



## *Antimicrobial Effect of Lemongrass and Ginger Essential Oils Against Nosocomial Infection Pathogens*

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### KEYWORDS

*Anti-bacterial agents, ginger, lemongrass oil, nosocomial infection, essential oil*

### ABSTRACT

**Background:** According to the 2011 WHO survey on nosocomial infection, 15% patients treated as in-patient had nosocomial infection globally. The causal pathogens had resistance to general antibiotics used by clinicians, therefore it is getting harder to treat patients with these infections. The study aims to investigate the antimicrobial effects of lemongrass dan ginger essential oils against nosocomial infection pathogens. **Methods:** The research is an experimental research. The strain used are *Pseudomonas aeruginosa* ATCC® 27853 and *Acinetobacter baumannii* ATCC® BAA-747. The antimicrobial assays were done triplicate using disk diffusion method and microdilution method. **Results:** From disk diffusion assay, lemongrass essential oil shows zone of inhibition against both pathogens while ginger essential oils doesn't show antimicrobial effect against both pathogens. The mean highest zone of inhibition is 11,33 mm on 100% concentration against *A.baumannii*. From microdilution assay, lemongrass and ginger oil show antimicrobial effect against both pathogens, with lowest MIC detected for lemongrass and ginger essential oils are <0.5µg/ml (against *A. baumannii*) and 32µg/ml (against *P. aeruginosa* and *A. baumannii*). **Conclusion:** Lemongrass and ginger essential oils had antimicrobial effects against nosocomial infection pathogens. Antimicrobial activity of lemongrass oil is more potent than ginger oil on both antimicrobial assay against both pathogens tested.

## INTRODUCTION

According to the 2011 WHO survey on nosocomial infection, 15% patients treated as in-patient had nosocomial infection globally (Khan, Baig and Mehboob, 2017). The frequency was higher on South East Asia (10%) and East Mediterranean (11.8%) regions (WHO, 2002). Increasing incidence of nosocomial infections caused several disadvantages such as increased patients' length of stay at hospital, increased socioeconomical burden, decreased patients' productivity, increased mortality rate, and increased microbes' resistance against antibiotic due to prolonged antibiotic use (Khan, Baig and Mehboob, 2017).

There are several factors affecting the incidence of nosocomial infections, such as infecting agents, patients' susceptibility to antibiotics, environmental factors, and antibiotic resistance (WHO, 2002). One of the most important problem in developing countries regarding this issue is antibiotic resistance. Misuse of antibiotics in human, livestock, and fishery become the main cause of antibiotic resistance. Unnecessary antibiotic prescription by doctors and antibiotic sales without prescriptions still become common practices in the society despite the rules (Hadi *et al.*, 2008). Also due to high demand of poultry products and fishery export commodities, agricultural sectors industries tend to cross the maximum limit of antibiotic usage in Indonesia (Van Boeckel *et al.*, 2015). These lead to massive impact to the microorganism's balance in nature.

Reports stated from researches done in hospitals around Indonesia had stated that the antibiotic resistance problem commonly occurred in nosocomial infection pathogen such as *Staphylococcus aureus* (Adrizain *et al.*, 2018), *Enterobacteriaceae* (Murni *et al.*, 2016; Adrizain *et al.*, 2018), *Pseudomonas aeruginosa* (Murni *et al.*, 2016; Adrizain *et al.*, 2018), *Acinetobacter baumannii* (Murni *et al.*, 2016; Adrizain *et al.*, 2018; Saharman *et al.*, 2018), etc. WHO listed 3 pathogens with critical priority status in the search of new antimicrobial agent, those are carbapenem-resistant *Acinetobacter baumannii* (CRAB), carbapenem-resistant *Pseudomonas aeruginosa*, and *Enterobacteriaceae* family that are resistant to carbapenem and third-generation cephalosporin (Tacconelli *et al.*, 2017).

South East Asia region had greater burden associated with CRAB infection compared to other developing countries. Nosocomial infection incidence caused by CRAB reached 64,91% out of all cases (Teerawattanapong *et al.*, 2018). Approximately 50,5% isolates containing *A. baumannii* taken from ICU in Ciptomangunkusumo hospital, Jakarta were considered as CRAB (Karuniawati, Saharman and Lestari, 2013). Samples derived from in the neonatal unit showed results of 84% CRAB isolates out of all *A. baumannii* samples (Tjoa *et al.*, 2013).

