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POTENTIAL OF LEMONGRASS LEAF EXTRACT (Cymbopogon citratus (DC.) Stapf) AS AN ANTIBACTERIAL INHIBITORY of Staphylococcus epidermidis

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ABSTRAK

Resistensi antibiotik akibat penggunaan antibiotik yang berkepanjangan dalam pengobatan infeksi bakteri telah menjadi perhatian dalam beberapa waktu terakhir. Daun serai dapur (*Cymbopogon citratus (DC.) Stapf*) merupakan salah satu bahan alami yang dapat digunakan sebagai alternatif antibakteri. Tujuan penelitian ini adalah mengetahui aktivitas antibakteri dari ekstrak etanol 70% daun serai dapur terhadap *Staphylococcus epidermidis* menggunakan metode difusi agar. Selain itu, Konsentrasi Hambatan Minimum (KHM) dan Konsentrasi Bunuh Minimum (KBM) ekstrak ditentukan dengan metode dilusi cair. Hasil penelitian ini menunjukkan bahwa konsentrasi ekstrak sebesar 1.000.000 ppm mememiliki zona hambat pada kategori *intermediate* jika dibandingkan dengan kontrol positif, yaitu klindamisin. Sementara itu, nilai KHM yang diperoleh adalah 1250 ppm, sedangkan nilai KBM adalah 5000 ppm. Hasil analisis statistik menunjukan bahwa tidak ada perbedaan signifikan dari diameter zona hambat yang terbentuk dari antara konsentrasi-konsentrasi yang diujikan.
Kata Kunci: *Stpahylococcus epidermidis,* Daun serai, Antibakteri

ABSTRACT

The concerns of antibiotic resistance have increased in recently years, this issue caused by long-term repeated use of antibiotics to treat bacterial illnesses. Lemongrass leaf (Cymbopogon citratus (DC.) Stapf) is one of the natural ingredients that can be used as an alternative antibacterial. The study objective is to determine the antibacterial activity of the ethanol extract of 70% cooking leaves against Staphylococcus epidermidis using diffusion methods. In addition, the Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of extracts were determined using liquid dilution methods. The results of this study show that extract concentrations of 1,000,000 ppm have a barrier zone in the intermediate category when compared with the positive control, that is, clindamycin. Meanwhile, the MIC value obtained is 1250 ppm, while the MBC value is 5000 ppm. The results of statistical analysis showed that there were no significant differences in the inhibition zone diameter formed between the test concentrations.
Keywords: *Antibacterial, Staphylococcus epidermidis, Lemongrass*

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INTRODUCTION

The prevalence of infections that occur in hospitals from all over the world ranges from 3-21% (an average of 9%) or about 1.4 million patients wherein the number of nosocomial infections in Indonesia reaches 15.74%[1]. Primarily, the main cause of that issue is *Staphylococcus epidermidis*. These bacteria are normal flora on human skin, but they can be opportunistic pathogens for individuals with low immune systems. In dealing with these issues, it is necessary to use appropriate antibiotics.

One of the topical antibiotics commonly used in acne treatment is clindamycin. In comparison, this antibiotic is more effective in treating acne than others, such as erythromycin and tetracycline, even though it can cause bacterial strains that are resistant to clindamycin in long-term use[2]. More than 2.8 million infections are found in the United States and at least 35,000 people have died because cannot be cured by resistant antibiotics[3]. Admittedly, it is necessary to discover an acne medicine that has a good working ability with minimal side effects. Exploring active compounds within natural ingredients as an antibacterial is a promising approach to address that issue.

Plenty of developing countries such as Indonesia use biodiversity as their medical treatment from generation to generation. The utilization of traditional and herbal medicines is widely found in ASEAN countries[4]. Admittedly, one of the plants that have an antibacterial effect is lemongrass (*Cymbopogon citratus (DC.) Stapf*). Extracts from its leaves contain saponins, flavonoids, polyphenols, alkaloids, and essential oils. Based on research, ethanol 90% extract from lemongrass stalks contains various secondary metabolites. In addition, it also has a Minimum Inhibitory Concentration (MIC) against *Staphylococcus aureus* is 4096 µg/mL and a Minimum Bactericidal Concentration (MBC) is over 4096 µg/mL[5]. Another study shows that ethanol 95% extract of lemongrass leaves contains flavonoids, phenols, saponins, glycosides, alkaloids, steroids, tannins, triterpenoids, and terpenoids. Furthermore, its MIC against *Staphylococcus aureus* is 150 mg/mL[6]. Moreover, ethanol 70% extract of lemongrass leaves has antibacterial activity against *Streptococcus mutans*

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and *Staphylococcus aureus*[7].

