Indonesian Journal of Life Sciences Vol. 01 | Number 02 | September (2019)
http://journal.i3l.ac.id/ojs/index.php/IJLS

RESEARCH ARTICLE

Synergistic Antibacterial Activities of Ginger and Lemongrass Essential Oils as an Alternative Prevention to Food-Borne Disease

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ABSTRACT

Background: Lemongrass and ginger are traditional food ingredients in Asian countries, including Indonesia, Thailand, India, and Malaysia. Although their single essential oil has been assessed for its antibacterial activities, no report has been done for their combination. **Material and methods:** Our study evaluated single and combination of these herbs for their antibacterial properties against food-borne bacteria *E. coli* (NEB® catalog No. C2989K), *B. subtilis* (ATCC 6633), *S. typhi* (ATCC 14028) and *S. aureus* (InaCC B4). Essential oil of lemongrass and ginger were obtained by steam distillation and their antimicrobial were evaluated using disk diffusion assay with chloramphenicol as the standard antibiotic. Synergistic activity was assessed using the combination of materials at two or four-fold dilution from their respected MIC value. **Results:** We confirmed that single lemongrass and ginger essential oils inhibited the bacteria growth with MIC value of about 1-5% and 2.5-5%, respectively. Moreover, their synergism activities were observed when both were mixed at two-fold dilution from their respective MIC. **Conclusion:** We conclude that the combination of the ginger essential oils and lemongrass essential oils may have potential as a natural preservative to prevent food-borne diseases.

Keywords: Ginger essential oil; Lemongrass essential oil; Antibacterial activity; Synergistic effect

INTRODUCTION

Food-borne diseases (FBD) have become major threats for human society, not only in the developing countries but also in other developed countries. According to the data released by WHO regarding the burden of FBD in 2010, about 600,000,000 cases of foodborne illnesses occurred world widely, in which about 420,000 foodborne deaths were estimated just in 2010 (Havelaar et al., 2015). The subregional grouping SEAR B (South East Asian Region B), which includes Indonesia, Sri Lanka, and Thailand, experienced 685 Disability Adjusted Life Years (DALYs) per 100,000 populations, in which approximately 50% were caused by diarrheal disease agents, such as *E. coli, Campylobacter spp., Shigella spp.,* and *V. cholerae*. Meanwhile, another

47% were caused by invasive infectious disease agents such as *S. typhi, L. monocytogenes,* and *T. gondii*. This situation requires careful attention, not to mention that food is one of the basic needs of human living.

One of the possible approach for this problem is to find preventive medicine for FBD by exploring Indonesia's natural resources. Ginger (*Zingiber officinale)* and lemongrass (*Cymbopogon citratus*) are plants which typically found in Indonesia and mainly used as the culinary herbs. Multiple researches have demonstrated the antibacterial activity of ginger (Malu et al., 2009; Ekwenye and Elegalam, 2005; Sebiomo et al., 2011) and lemongrass (Naik et al., 2010; Hammer, Carson and Riley, 1999; Prabuseenivasan, Jayakumar and Ignacimuthu, 2006). However, there is no study to date, that compare the antibacterial activity of each extract and the combination of these extracts.

The combination of two or more herbal extracts may result in synergism, additives or antagonism. Study by Kamatou et al. (2006) showed that combination between *Salvia chamelaeagnea* and *L. leonurus* led to increased antibacterial activity compared to their individual antibacterial activity. Additionally, another study also reported the synergistic antimicrobial activity between rosemary and lemon iron bark extracts and also galangal and lemon iron bark extracts (Weerakkody et al., 2011). Due to the synergistic effect, the concentration of each extract needed to show antibacterial activity would be reduced, and may be used to prevent FBD without compromising the taste of the food itself.

This study aims to evaluate the antimicrobial activity from each extract of ginger and lemongrass together with their combinations towards several types of bacteria which are commonly cause FBD. These bacteria are *Salmonella typhi, Escherichia coli, Bacillus subtilis,* and *Staphylococcus aureus*. Based on the result, this study would open a new perspective of synergistic antimicrobial potential of Indonesian traditional herbs for prevention of food-borne diseases.

MATERIAL AND METHODS

Materials

Ginger and lemongrass were obtained from traditional market in North Jakarta, Indonesia. Polysorbate 80 (Tween 80) and Mueller-Hinton Broth were purchased from Merck (Germany). Chloramphenicol were purchased from Bio Basic (Canada). *E. coli* (NEB® catalog No. C2989K)*, B. subtilis* (ATCC 6633)*, S. typhi* (ATCC 14028) and *S. aureus* (InaCC B4) were generously provided from the bacterial collection of Indonesia International Institute for Life Sciences, Indonesia. Whatman paper no. 1 was obtained from Whatman (Sigma-Aldrich). Bacteriological agar was obtained from Becon (UK).

