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Antibacterial activity of emulgel essential oil from scented lemongrass (*Cymbopogon nardus* (L.) Rendle) against *Staphylococcus aureus* ATCC-29213

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ABSTRACT. Lemongrass (*Cymbopogon nardus* (L.) Rendle) is one of plants with many benefits, which the main compound is able to inhibit bacterial activity. Utilizing citronella essential oil in an emulgel dose form can help treat *Staphylococcus aureus*. This study aims to determine the antibacterial effect of essential oil concentrations of citronella emulgel preparations at concentrations of 10%, 20%, 30%, and negative control against *S. aureus*. The used method was agar diffusion by means of wells. Data were analyzed by One-Way ANOVA test and Post Hoc test in SPSS ver. 26. Lemongrass essential oil emulgel was proven to be able to inhibit the growth of *S. aureus* as evidenced by the inhibition zone formed. The diameter of the inhibition zones for the 10%, 20%, 30% concentrations were 5.367 mm, 7.867 mm, and 11.833 mm, respectively. While the emulgel base as a control was 0 mm. The results of the ANOVA test showed a significant value of 0.000<0.05, so there was a significant difference between the inhibition zone and the 95% confidence level. Based on the Post Hoc test, the inhibition of the three groups of emulgel formulations of citronella essential oil was significantly different from the control group. The three emulgel concentration groups proved to be significantly different, in which citronella essential oil emulgel with a concentration of 30% had the best antibacterial activity. This finding revealed the optimum concentration for the emulsion of lemongrass essential oil to promote antibacterial activity.

Keywords: anti-acne formulation; citronella emulgel; essential oil; inhibition zone; *Staphylococcus aureus*

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INTRODUCTION

Acne is a common skin condition that affects 85% of young people and adults between the ages of 11 and 30. Around 80-85% of acne sufferers in Indonesia are young people aged around 15-18 years, around 12% are women over 25 years and around 3% are around 35-44 years old (Achmad *et al*., 2022). Generally, acne is caused by several factors including genetics, food, hormones, climate, and bacterial infection (Albuquerque *et al*., 2014; Bhadra & Deb, 2020). One of the causes of inflammation in acne is the bacteria *Staphylococcus aureus* (Totté *et al*., 2016; Weber *et al*., 2019), *S. aureus* is a normal flora that exists on the skin, respiratory tract, and human digestive tract, as well as in the air and the surrounding environment. *S. aureus* and *Propionibacterium acnes* were the main actors in the skin disease caused by infection or inflammation of the pilosebaceous unit (Fitz-Gibbon *et al*., 2013; Platsidaki & Dessinioti, 2018; Ramadani *et al*., 2022). Since the most frequent bacteria isolated from acne patients were *S. aureus*, it is possible that acne vulgaris is mainly caused by *S. aureus* rather than *P. acne* (Dhillon & Varshney, 2013). In general, the acne treatment used contains the antibiotics tetracycline, erythromycin, and clindamycin which can cause side effects such as irritation (Tripathi *et al*., 2013). Also, long-term use of antibiotics can cause resistance and organ damage. Therefore, acne treatment using natural ingredients aims to minimize unwanted side effects from the use of these antibiotics (Tripathi *et al*., 2013; Fox *et al*., 2016).

Indonesia has a variety of medicinal plants. At least 80% of the medicinal plant species in South East Asia can be found in Indonesia, whether they are native or introduced (Cahyaningsih *et al*., 2021). One of the plants known to the public as a traditional medicine is lemongrass (*Cymbopogon nardus* (L.) Rendle). Distillation of citronella leaves and stems produces essential oil which in the

world of trade is known as citronella oil (Kusumaningrum *et al*., 2020). The chemical content of citronella essential oil is citronellol, geraniol, and citronellol (Wany *et al*., 2014; Eden *et al*., 2018). The antibacterial activity of essential oils from citronella using the microdilution method showed that the essential oils were able to inhibit all test bacteria, including *Escherichia coli*, *S. aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Shigella flexneri*, and *Bacillus cereus* (Mirnejad *et al*., 2013; Puspawati *et al*., 2016; Wu *et al*., 2019).

Citronella essential oil is difficult to dissolve in water (Natrajan *et al*., 2015), hence it is best used as an emulgel dosage form. Emulgel helps unite the hydrophobic active ingredients in the oil phase then the oil globules are dispersed in the water phase (O/W emulsion) which can then be mixed in the gelling agent (Ajazuddin *et al*., 2013). Emulgel has good stability because the addition of a gelling agent increases the stability of the emulsion. When applied to the skin, emulgel offers a number of advantages, including the fact that it is non-greasy, has good dispersion, can be washed with water, is soft and comfortable to use, has a longer shelf life, tisotropic properties, and is environmentally friendly (Yadav *et al*., 2016; Sah *et al*., 2017). However, the use of citronella-based emulgel formulations is still quite restricted.

