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Chemical Composition and Antimicrobial Study of Essential Oil of Lemongrass (*Cymbopogon citratus*)

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ABSTRACT

***Objectives**: This study aimed to analyze the constituents of essential oil of the aerial parts of lemongrass (Cymbopogon citratus) and evaluate its minimum inhibitory concentration (MIC) against five food-borne microorganisms (Bacillus subtilis, Staphylococcus aureus, Salmonella typhimurium, Vibrio cholera and Escherichia coli).*

***Methodologies**: The essential oil of lemongrass was obtained by hydro distillation. Its chemical constituents were analyzed with gas chromatography/mass spectrometry (GC/MS). Its MIC against the tested microorganisms was determined with macro dilution method.*

***Results**: The study showed that essential oil of lemongrass consisted of 40 components, with geranial (45.058%) and neral (33.100%) as major constituents. The MIC of essential oil of lemongrass against B. subtilis and S. aureus*

was 31.25 µg/mL and 125 µg/mL, respectively. Somehow it was inactive against S. typhimurium, V. cholera, and E. coli.

Conclusions: *The essential oil of lemongrass was mainly consisted of neral and geranial. These two aldehydes compounds were responsible for moderate to strong antimicrobial activity of essential oil of lemongrass against B. subtilis and S. aureus.*

Keywords: *essential oil, lemongrass, chemical constituents, MIC, food borne microorganisms*

INTRODUCTION

Lemongrass (*Cymbopogon citratus*) is a perennial grass with thin, long leaves and grown in tropical region. It is a common medicinal and aromatic plant. The genus Cymbopogon is known for their essential oils, and has been used for flavour, fragrances, cosmetics, perfumery, soaps, detergents, and pharmaceuticals [1, 2]. Essential oil of lemongrass was active as inhibitor of mycotoxin production and antimicrobial [3–6]. Related to its antimicrobial activity, essential oil are considered to be safer and more effective against the microorganisms by functioning on multi-targeted sites and thus be useful to control of food-borne illness [7]. In Indonesia, *Vibrio cholera* and *Salmonella typhimurium* are the common causes of food-borne illness, along with food poisoning caused by *Escherichia coli*, *Bacillus* spp., and *Staphylococcus aureus* [8, 9].

It has been reported that essential oils containing aldehydes or phenols showed the highest antibacterial activity, followed by another groups of essential oils, while volatile oils containing terpene hydrocarbons were usually inactive [10]. In this study, we evaluated the chemical constituents of essential oils of lemongrass, determined its minimal inhibitory concentration (MIC) against common food borne microorganisms in Indonesia, and connected those two aspects to explain its potency as an antimicrobial agent for food preservatives.

MATERIALS AND METHODS

Plant materials

The aerial parts of lemongrass were purchased from a local market at Purbalingga, Central Java, Indonesia. The leaves were dried at room temperature and ground into a fine powder using grinding machine.

Hydro distillation of essential oils

Powder of dried leaves were subjected to hydro distillation using a Clevenger apparatus for 4 hours for the isolation of essential oils according to the method recommended [11]. The volume of the extracted essential oil was recorded. The extracted essential

oils were dehydrated over anhydrous sodium sulfate and stored at 0ºC in air-tight glass vials until used for further analysis.

Microorganisms

Five bacterial strains were obtained from the American type culture collection (ATCC; Rockville, MD, USA) as well as the culture collection of the Assessment Service Unit, Airlangga University, Surabaya, Indonesia. They were *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Salmonella enterica typhimurium* ATCC 14028 and *Vibrio cholera* ATCC 9027. All microorganisms were stocked in appropriate conditions and regenerated before used.

Analysis of chemical constituents

The volatile composition of essential oils was analyzed using GC-MS system (Agilent 6980N GC System coupled to Agilent 5973 inert MSD detector), equipped with a ZB-5 capillary column (30 m x 0.25 mm x 0.25 µm). The carrier gas was helium at flow rate of 1.3 mL/min, and 2 µL of sample was injected. The electron impact technique (70 eV) was used. The injector and detector temperatures were 250 °C and 230 °C.