Carbapenem-resistant pathogens infections used to be treated with colistin, but due to frequent use, the case of colistin resistance had also emerged (Antunes, Visca and Towner, 2014; Qureshi *et al.*, 2015). The damaging side effects to the kidney by colistin had also

become challenge in treating patients with carbapenem-resistant pathogens' infections.

## METHODS

### Essential oils and chemicals

Lemongrass essential oil (Pavettia) and ginger essential oil (Nusa Aroma) were diluted to the desired concentrations for both antimicrobial assays using dimethyl sulfoxide (MERK) and Mueller-Hinton broth (MERK).

### Bacterial isolates

Bacterial isolates were provided by Microbiology Laboratory, Faculty of Medicine and Health Science, Atma Jaya Catholic University of Indonesia. The bacterial strains used are *Pseudomonas aeruginosa* ATCC® 27853 and *Acinetobacter baumannii* ATCC® BAA-747. These strains were maintained at 4°C in 15% glycerol and were subcultured in Mueller-Hinton agar at 37°C before the assays.

### Sterility test

Both essential oils and DMSO were inoculated on Mueller-Hinton agar and incubated at 37°C for 18-24 hours for sterility test. All tests showed no growth on the plates.

### Disk diffusion assay

The zone of inhibition was determined using disk diffusion assay with modifications (Aumeeruddy-Elalfi, Gurib-Fakim and Mahomoodally, 2016; Clinical and Laboratory Standards Institute, 2017). The assays were done in triplicates.

Bacterial suspensions were prepared by direct colony suspension method. The turbidity of initial

suspension was adjusted to 0,5 McFarland standard by densitometer (MERK). The bacteria were inoculated on Mueller-Hinton agar plates using sterile cotton swabs (MERK) in medium size.

On sterile petri dish, disks were impregnated with 10 µl of diluted essential oils. The disks were immediately placed on the inoculated plates, then left for 15 minutes in the room temperature to allow the diffusion of the disk contents.

Meropenem 30 µg was used as positive control. Blank disk impregnated with 10 µl DMSO was used as negative control.

### Microdilution assay

The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the essential oils were determined using the microdilution method with modification (Clinical and Laboratory Standards Institute, 2012; Aumeeruddy-Elalfi, Gurib-Fakim and Mahomoodally, 2016). The assays were done in triplicates. Bacterial suspensions were prepared by direct colony suspension method. The turbidity of initial suspension was adjusted to 0,5 McFarland standard by densitometer. Initial bacterial suspensions were 1:100 diluted in Mueller-Hinton broth with 1% DMSO.

Twofold serial dilutions of essential oils were made in a concentration range from 256 µg/ml to 0,5 µg/ml in sterile 96-well plates (Biologix) containing Mueller-Hinton broth with 1% DMSO. A 50 µl of diluted bacterial suspensions was added to each well to give a final concentration of 5 x

10<sup>5</sup> CFU/ml. The inoculated plates were incubated at 37°C for 18-24 hours. MIC was defined as the lowest concentration of tested compound that showed no growth after incubation period.

MBC was determined by plating 100 µl of samples from MIC, MICx2, and MICx4 wells on Mueller-Hinton agar. The plates were incubated at 37°C for 18-24 hours. At the end of incubation period, the lowest concentration with no growth was defined as MBC.

Meropenem 125mg/ml dissolved in Mueller-Hinton broth with 1% DMSO was used as positive control. Uninoculated Mueller-Hinton broth with 1% DMSO was used as negative control.

## RESULT

Results from disk diffusion assay was presented on Table 1. Lemongrass oil showed zone of inhibition against both pathogens tested, but only showed zone of inhibition against *P. aeruginosa* at 100% concentration. Zone of inhibition of lemongrass oil are smaller against *P.aeruginosa*, indicating that the bacteria is more resistant to lemongrass oil compared to *A. baumannii*. Lemongrass oil showed zone of inhibition against *A.baumannii* at all concentrations. Zone of inhibition decreased in diameter along with the decreasing oil concentration on the disk. On the other hand, ginger oil showed no zone of inhibition against both pathogens tested. If we compared zone of inhibition formed by meropenem, both essential oils had smaller zone of inhibition, suggesting lower antimicrobial activity against both pathogens.