It is necessary to develop an anti-acne emulgel formulation with citronella essential oil. This study emphasizes the use of citronella plants in various emulgel variations, which provide innovative input from previous studies. *S. aureus* were also employed to assess the anti-acne effectiveness of emulgel formulations. This study aims to determine the antibacterial effect of essential oil content of citronella emulgel preparations at concentrations of 10%, 20%, 30%, and negative control against *S. aureus*. This study is expected to provide insight into the potency of essential oils as antimicrobial agents, which can be used to create commercial products, medicines, and therapeutic procedures.

MATERIALS AND METHODS

Emulgel making and antibacterial activity tests were carried out in the Laboratory of Microbiology, Study program of Biology Education, Universitas Sebelas Maret. *Staphylococcus aureus* was purchased from PT. Agritama Sinergi Inovasi (AGAVI), Bandung with the staining code ATCC-29213 (Fig. 1), and citronella essential oil Tetesan Atsiri was purchased from Bogor.

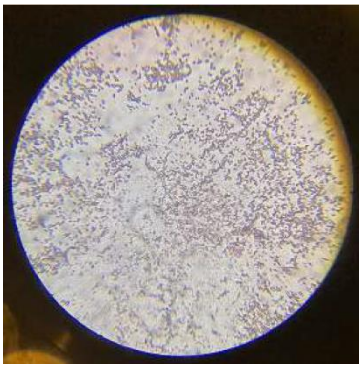

Fig. 1. Gram staining result on *Staphylococcus aureus* ATCC-29213 cultured by PT. Agritama Sinergi Inovasi

Emulgel preparations. All the necessary components were weighed, followed by the addition of 90 cc of distilled water and carbopol until it swelled. The pH was determined after expansion, and TEA was then added to create a neutral pH of 7. In a glass beaker, the oil phase was combined with span 20, Ol Olive, and citronella essential oil 1. In a glass beaker, the water phases were thoroughly combined (nipagin, nipasol, and BHT were dissolved in propylene glycol, followed by the addition of tween and the remaining water). The water and oil phases are homogeneous within each of them, therefore they are combined (the oil phase was added to the water phase), then stirred to create an emulsion. After that, a gel base was added to the concoction in a glass beaker, which was thoroughly

agitated. The storage container was then shut tightly. It was shielded from direct sunlight and kept at ambient temperature. The table 1 depicts the emulgel preparation formula (Farida, 2019).

Table 1. Emulgel preparation formula

No.	Material Name	Control (%)	F1 (%)	F2 (%)	F3 (%)
1	Lemongrass essential oil	-	10	20	30
2	Carbomer	1	1	1	1
3	Triethanolamine	qs	qs	qs	qs
4	Polysorbate 80	3.65	3.65	3.65	3.65
5	Sorbitan monolaurate	4.35	4.35	4.35	4.35
6	Propylene glycol	10	10	10	10
7	Methyl paraben	0.1	0.1	0.1	0.1
8	Propyl paraben	0.1	0.1	0.1	0.1
9	Oleum olive	0.5	0.5	0.5	0.5
10	Butylhydroxytoluene	0.1	0.1	0.1	0.1
11	Aquadest	Ad 100	Ad 100	Ad 100	Ad 100

Bacterial rejuvenation. The test bacteria were rejuvenated on Mueller Hinton Agar (MHA) media which had been solidified by taking 1 ose of bacteria and then the bacteria were incubated at 37°C for 24 h (Sudagung *et al*., 2015).

Preparation of McFarland standard. McFarland's solution was used as a standard in the manufacture of bacterial suspensions. About 0.1 mL of 1.175% $BaCl_2$ solution was mixed with 9.9 mL of 1% H_2SO_4 solution and shaken homogeneously (Hombach *et al*., 2015; Pajan *et al*., 2016).

Preparation of bacterial suspension. *S. aureus* were removed from the rejuvenation process using sterile oil, placed in tube A, and shaken for about 10 seconds. It was compared to the McFarland standard 0.5. Bacterial dilution was done by adding 1 ml of tube A to 10 ml of sterile, distilled water and homogenising the mixture (tube B). About 1 ml of tube B was taken, 10 ml of MHB added, and tube C was homogenized after that (Farida, 2019; Ghasemi *et al*., 2020).

Preparation of MHA media. To make the media, 38 g of MHA were weighed, then 1.000 ml of distilled water was added and stirred with a stirring rod until the mixture was homogenous. The media was subsequently sterilized in an autoclave at 121°C for 15 min (Sudagung *et al*., 2015).

