Identification of Active Compounds and Analysis of Antibacterial Activity of Blended Essential Oil From Citronella *(Cymbopogon winterianus jowitt)*, Cananga *(Cananga odorata)*, and Ginger *(Zingiber officinale)*

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Abstract. Essential oil is an aromatic liquid obtained from various parts of plants. The growing need for essential oil functions has led to the proliferation of blended essential oil products aimed at achieving specific compositions and functions. Different terpenoid components in essential oils can interact to either reduce or enhance antimicrobial effectiveness. In this study, a mixture of three types of essential oil, citronella oil, cananga oil, and ginger oil that obtained through steam distillation with a composition of 4:5:1 will be investigated. The purpose of this blending is to observe the compound ratios using GC-MS (Gas Chromatography-Mass Spectrometry) analysis and to compare the antibacterial capabilities against the Gram-positive bacterium *Staphylococcus aureus* and the Gram-negative bacterium *Escherichia coli* using the Kirby-Bauer Disc Diffusion Method. The results of this research reveal that the major compound in citronella oil is *geraniol* at 27.87%, cananga oil contains 25.08% *caryophyllene*, and ginger oil consists of 15.95% *champene*. The oil resulting from the blend of these three oils is composed of five major compounds: *geraniol* (10.95%), *caryophyllene* (8.42%), *citronellol* (5.35%), *germacrene D* (4.82%), and *cis-a-bergamotene* (5.90%). Moreover, antibacterial testing demonstrates that the blended essential oil exhibits the highest antibacterial efficacy against *Staphylococcus aureus*, but not against *Escherichia coli*. Therefore, this blending demonstrates a synergistic effect on the antibacterial capability against *Staphylococcus aureus* while exhibiting an antagonistic effect against the antibacterial capability against *Escherichia coli*.

Keywords: Antibacterial, Blended essential oil, GC-MS, Kirby-Bauer Disc Diffusion Method.

1. Introduction

Essential oil is an aromatic and volatile liquid obtained from plant materials, including flowers, roots, bark, leaves, seeds, peels, fruits, wood, and whole plants [1]. Each type of essential oil has a different aroma and composition. Besides their strong aroma, essential oils and other plant extracts possess antibacterial, antifungal, and antiviral properties and have been recognized worldwide as potential sources of new

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antimicrobial compounds, alternatives for treating infectious diseases, and agents that promote food preservation [2].

The growing demand for essential oil functions has led to the proliferation of blended essential oil products, aimed at achieving specific compositions and functions. Mixing two or more essential oils results in different compounds, and these blends can be highly specialized and potent, potentially enhancing efficacy without increasing the dosage [3]. One method of blending essential oils is through "blending by notes," with a composition ratio of 20-40% top notes, 50-70% middle notes, and 10% base notes [4].

In addition to aiming for the desired aroma, blending essential oils can also be used to enhance antimicrobial functions. According to Bassolé & Juliani [5], different terpenoid components in essential oils can interact to either reduce or increase antimicrobial effectiveness. The interaction among these essential oil compounds can result in four types of effects, including additive effect, which occurs when the combined effect is equal to the total effect of each component individually. Antagonistic effect, occurs when one or both components have a lower effect when used together compared to when used individually. Synergistic effect, occurs when the combined effect of these substances is greater than the total effect of each component individually. No interaction effect, defined as the lack of a significant impact from the mixture, meaning that the combination doesn't significantly alter the effects of the individual components.

Citronella oil is an Indonesian essential oil commodity known for its high antibacterial activity. Citronella (Cymbopogon winterianus Jowitt ex Bor) extracted using various methods yields a range of 0.89-1.50% yield, with compound compositions including citronellal at 2.2–55.4%, geraniol at 14.2–53.0%, citronellol at 8.2–16.4%. In terms of antimicrobial activity, the Minimum Inhibitory Concentration (MIC) ranges from 125–1000 μg/mL. Generally, citronella oil is found to be quite active against both Gram-positive and Gram-negative bacteria [6].