**Table 1. Zone of inhibition (mm) of lemongrass and ginger oil against nosocomial infection pathogens**

Disk Content	<i>Pseudomona s aeruginosa</i>	<i>Acinetobacte r baumannii</i>
LGO 100%	7 ±0.4	11.33±1.2
LGO 75%	NA	11±0.8
LGO 50%	NA	10.33±0.5
LGO 25%	NA	8.17±0.2
GO 100%	NA	NA
GO 75%	NA	NA
GO 50%	NA	NA
GO 25%	NA	NA
Meropene m 10 µg	22.33±1.2	20.33±0.9

Abbr. LGO lemongrass oil; GO ginger oil.

Results from microdilution assay was presented on Table 2 and Table 3. Surprisingly, we found that MIC value between lemongrass and ginger oil against *P.aeruginosa* had little differences. MIC of lemongrass oil against *P.aeruginosa* is 16 µg/mL, while MIC of ginger oil against same pathogen is 32 µg/mL.

**Table 2. MIC (µg/mL) of lemongrass and ginger oil against nosocomial infection pathogens**

Oils	<i>Pseudomonas aeruginosa</i>	<i>Acinetobacter baumannii</i>
Lemongrass	16	<0.5
Ginger	32	32

The difference is only two-fold. The results is quite different compared

to the disk diffusion assay results, where ginger oil showed no zone of inhibition against *P.aeruginosa*. Lemongrass oil are considered to be very potent against *A.baumannii*. MIC and MBC of lemongrass oil are <0.5 µg/mL, which are very low.

**Table 3. MBC of lemongrass and ginger oil against nosocomial infection pathogens**

Oils	<i>Pseudomonas aeruginosa</i>	<i>Acinetobacter baumannii</i>
Lemongrass	64	<0.5
Ginger	32	32

## DISCUSSION

Antimicrobial activity of lemongrass oil shown to be more effective toward Gram positive bacteria. Gram negative bacteria generally more resistant towards antimicrobial activity of essential oil. On a study done by Naik et al. about antimicrobial effect of lemongrass oil, the most sensitive bacteria was *S. aureus*, while the most resistant bacteria was *P. aeruginosa* (Naik et al., 2010).

In-depth study about lemongrass oil (Adukwu et al., 2016) stated that antimicrobial effect of citral as a pure component was more potent compared to lemongrass oil. MIC of citral ranged from 0.06% to 0.25%, while MIC of lemongrass oil ranged from 0.25% to 1%. Even thou the differences weren't very significant, but we can conclude that citral is the main component showing antimicrobial activity against pathogenic bacteria.

While there was no zone of inhibition of ginger oil against both

pathogens tested, MIC and MBC of ginger oil showed that ginger oil had quite good antimicrobial activity against both pathogens, but still less superior than lemongrass oil. The results from both methods might be different significantly because of several factors such as growth of microbes, the amount of oil exposure with microbes, oils' solubility, and type of solvent used (Naik et al., 2010). MIC and MBC of ginger oil shown to be more potent compared to ginger methanol extract (Nikolic et al., 2014; Hasan, Danishuddin and Khan, 2015) or crude extract (Hasan, Danishuddin and Khan, 2015). On study by Nikolic et al. (Nikolic et al., 2014), MIC of ginger methanol extract was above 10 mg/mL, while the MBC was 20 mg/mL. This is very high compared to our results. This probably due to the amount of antimicrobial substance extracted from ginger.

Antimicrobial activity of essential oil does rely on the chemical structure of the main antimicrobial component as well, whether it is hydrophobic or hydrophilic. To reach target, antimicrobial substance must go through lipopolysaccharide (LPS) of the cell membrane. Hydrophilic substances could pass the membrane freely compared to hydrophobic substances (Araz et al., 2018). Gram positive bacteria doesn't have LPS, therefore antimicrobial substances could easily pass the membrane. However, another study stated that there could be other targets that suited hydrophobic substance better, such as plasma membrane (Rialita et al., 2015). This explains the inhibition effect of essential oil against Gram negative bacteria. Essential oil is hydrophobic, therefore can interact with plasma membrane by

disrupting permeability and fluidity of cell membrane.

*Research Journal: Health and Natural Sciences.* 9(1): 1-5. doi: 10.5206/wurjhns.2018-19.2.

## CONCLUSION

Lemongrass and ginger essential oils had antimicrobial effects against nosocomial infection pathogens. Antimicrobial activity of lemongrass oil is more potent than ginger oil on both antimicrobial assay against both pathogens tested.

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