Ginger extraction

Ginger was cleaned and cut into thin pieces. Thin pieces of ginger were placed in a steam apparatus, which was heated using an electrical heater. Vapor of essential oil was condensed and automatically separated with water condensate due to difference in density. Essential oil of ginger was collected and used for further testing.

Lemongrass extraction

Lemongrass was dried in room temperature for several days before thinly cut. Similarly, thin pieces of lemongrass were placed in a steam distillation apparatus to extract lemongrass essential oil.

Antibacterial assay

Antibacterial activity of ginger essential oil (GEO) and lemongrass essential oil (LEO) was

assessed separately using disk diffusion method, as discussed elsewhere (Burt, 2004). The fractions and essential oils were diluted until the intended concentrations (5%, 10% and 15%) in 5% of Tween-80. Mueller-Hinton Agar (MHA) was used as the media, and was prepared by mixing 2.1% Mueller-Hinton Broth (MHB) and 1.7% bacteriological agar in distilled water before sterilization using autoclave at 121^0C for 15 minutes. Whatman filter papers were cut into circled papers with a size of 5 mm in diameter prior sterilization process. Each paper disk was immersed in the tube containing tested materials at least 1 hour prior to assay. Immersed disks were separately placed in petri dishes that had been inoculated with 100 µl of the cultures of either *E. coli, B. subtilis, S. typhi* or *S. aureus* that was equal to 0.5 MacFarland standard (1.5×10^8 CFU/ml). Chloramphenicol stock solution (25 mg/ml) was prepared in ethanol and diluted to a working concentration of 250 µg/ml with distilled water. Chloramphenicol was also tested accordingly as a positive control. Petri dishes were then incubated at 37 °C for 24 hours. All of the antibacterial assay were performed in quadruplicate. The inhibition zone was observed from each of test material and the diameter of inhibition was measured.

Determination of minimum inhibitory concentration (MIC)

MIC was determined by using disk diffusion method as explained by Wiegand et al. (2008) and Ganjewala et al. (2014). The essential oils were diluted at concentration of 0.5%, 1%, and 2.5% in 5% of Tween-80. 5 mm diameter of Whatman filter papers were immersed in each tube containing different concentration of extracts for at least 1 hour prior to assay. The immersed paper discs were placed over the MHA plates seeded with 100 µl of the cultures of either *E. coli, B. subtilis, S. typhi* or *S. aureus* that was equal to 0.5 MacFarland standard (1.5×10^8 CFU/ml). Each experiment was performed in quadruplicate. Thereafter, plates were placed at 37 °C for 24 hours. The diameter of inhibition in each test materials' concentration was measured and recorded. The MIC was defined as the lowest concentration of test material which inhibit the growth of the bacteria.

Synergistic assay

Synergism of the test materials was evaluated using disk diffusion assay by mixing each test materials at concentration of two and four times below their MIC for each type of bacteria. The synergistic effect was determined by the presence of inhibition zone at concentration lower than the individual's materials MIC.

Statistical analysis

The data recorded during the investigation were statistically analyzed by one-way ANOVA technique. The P values of 0.05 was used to determine the significance level.

RESULTS AND DISCUSSION

Ginger and lemongrass are two of the common herbs used in many household activities in Indonesia, including for culinary purposes and traditional herbal medicines. Both of them were often combined in different types of food and drinks to obtained favor, taste or smell. In this study we have performed antibacterial testing of GEO, LEO and the combination between GEO and LEO (CEO) against four types of food-borne bacteria, which are *S. aureus*, *B. subtilis*, S. *typhi* and *E. coli*. The antibacterial activities were compared with standard antibiotic chloramphenicol, which revealed that all bacteria used were sensitive with the standard antibiotic.

Both GEO and LEO were obtained by steam distillation of fresh ginger and dried lemongrass, respectively. The extraction yield

of fresh ginger and dried lemongrass was 0.125 % and 0.2 %, respectively. Each essential oil was diluted with 5% Tween 80 to give the predetermined concentration before testing.

GEO and LEO showed antibacterial activity against all four types of food-borne bacteria, as shown in Table 1. In order to determine the MIC (minimum inhibitory concentration) of GEO and LEO, the concentration of each essential oil was lower down two fold until no zone of inhibition observed. As a positive control, the zone of inhibition of chloramphenicol at concentration of 250 µg/ml was also measured (Table 1).