Another essential oil commodity in Indonesia known for its many benefits and high antibacterial content is cananga oil produced from the flowers of Cananga odorata. According to previous research, the main components of cananga oil produced in this study are caryophyllene (36.44%), â-linalool (5.97%), á-caryophyllene (9.61%), germacrene (17.23%), and benzyl benzoate (7.18%) (Pujiarti et al., 2016). The antibacterial ability of cananga oil has been demonstrated in other research. GC-MS analysis has shown the presence of linalool (21.78%), acetic acid (15.77%), β-ocimene (13.53%), piperonal (10.02%), and eugenol (7.42%), which are recognized as bioactive compounds with antimicrobial activity against foodborne microorganisms, including *Salmonella typhi, Bacillus subtilis, Staphylococcus aureus,* and *Escherichia coli* [7].

Ginger oil, as one of the essential oil commodities in Indonesia, also possesses antibacterial capabilities. Previous research on the antibacterial activity of ginger has shown that the main components of ginger oil extracted include zingiberene and α-curcumene. The diameter of the inhibition zone (DIZ) of ginger essential oil against S. aureus is 17.1 mm, with a minimum inhibitory concentration (MIC) of 1.0 mg/mL and a minimum bactericidal concentration (MBC) of 2.0 mg/mL. For E. coli, the DIZ is 12.3 mm, with MIC and MBC values of 2.0 mg/mL and 4.0 mg/mL, respectively [8].

Based on previous research, citronella oil, cananga oil, and ginger oil have different compositions and antibacterial activities against Gram-positive and Gram-negative bacteria. Additionally, these three commodities are readily available. Therefore, these three ingredients were selected, and this research was conducted to determine the compound composition and antibacterial effectiveness against the Gram-positive bacterium *Staphylococcus aureus* and the Gram-negative bacterium *Escherichia coli* from a mixture of citronella oil, cananga oil, and ginger oil.

2. Material and methods

2.1 Material

The materials used in the distillation process include citronella (Cymbopogon winterianus Jowitt) obtained from the Shafaluna Citronella Plantation in Bantul, cananga flowers, and red ginger rhizomes obtained from the Bringharjo Market in Yogyakarta. Additionally, the materials used in the antibacterial testing include *Staphylococcus aureus bacteria*, *Escherichia coli bacteria*, a 0.9% NaCl solution, and Mueller Hinton agar powder.

2.2 Equipment

The equipment used in this research includes a steam distillation apparatus, GC-MS (Gas Chromatography-Mass Spectrometry), autoclave, incubator, scale, refractometer, and pycnometer.

2.3 Methods

The extraction of essential oils is carried out using steam distillation at a temperature of 100°C for 4 hours. The resulting oil is tested for its refractive index using a refractometer, and its density is measured using a pycnometer. The mixing ratio of citronella oil, cananga oil, and ginger oil to be used is 4:5:1, determined based on the maximum ratio for top notes and base notes. A completely randomized design (CRD) is used in the antibacterial test with the Kirby Bauer Disc Diffusion Method. Subsequently, data analysis is performed using one-way ANOVA and t-test.

3. Result and discussion

3.1 Distillation and essential oil blending

Table 1 shows the average yields from steam distillation of citronella, cananga, and ginger. Citronella oil produced the highest yield at 0.93±0.01%, cananga oil had an average yield of 0.47±0.06%, and the average yield of ginger oil obtained in this study was 0.14±0.02%. The oil mixture is prepared with a composition of citronella oil: cananga oil: ginger oil in a ratio of 4:5:1. The resulting oil blend has a density of 0.895, a refractive index of 1.489, and a yellow color.