Antibacterial activity test. The agar well diffusion method was used to test the antibacterial activity. In a sterile petri dish with approximately 1 ml of bacterial suspension already present, MHA medium solution 20 ml was added. The mixture was homogenized by spinning it at room temperature. Wells produced up to 0.5 g in size were filled with the emulgel formulation, which was then incubated for 24 h at 37°C. The clear zone surrounding the well area was then measured using a ruler (Farida, 2019).

Data analysis. With using SPSS ver. 26, one-way ANOVA and a post hoc test were used to analyze the data. The estimated p value can be used to determine which formula has a significant difference. A significant difference is indicated if the estimated p value is < 0.05. To determine whether data were different, least significant different (LSD) tests were conducted afterward.

RESULTS AND DISCUSSION

The oil phase of the lemongrass emulgel consists of span 20, which functions as an emulsifier, olive oil, which functions as an emollient (de Meza, 2013), and citronella essential oil, which is the active ingredient. The aqueous phase of the lemongrass emulgel consisted of tween 80, propylene glycol, methylparaben, and the rest of the water. To facilitate the solubility of butylhydroxytoluene (BHT) and propyl paraben, they are dissolved in propylene glycol. Tween 80 functions as an emulsifier (Espert *et al*., 2019), propylene glycol functions as a humectant to maintain the water content in the preparation and absorbs moisture to maintain the stability and physical properties of the preparation during storage. Propylparaben and methylparaben function as preservatives to prevent the growth of microbes and fungi. The purpose of combining propyl paraben and methyl paraben is

to preserve the oil and water phases because in the emulgel formula there are water and oil phases. BHT is used as an antioxidant to prevent oxidation, which causes the essential oil of citronella to become rancid. The combination of emulsifiers tween 80 and span 20 is able to create stable emulsions because tween is an ester of polyoxyethylene sorbitan fatty acid, which is soluble in water, while span 20 is soluble in oil (Mosca *et al*., 2013; Campolo *et al*., 2020). Propylene glycol is used as an emulgel for acne because it simultaneously serves three purposes: as a humectant, an enhancer of hydration, and an antibacterial. This is expected to increase *S. aureus* inhibition. The decision was taken to use olive oil as an emollient since it also serves as an antimicrobial. Because the concentration used is low (0.5%), it is not anticipated to exacerbate acne. Emulgel preparations that have been made are then used for physical characteristics and antibacterial tests.

Emulgel physical characteristics and antibacterial test result. An evaluation of the physical properties of the citronella essential oil emulgel has been carried out which includes organoleptic tests. Tests were carried out on each formula and its replication. According to the results of the organoleptic test, the citronella essential oil emulgel formulations I, II, and III all have a soft texture, the same colour as yellowish-white, and a fresh lemongrass odour (Fig. 2). This is in line with Farida (2019), which describes the substance as an emulgel with a delicate texture, a yellowish-white colour, and a characteristic odour of the plant species.

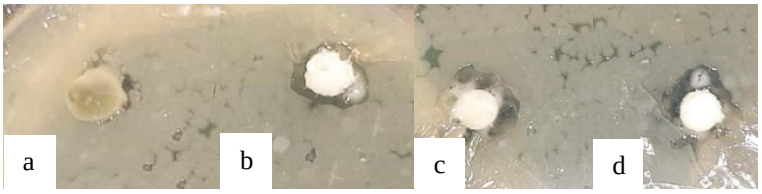

Fig. 2. Inhibition zone of lemongrass essential oil emulgel: a. 0% (emulgel preparation without citronella essential oil); b. 10%; c. 20%; d. 30%

The diameter of the inhibition zone increased as the concentration of citronella essential oil increased (Fig. 3). This indicated that the citronella essential oil emulgel at concentrations of 10%, 20%, and 30% was able to affect the metabolism of *S. aureus*. The average inhibition zone of the 30% lemongrass essential oil emulgel concentration was the largest at 11.833 mm, then decreased in the 20% lemongrass essential oil emulgel concentration which was 7.867 mm, and also decreased in the 10% lemongrass essential oil emulgel which was 5.367 mm. This study is in accordance with Bota *et al.* (2015) that citronella essential oil has an antibacterial activity against *S. aureus*.

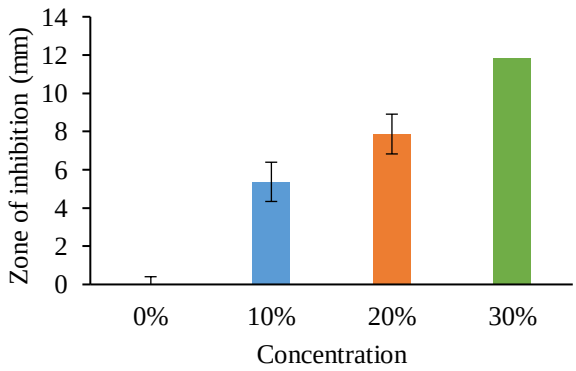

Fig. 3. The essential oil inhibition zone, where the error bars represent the standard deviation

To ascertain whether there was a significant difference in the antibacterial activity of citronella essential oil emulgel in each formula between varying concentrations and negative controls, data analysis used the One-Way ANOVA method with a 95% confidence level = 0.05. There was a significant difference between the concentration of citronella essential oil concentration in the emulgel. Based on the results of the one-way ANOVA test, H_0 was rejected, which means that there

was a significant difference between the concentrations of citronella essential oil in the emulgel. The emulgel of citronella essential oil with concentrations of 10%, 20%, and 30% showed antibacterial activity of *S. aureus* (Table 2).

