroxyl group in phenolic structure which having antibacterial properties to inhibit food-borne pathoges as reported by Dorman and Deans [2]. The mechanism of action generally was considered by disturbing the cytoplasmic membrane and the proton motive force (PMF), active transport, electron flow and coagulation of cell contents [3]. Futher, the alkyl substitution into the phenol nucleus might enhance the activity of antimicrobial by altering the distribution ratio between the nonaquaeous and aqueous phases (including bacterial phases) which reduce the surface tension or altering the species selectivity [2]. Moreover, the hydrophobicity of essential oils enables to make the partition on the lipids of bacterial membrane and mitochondria which disturb the structure, render the membrane to be more permeable and promote the leakage of ions and other cell contents [3].

Lemongrass oil composed by terpenes (carvacrol, citral, linalool, and geraniol), phenolics (flavonoids and phenolic acids) and terpenes which have been effective as antimicrobial against pathogenic bacteria and deteriorative bacteria [13]. However the presence of citral and geraniol in lemongrass oil were more effective to inhibit *B. cereus* compared to those of *S. aureus* [14]. This study also observed the same results that antimicrobial activity of lemongrass oil was stronger on *B. cereus* than those of *S. aureus* as shown in table 1. The previous studi reported that the antibacterial activity of lemongrass oil components depended on the compositions, concentration, and cell target sites [14]. This study also observed that antimicrobial activity of components in lemongrass oil was less to against Gram-negative bacteria compared to those Gram-positive bacteria. In similar, Aiemsaard reported that citral more effective against Gram-positive bacteria (*S. aureus*, *S. agalactiae*, and *B. cereus*) than Gram-negative bacteria (such as *E. coli*) [14]. Citral and geraniol has been reported as the major bioactive components of lemongrass oil [15].

This present study found that inhibition zone of ginger oil on *S. aureus* was relatively larger than those of *S.* Typhimurium and *B. cereus* as shown in table 1. Inhibition zone on *S. aureus* was observed for 4.33 ± 0,58 mm. However the inhibition zone of. *S.* Typhimurium and *B. cereus* was found 2.29 ± 0.36 and 1.92 ± 0,36, respectively. Ginger oil has been reported to be composed by α-curcumene, α-zingiberene, β-bisabolene, β-sesquiphellandrene, cineole, 2,2-dimethyl-3-methylenenorbornane and rosefuran epoxide [8; 9]**.** Nader reported that extract ginger oil was contained by alkaloid, glicosides, terpenoid, flavonoid, phenolic [18]. Stoyanova [17] revealed that antimicrobial activity of the components in ginger oil was very weak on the Gram-negative and Gram-positive bacteria. Bassole and Juliani [4] observed that the low antimicrobial activity of ginger was promoted by the small amount of aldehydes and phenols constituents (3.804). However the large amount of terpene alcohols (32.508%) and terpene hidrocarbons (59.416%) was measured by previous study which may cause the weaker antimicrobial antivity [4].

Galangal oil was observed to be effective more on Gram-positive than those of Gram-negative. This present study found that the antimicrobial activity in galangal oil was less compared to clove bud oil, lemongrass oil, and ginger oil. This is in similar that previous study. Bassole and Juliani [4] revealed that the relatively low antimicrobial activity of galangal oil was related to their composition of chemical. Galangal oil was composed by cineole, 4-allylphenyl acetate, α-farnesene, (2,6-dimethylphenyl)borate and α-pinene [16]. This means that galangal oil composed by high terpende alcohols, ketones, esters and terpene hydricarbons but low aldehydes and phenols. The study revealed that ketones or esters, acetate had much weaker antimicrobial activity [4].

In similar, antimicrobial activity of turmeric oil was found to be weaker than those of clove bud oil, lemongrass oil dan ginger oil. Turmeric oil and galangal oil showed have the same antimicrobial activity to against *S.* Typhimurium, *B. cereus* and *S. aureus*. Turmeric oil was composed by the eucalyptol as the major component [19]. Eucalyptol was grouped as was ketones which had weak in antimicrobial activity [4]. Other compounds were α-pinene (1.50%), β-phellandrene (2.49%), β-pinene (3.57%), limonene (2.73%), 1,3,8-p-menthatriene (1.76%), ascaridole epoxide (1.452%), 2-methylisoborneol (2.92%), 5-isopropyl-6-methyl-hepta-3, dien-2-ol (2.07%) [19].

Candlenut oil showed low antimicrobial activity to inhibit *S. aureus* but it was not effective to inhibit *S.* Typhimurium and *B. cereus*. The previous study found that candlenut oil composed by flavonoids, cardiac glycosides, saponins, phenolic compounds, phytosteroids, tannins, triterpenoids, anthraquinones, and alkaloids [13; 14; 15; 16]. Mpala *et al.* [20] reported that candlenut (*A. moluccanus*) extracts to block microbial triggers of three bacteria (*Proteus* spp., *K. pneumonia, S. pyogenes*). In this present study, candlenut did not showed antimicrobial activity to inhibit *S.* Typhimurium and *B. cereus* which has not being reported by other researchers.

4. Conclusion

Clove bud oil showed the highest antimicrobial activity to inhibit the growth of S. Typhimurium, *B. cereus* and *S. aureus*. It was followed by lemongrass oil and ginger oil. These essential oils showed promising antimicrobial activity against *S.* Typhimurium, *B. cereus* and *S. aureus*.

5. Acknowledgment

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