Determination of antimicrobial activity

The MIC was examined by broth dilution method in nutrient broth using a method previously described with modification [12]. Briefly, active cultures for MIC determination were prepared by transferring loopful of cells from the stock cultures to flasks and inoculated in nutrient broth (NB) medium and incubated at 37 ºC for 24 hours. The cultures were diluted with NB broth to achieve an optical density of 10^7 CFU/mL at the wavelengths of 600 nm by UV/Vis Spectrophotometer. Essential oils were diluted to get the final concentration ranging from 0 to 1000 µg/mL in NB medium. Finally, 20 µL inoculums of each bacteria strain (10^7 CFU/mL) were inoculated and the tests were performed at a final volume of 5.0 mL. The plates were incubated at 37 ºC for 24 hours. The lowest concentration of the test samples, which did not show any visual growth of tested organisms after macroscopic evaluation, was determined as MIC, which was expressed in µg/mL.

RESULTS AND DISCUSSION

The rendement of hydro distillation of essential oil of aerial parts of lemongrass was 0.645%. The GC/MS analysis resulted in 39 constituents that representing of 99.035 % of the essential oil compounds. The retention time and percentage of respective constituent of essential oil of lemongrass is presented in Table 1.

Table-1: Chemical constituents of essential oils of Lemongrass

No	Compound Name	Retention time (min)	Percentage (%)
1	6-methyl-5-hepten-2-one	8.774	1.469
2	β-myrcene	8.927	4.544
3	cineole	10.601	0.120
4	α-ocimene	11.174	0.535
5	β-ocimene	11.694	0.260
6	durenol	14.399	0.144
7	β-linalool	14.590	0.212
8	(+)-camphor	16.745	0.427
9	β-citronellal	17.700	0.252
10	(+)-verbenol	18.442	0.433
11	rose furan epoxide	19.076	0.405
12	(+)-pulegone	19.573	0.566
13	neral	23.539	33.100
14	geranial	25.640	45.058
15	methyl crotonic	30.676	0.575
16	geraniol acetate	31.135	0.799
17	β-caryophyllene	32.083	0.796

18	α-bergamotene	32.831	0.649
19	(-)-germacrene C	33.290	0.215
20	β-farnesene	33.680	0.193
21	γ-muurolene	34.146	0.096
22	(-)-germacrene D	34.238	0.534
23	β-selinene	34.314	0.326
24	α-curcumene	34.429	0.619
25	δ-selinene	34.604	0.308
26	β-bulnesene	34.841	0.129
27	β-cadinene	34.910	0.296
28	α-bulnesene	35.055	0.190
29	α-amorphene	35.300	0.710
30	δ-cadinene	35.606	0.776
31	δ-selinene	35.988	0.141
32	caryophyllene oxide	37.187	0.179
33	germacrene-D-4-ol	37.547	1.017
34	epicurzerenone	37.860	0.312
35	α-gurjunene	38.792	1.313
36	β-gurjunene	39.151	0.085
37	germacrone	40.023	0.153

38	α-springene	47.833	0.546
39	geranyl linalool	48.956	0.553
	Total		99.035

The major constituents of essential oil of lemongrass were geranial (45.058%), neral (33.100%), and β-myrcene (4.544%) (Figure 1). Geranial and neral were reported as main constituents of essential oil of lemongrass elsewhere, somehow the percentage was slightly different than that of our study. A previous report showed that the percentage of geranial, neral, and myrcene in essential oil of lemongrass obtained from Africa was 27.04, 19.93, and 27.04 %, respectively [13]. Similar study conducted in Kenya reported that geranial (39.53%), neral (33.31%), and myrcene (11.41%) were the main constituents of essential oil of local lemongrass [14]. Another study reported that essential oil of lemongrass collected in Brazil majorly contained geranial (42.92%) and neral (30.91%) [15]. The differences in chemical constituents of essential oil of lemongrass from the studies mentioned were caused by the different place where the specimens were collected. A study explained that composition of essential oils from a particular species of plant can differ between harvesting seasons and geographical sources [16].