Table 2. The results of the analysis of the inhibition zone of *Staphylococcus aureus* using the One-Way ANOVA

	Sum of Squares	df	Mean square	F	Sig.
Between Groups	161.282	3	53.761	87.416	.000
Within Groups	4.920	8	.615		
Total	166.202	11			

The results of the One-Way ANOVA show that H_0 is rejected, then the next step is to carry out further tests (Post Hoc Tests), which presented in Table 3.

Table 3. Least Significant Different (LSD) Post hoc test results

(I) Concentration of citronella essential oil in emulgel	(J) Concentration of citronella essential oil in emulgel	Mean difference (I-J)	Std. error	Sig.	95% Confidence interval	
					Lower bound	Upper bound
0%	10%	-4.3667*	.6403	.000	-5.843	-2.890
	20%	-6.3667*	.6403	.000	-7.843	-4.890
	30%	-10.1667*	.6403	.000	-11.643	-8.690
10%	0%	4.3667*	.6403	.000	2.890	5.843
	20%	-2.0000*	.6403	.014	-3.477	-.523
	30%	-5.8000*	.6403	.000	-7.277	-4.323
20%	0%	6.3667*	.6403	.000	4.890	7.843
	10%	2.0000*	.6403	.014	.523	3.477
	30%	-3.8000*	.6403	.000	-5.277	-2.323
30%	0%	10.1667*	.6403	.000	8.690	11.643
	10%	5.8000*	.6403	.000	4.323	7.277
	20%	3.8000*	.6403	.000	2.323	5.277

Notes: *= The mean difference is significant at the 0.05 level

Based on the post-hoc follow-up test, the inhibition of the three citronella essential oil emulgel formulation groups (10%, 20%, and 30%) significantly differed from the control group. Furthermore, the three groups of emulgel concentrations were shown to be significantly different, which means that the citronella essential oil emulgel with a concentration of 30% had greater *S. aureus* antibacterial activity than the citronella essential oil emulgel with a concentration of 20%. Furthermore, citronella essential oil with a concentration of 20% had greater antibacterial activity against *S. aureus* than emulgel of citronella essential oil with a concentration of 10%. Thus, there was a significant difference in inhibition between groups of citronella essential oil emulgel formulations.

In the citronella emulgel formulation, the primary compounds in citronella oil that have antibacterial properties are citronellol, geraniol, and citronellol. These terpenoids are members of the monoterpene group and are formed by the elements carbon, hydrogen, and oxygen with the formula elements C_{10}, $H_{16,18,20}$, and O, respectively (Bota *et al*., 2015; Kiani *et al*., 2022; Andaririt, 2023). Most of the antibacterials are known as secondary metabolites belonging to the phenolic and terpenoid groups in the essential oil fraction. The terpenes included in citronella essential oil have bactericidal effects on a number of bacterial species, including *S. aureus, S. epidermidis, P. aeruginosa*, and *E. coli* (Guimarães *et al*., 2019; Usach *et al*., 2020; Kaltschmidt *et al*., 2020; Dewi *et al*., 2023). In addition, geraniol, a monoterpene molecule that takes the form of an alcohol, may kill bacteria by denaturing proteins, such as *S. aureus, Streptococcus mutans, E. coli*, and *Salmonella enterica* (Manu, 2016; Lukita *et al*., 2021). Phenolic chemicals and terpenoids harm the bacterial cell wall's structure and interfere with protons' ability to move freely through the membrane and their strength (Murnová & Dercová, 2014; Zengin & Baysal, 2014). Therefore, the bacterial cell wall will be damaged due to a decrease in permeability which allows disruption of the transport of important

organic ions that will enter the bacterial cell, which can result in disruption of metabolism or the death of bacterial cells. Others chemical compounds in this product still require to be further studied.

CONCLUSION

The emulgel of citronella essential oil with concentrations of 10%, 20%, and 30% has antibacterial activity of *Staphylococcus aureus*. The inhibition of the three citronella essential oil emulgel formulation groups had significant differences with the control group. The citronella essential oil emulgel with a concentration of 30% concentration has the best antibacterial activity. Future research is expected to examine the chemical content of citronella essential oil emulgels.

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