Table 1. Zone of inhibition of ginger essential oil, lemongrass essential oil and chloramphenicol against four types of food-borne bacteria

Zone of inhibition (mm)				
Ginger essential oil	***S. typhi***	***E. coli***	***B. subtilis***	***S. aureus***
0.5%	ND	ND	ND	ND
1%	ND	ND	ND	ND
2.5%	ND	6.75±0.25	7±0	6.75±0.48
5%	6.75±0.25	7±0	7.25±0.25	7±0
10%	7.25±0.48	7±0	10.75±0.75	7.75±0.25
15%	7±0.41	7.75±0.25	11±0.71	10.75±0.75
Lemongrass essential oil	***S. typhi***	***E. coli***	***B. subtilis***	***S. aureus***
0.5%	ND	ND	ND	ND
1%	ND	6.5±0.29	7.25±0.25	ND
2.5%	7±0	6.75±0.25	7.5±0.96	ND
5%	7±0	7.5±0.29	8.5±0.29	9.75±0.25
10%	8±0	8±0	10.75±1.1	17.75±1.44
15%	9±0.58	8.5±0.29	13.25±1.25	16.5±1.06
Chloramphenicol	***S. typhi***	***E. coli***	***B. subtilis***	***S. aureus***
250 µg/ml	15.5±0.87	11.5±0.87	16±0.58	13.5±0.5

ND: Not Detected; Value are given as mean of quadruplicate ± SE.
The zone of inhibition including 5 mm paper disk.

GEO already showed antibacterial activity for *E. coli, B. subtilis* and *S. aureus* at concentration of 2.5%, while the activity against *S. typhi* was observed at concentration of 5%. LEO exhibited antibacterial activity for *E. coli* and *B. subtilis* at concentration of 1%, *S. typhi* at concentration of 2.5% and *S. aureus* at concentration of 5%. This result might indicate that LEO was more potent than GEO in inhibiting the growth of *S. typhi, E. coli* and *B. subtilis*. However, GEO had lower MIC against *S. aureus*, which was 2.5%. Nevertheless, at concentration of 15%, LEO was more effective than GEO, as indicated by larger inhibition zone of LEO against *S. aureus*. The exact reason is not yet understood, but it might be attributed to the proportion of bioactive compound which is responsible for antibacterial effect. LEO has been reported to contain high concentration of citral compared to GEO (Lis-Balchin, Deans and Eaglesham, 1998; López et al., 2017), while the antibacterial effect of citral had been extensively studied (Onawunmi, 1989). Both GEO and LEO displayed concentration-dependent antibacterial activity, as the zone

of inhibition increased as the concentration increased. These data were aligned with the previous report about antibacterial activity of GEO (López et al., 2017) and LEO (Naik et al., 2010).

When compared with chloramphenicol standard, only LEO at concentration of 10% and 15% were exceeded the standard used against *S. aureus* (Table 1). Larger inhibition zone of GEO and LEO at concentration of 15% towards *B. subtilis* and *S. aureus* indicated that both GEO and LEO were more potent on gram positive bacteria, which was consistent with other previous reports (Naik et al., 2010; Gull et al., 2012).

After obtaining the MIC of each essential oil, the synergistic antibacterial activity of GEO and LEO was evaluated against the previous four types of food-borne bacteria. GEO and LEO was mixed at concentration about two and four fold below their respected MIC for each bacteria. The synergistic effect was assessed using disk diffusion method, and the data were recorded as the zone of inhibition (Table 2).

Table 2. Zone of inhibition of individual ginger essential oil, lemongrass essential oil and combined essential oil at concentration below MIC against four types of bacteria

Bacteria	Zone of Inhibition (mm)		
S. typhi	LEO 1%	GEO 2.5%	LEO 1%+ GEO 2.5%
	ND	ND	6.5 ± 0.57
S. typhi	LEO 0.5%	GEO 1%	LEO 0.5%+ GEO 1%
	ND	ND	6.5 ± 0.57
S. aureus	LEO 2.5%	GEO 1%	LEO 2.5%+ GEO 1%
	ND	ND	7.25 ± 0.5
S. aureus	LEO 1%	GEO 0.5%	LEO 1%+ GEO 0.5%
	ND	ND	ND
B. subtilis	LEO 0.5%	GEO 1%	LEO 0.5%+ GEO 1%
	ND	ND	9.5 ± 1
B. subtilis	LEO 0.25%	GEO 0.5%	LEO 0.25% + GEO 0.5%
	ND	ND	ND
E. coli	LEO 0.5%	GEO 1%	LEO 0.5%+ GEO 1%
	ND	ND	7.5 ± 1
E. coli	LEO 0.25%	GEO 0.5%	LEO 0.25% + GEO 0.5%
	ND	ND	ND

ND: Not Detected; Value are given as mean of quadruplicate ± SE.
The zone of inhibition including 5 mm paper disk.
Each data was analyzed using one-way ANOVA with $P < 0.05$.