Table 1. Essential oil yield, specific gravity and refractive index

Essential Oil	Yield (%)	Specific gravity	Refractive index
Citronella	0,93±0,01	0,883	1,474±0,001
Cananga	0,47±0,06	0,906	1,498±0,001
Ginger	0,14±0,02	0,879	1,848±0,001

Fig. 1. Blended essential oil

3.2 GC-MS analysis

Compound analysis using GC-MS was conducted to identify the compounds present in citronella oil, cananga oil, ginger oil, and the blended essential oil. The GC-MS chromatogram for citronella oil showed 106 peaks. The major compounds in citronella oil include geraniol 27.87%, citronellol 13.95%, citronellal 12.32%, citral 4.47%, and D-limonene 4.46%.The chromatogram produced in the testing of cananga oil showed 125 peaks, with the major compounds being caryophyllene 25.08%, humulene 8.20%, germacrene D 7.13%, linalyl acetate 6.45%, and cis-a-bergamotene 5.42%. The GC-MS chromatogram of ginger oil also displayed 125 peaks, with the main compounds being camphene at 15.95%, (1S,5S)-2-Methyl-5-((R)-6-methylhept-5-en-2-yl)bicyclo[3.1.0]hex-2-ene at 14.53%, benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- at 13.35%, 2,6-octadienal, 3,7-dimethyl-, (Z)- at 7.67%, and 1,3,6,10-dodecatetraene, 3,7,11-trimethyl-, (Z,E)- at 6.27%. The GC-MS testing conducted on the mixed essential oil showed a chromatogram with 95 peaks. The main components of the resulting mixture of essential oils include geraniol at 10.95%, caryophyllene at 8.42%, citronellol at 5.35%, germacrene D at 4.82%, and cis-a-bergamotene at 5.90%.

Table 2 displays the 15 main compounds obtained from the composition of the blended essential oil and a comparison of the quantities of compounds contained in each of the citronella cananga, and ginger essential oils.

Table 2. Blended essential oil main compound

No	Compound name	Compound formula	% blended EO	%Citronella EO	%Cananga EO	%Ginger EO
1	*Geraniol*	$C_{10}H_{18}O$	10,95%	27,87%	1,30%	1,14%
2	*Caryophyllene*	$C_{15}H_{24}$	8,42%	-	25,08%	-
3	*Germacrene D*	$C_{15}H_{24}$	4,82%	1,83%	7,13%	-
4	*Citronellol*	$C_{10}H_{20}O$	5,35%	13,95%	-	-
5	*cis-a-Bergamotene*	$C_{15}H_{24}$	5,90%	-	5,42%	0,18%
6	*2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-*	$C_{15}H_{26}O$	5,87%	-	2,17%	-
7	*Citronellal*	$C_{10}H_{18}O$	4,16%	12,32%	-	-
8	*Benzyl Benzoate*	$C_{14}H_{12}O_2$	3,34%	-	3,93%	-
9	*Humulene*	$C_{15}H_{24}$	2,95%	-	8,42%	-
10	*D-Limonene*	$C_{10}H_{16}$	2,48%	4,46%	-	-
11	*Camphene*	$C_{10}H_{16}$	2,34%	2,40%	-	15,95%
12	*?-Muurolene*	$C_{15}H_{24}$	2,27%	-	-	-
13	*Geranyl acetate*	$C_{12}H_{20}O_2$	1,83%	4,04%	2,80%	0,96%
14	*Linalool*	$C_{10}H_{18}O$	1,73%	1,05%	6,45%	0,79%
15	*1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z,E)-*	$C_{15}H_{24}$	1,70%	-	1,84%	6,27%

3.3 Kirby-Bauer disc diffusion method

The antibacterial testing was conducted with 5 repetitions against *S. aureus* and *E. coli bacteria*. The results of the experiments are obtained as shown in Table 3.

Table 3. Inhibition zone

Essential Oil	Inhibition Zone (mm)	
	S.aureus	*E.coli*
Serai wangi	$4,67 \pm 0,75^a$	$6,85 \pm 2,23^a$
Kenanga	$2,75 \pm 0,60^a$	0
Jahe	$2,49 \pm 1,08^a$	$7,32 \pm 2,27^a$
Campuran	$11,18 \pm 1,97^b$	0

Note: Different letters in the same row indicate significant differences ($p > 0.05$).