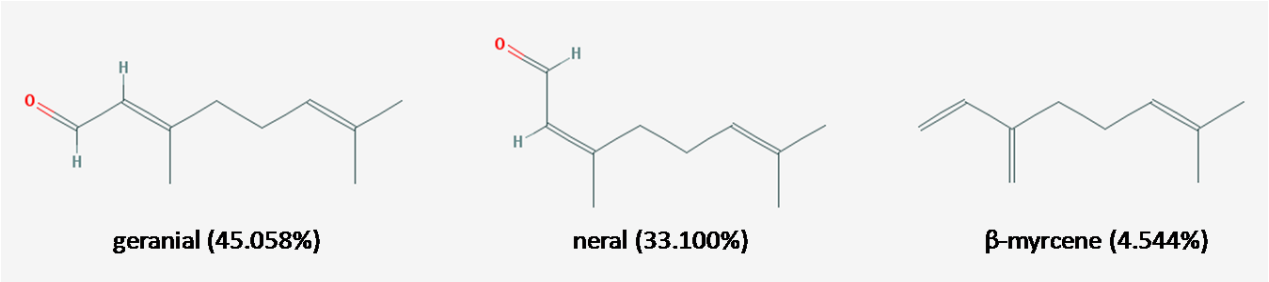

Figure-1: Major constituents of essential oil of lemongrass

The antimicrobial activity of essential oils of lemongrass was shown by their MIC against *B. subtilis, E. coli, S. aureus, S. typhymurium,* and *V. cholera* (Table 2). Its MIC against those foods borne microorganisms ranged from 31.25 to 1000 µg/mL. The MIC of essential oil of lemongrass against *B. subtilis* and *S. aureus* was low, they were 31.25 and 125 µg/mL, respectively. Nevertheless, its MIC against *S. typhimurium, V. cholera,* and *E. coli* was relatively high (1000 µg/mL). This result showed that essential oil of lemongrass possessing strong inhibitory activity against *B. subtilis* and *S. aureus*, but it was not active against *S. typhimurium, V. cholera*, and *E. coli*. Our result was in agreement with a study from Brazil, that essential oil of lemongrass was more active inhibiting the growth of *S. aureus* (MIC=16.35 mg/mL), and *Enterococcus* sp. (MIC=15.90 mg/mL) than against *E. coli* MIC=30.60 mg/mL) and *Salmonella* sp. (MIC=30.65 mg/mL) [17]. Our result also confirmed the previous report that

essential oil of lemongrass collected from Thailand was active against *B. cereus*, *S. aureus*, and *E. coli*. Nevertheless, *E. coli* was the least susceptible bacteria [18] Another report stated that MIC of essential oil of lemongrass from India against *S. aureus* and *B. subtilis* were same (0.06%), and it was significantly different than that of it against *E. coli* (MIC=0.12%) [4].

Table 2. MIC of essential oils of lemongrass against food borne microorganisms

No	Microorganisms	MIC (µg/mL)
1	*B. subtilis*	31.25
2	*E. coli*	1000
3	*S. aureus*	125
4	*S. typhymurium*	1000
5	*V. cholera*	1000

The essential oil of lemongrass inhibited bacterial growth by mechanism of attacking multiple targets in the bacterial cell, included inhibiting the biofilm formation, promoting intracellular material leakage and morphological changes. These effects was depending on the concentration used as well as the amount of its components [18]. In general, essential oils containing aldehydes or phenols showed the highest antibacterial activity [19]. Essential oil of lemongrass used in this study was mainly consisted of aldehydes (80.253%). Hence, aldehyde compounds such as β-citronellal, neral, and geranial were responsible for its antibacterial activity. Aldehydes as antimicrobial agent acted in dose-dependent manner. At low concentration, they may cross link amino groups in the cell wall and cytoplasm as well as inhibit enzymes with a thiol group at the cytoplasmic membrane. They may also cause coagulation of cytoplasm at intermediate concentrations [18].

CONCLUSION

The essential oil of lemongrass was mainly consisted of neral and geranial. These two aldehydes compounds were responsible for moderate to strong antimicrobial activity of essential oil of lemongrass against *B. subtilis* and *S. aureus*.

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