Based on the result, the combination between GEO and LEO showed synergistic antibacterial effect against *S. typhi*, *S. aureus, B. subtilis* and *E. coli*. At least all two-fold-dilution-CEOs exhibited significant difference compared to the individual essential oils. Interestingly, four-fold-dilution-CEO showed synergistic antibacterial activity against *S. typhi*, which is the Gram-negative bacteria, as shown in Table 2. However, there was no concentration-dependent response observed, as four-fold-dilution-CEO produced similar antibacterial activity with the two-fold-dilution-CEO.

GEO and LEO have been thoroughly investigated for their antibacterial properties. According to the report published by López et al (2017), GEO contained 53.57% sesquiterpenes and 21.87% monoterpenes, with one of the most abundant sesquiterpenes was zingiberene. Due to its tendency to inhibit the growth of Gram-positive bacteria, GEO has been predicted to attack the cell wall of bacteria (Burt, 2004). This may interfere the integrity of the cell wall, allowing water from extracellular to enter the bacteria which ultimately cause the bacteria to swell and burst. In addition, GEO might also alter the bacterial plasma membrane, which explained its inhibitory activity on Gram-negative bacteria. GEO has been suggested to change the permeability and fluidity of bacterial plasma membrane, causing leakage of ion and other cell contents, and lead to death.

Meanwhile, LEO also has been found to be effective in inhibiting Gram-positive bacteria compared to Gram-negative bacteria (Naik et al., 2010). The composition of LEO varies according to the geographical factors, but mostly includes terpenes, alcohols, ketones, esters and aldehydes (Shah et al., 2011). Citral and geranial are two of predominant components in LEO. Similar to GEO, LEO has been demonstrated to alter the stability of bacterial plasma membrane, causing loss of integrity (Burt, 2004). In addition, citral has been proposed to bound to amino groups in the cell wall and cytoplasm, while also inhibit enzymes in cytoplasmic membrane at low concentration (Aiemsaard et al., 2011).

There have been many studies demonstrating the synergistic effect of several essential oils (Ultee et al., 2000; Delaquis et al., 2002; Rivera-Carriles et al., 2005; Lambert et al., 2001). Synergism occurs when the effect of combining two substances or more results in greater than the sum of individual's effect. In this case, GEO and LEO showed synergism by demonstrating inhibitory effect when they were mixed below their respected MIC. It is somehow expected due to their similar mechanism in inhibiting bacterial growth. Generally, the synergistic effect might come from the inhibition of important enzymes and biochemical pathways, alterations in the cell wall, and enhancement of the uptake of other antibacterial agent (Rivera-Carriles et al., 2005). However, the exact synergistic mechanism between GEO and LEO is not within the scope of this report. Nevertheless, their synergistic antibacterial effect most likely is the result of complex synergism between distinct components inside the essential oils.

As ginger and lemongrass are commonly used in the mixture of variety of foods and beverages, knowing their synergistic antibacterial effect might be an additional value to increase the lifetime of foods. Therefore, this combination can be exploited as an alternative in food preservatives to prevent food-borne diseases. Furthermore, this study has opened a new possible essential oils combination with minimum required amount for accountable antibacterial activities.

CONCLUSION

Each individual ginger essential oil and lemongrass essential oil showed antibacterial activities towards *B. subtilis*, *S. aureus, E. coli* and *S. typhi*. Combination of ginger essential oil and lemongrass essential oil at concentration two fold below their respective MIC showed synergistic antibacterial effect for *B. subtilis*, *S. aureus, E. coli* and *S. typhi*. Moreover, combination of ginger essential oil and lemongrass essential oil at four-fold-dilution below their respective MIC also showed synergistic antibacterial effect for *S. typhi*. This result offers an alternative way to use combination of ginger and lemongrass

essential oil as natural preservative to prevent food-borne diseases.

ACKNOWLEDGMENT

The author gratefully thanks Indonesia International Institute for Life Sciences (i3L, Indonesia) for the financial support in this project.

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