Next, one-way ANOVA and t-tests were conducted. The hypothesis used was H0 = the type of oil does not have a significantly different effect on bacterial inhibition, and

H1= the type of oil has a significantly different effect on bacterial inhibition, specifically against S. aureus. The results of the one-way ANOVA on the bacterial inhibition ability against S. aureus showed that H0 was rejected, and H1 was accepted. Post hoc tests indicated a significant difference between the blended essential oil and citronella, cananga, and ginger oils.

The highest inhibition ability against *S. aureus* is exhibited by the blended essential oil with an average of 11.18 mm. This indicates an improvement in the inhibition ability against S. aureus in the blended essential oil. The enhancement in the inhibition ability suggests that the composition of the mixture shows a synergistic effect on the inhibition of *S. aureus bacteria*. Synergistic effect is a condition where the combined effect of substances is greater than the sum of individual effects [5].

The results of the test against *E. coli bacteria* show that cananga oil produced using steam distillation and the blended essential oil did not produce inhibition zones. However, citronella oil yielded an average inhibition zone of 6.85±2.23 mm, and ginger oil had an average inhibition zone of 7.32±27 mm.

The data was processed using the t-test method because there are only two data groups: inhibition by citronella oil and ginger oil. Based on the two-sided t-test, a p-value of 0.749 was obtained. This value is greater than 0.05, so H0 is accepted or it can be concluded that there is no significant difference in the inhibition ability against E. coli bacteria between citronella oil and ginger oil.

The results of the antibacterial test on the blended essential oil did not show inhibition against *E. coli bacteria*. Therefore, the mixture resulted in a decrease in the inhibition ability of citronella oil and ginger oil against *E. coli bacteria*. This decrease is referred to as an antagonistic effect, where the effect of one or both compounds is less when applied together than when applied individually [5].

The antimicrobial effectiveness of essential oils is largely determined by the type and concentration of chemical components present in them. These various components have different mechanisms of action. For example, phenolic compounds primarily function to disrupt the cell membrane structure and its permeability. The hydroxyl groups found in phenolic compounds can inhibit the activity of enzymes in microorganisms, including enzymes in the tricarboxylic acid cycle. On the other hand, terpenoids influence the composition of fatty acids in the cell membrane, altering membrane permeability levels and leading to the release of intracellular substances [9].

The difference in antibacterial effects on *E. coli* and *S. aureus* can be attributed to several factors. The same chemical composition can yield different effects when applied to various types of microorganisms because the composition and thickness of the cell membrane differ among microbial species, such as Gram-positive and Gram-negative bacteria. Generally, Gram-positive bacteria are more susceptible to essential oils than Gram-negative bacteria, or in other words, Gram-negative bacteria have a higher level of resistance to essential oils [10].

The cell wall of Gram-positive bacteria primarily consists of peptidoglycan and is thinner, allowing hydrophobic molecules in essential oils to easily penetrate and disrupt the cell wall and bacterial cytoplasm. In contrast, Gram-negative bacteria have a more complex cell wall with a thin peptidoglycan layer, covered by an outer membrane containing lipopolysaccharides. Lipopolysaccharides and other complex structures in the outer membrane serve as barriers that make Gram-negative bacteria more resistant to essential oils and other natural antimicrobial compounds. Additionally, Gram-negative bacteria have porin proteins in the outer membrane that facilitate the passage of hydrophilic molecules, making them relatively resistant to hydrophobic antibiotics [11].

4. Summary

The main compound content in citronella oil is composed of geraniol at 27.87%, cananga oil consists of Caryophyllene at 25.08%, and ginger oil is primarily composed of Camphene at 15.95%. The main chemical compound content of the blended essential oil includes geraniol at 10.95%, caryophyllene at 8.42%, citronellol at 5.35%, germacrene D at 4.82%, and cis-a-Bergamotene at 5.90%. There is a significant difference in the inhibition ability of citronella, cananga, ginger, and the blend against *Staphylococcus aureus bacteria*, but there is no significant difference against *Escherichia coli*. Furthermore, the blended oil has a synergistic effect on *Staphylococcus aureus bacteria* but exhibits an antagonistic effect on the inhibition of *Escherichia coli bacteria*.

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