Based on the studies above, the compounds contained in the ethanolic extract of lemongrass leaves have the potential to be antibacterial of the genus *Staphylococcus* but there isn't any study has found the activity of this plant against the *Staphylococcus epidermidis*. The use of 70% ethanol as a solvent is also commonly used in various studies[8,9,10,11], some of which show high resistance[9,10]. Hence, this study was conducted to address that issue.

METHOD

The materials used in this study were distilled water, *Staphylococcus epidermidis* bacteria, ethanol 70%, $FeCl_3$ 1%, 2 N HCl, HCl P, H_2SO_4 P, H_2SO_4 2N, NaOH 1 N, chloroform, McFarland standard solution. 108 cells/mL, crystal violet paint, iodine, safranin, hydrogen peroxide (H_2O_2) 10%, Nutrient Broth media, Nutrient Agar (NA) media, Mueller Hinton Agar (MHA) media, dimethylsulfoxide (DMSO), clindamycin, paper disc blank, Magnesium powder, ammonia, Dragendorff reagent, Mayer reagent, NaCl 0.9%, and aqua pro injection and lemongrass leaves (*Cymbopogon citratus* (DC.) Stapf).

Extraction and Phytochemical Screening

The obtained sample was determined in number of B-664/V/DI.05.07/3/2022. The sample was extracted by maceration method in ethanol 70%. The filtrate concentrated with a rotary evaporator to obtain a thick extract and dried in an oven at a temperature of 50°C [12]. The value of % extract yield is then calculated its value. Phytochemical screening of the extracts carried out by testing for saponins, flavonoids, alkaloids, tannins, steroids and triterpenoids, phenols, and quinones.

NA and MHA

The Nutrient Agar (NA) medium was made by dissolving 14 g of it in 500 mL of boiled distilled water. Further, the solution was placed into a 500 mL Erlenmeyer flask, tightly closed with cotton, and sterilized in an autoclave at 121^{o}C at 15 psi pressure for 15 minutes[13]. Likewise, the Mueller Hinton Agar (MHA) medium was made by dissolving 38 g of it in 1000 mL of boiled distilled water. The solution was put into two erlenmeyer flasks scale and tightly closed

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with cotton and sterilized using an autoclave at 121^{o}C pressure 15 psi for 15 minutes[14].

Bacteria Suspension

Staphylococcus epidermidis bacteria were rejuvenated on Nutrient Agar (NA) medium in a petri dish and incubated for 24 hours at 37^{o}C. After 24 hours, one ose of bacteria from the NA medium was taken and suspended in 0.9% NaCl. Further, its turbidity is compared with a McFarland solution standard 0.5 (108 cells/mL)[14].

Antibacterial Assay

The antibacterial activity of the extract 70% ethanol of lemongrass (*Cymbopogon citratus* (DC.) Stapf) leave was measured through three stages of processing. First, the extract dissolved in various concentrations 200,000 ppm, 400,000 ppm, 600,000 ppm, 800,000 ppm, and 1,000,000 ppm. Second, the extracts were dripped onto a paper disc with a diameter of 6 mm, respectively, and then placed into a petri dish containing MHA medium that had been inoculated previously with *Staphylococcus epidermidis*. In addition, the positive control used was clindamycin (2µg in 20 µl)[15]. and the negative control used was 20 µl dimethyl sulfoxide (DMSO) 10% (distilled sterile aquadest). Third, after being stored for 24 hours at 37^{o}C, the diameter of the resistance was measured. The antibacterial activity identified by the clear zone that indicated bacterial growth is zero.

MIC and MBC Determination

The minimum inhibitory concentration is determined using the broth dilution method[16]. Initially, extract 70% ethanol of lemongrass leaves was added to tubes with a concentration of 5000 ppm, 2500 ppm, 1250 ppm, 626 ppm, 312.5 ppm, 156.25 ppm, 78.125 ppm, 39.06 ppm, and 19.53 ppm, respectively. Then, other tubes added a negative control containing bacterial suspension, positive control containing clindamycin and a blank solution containing extract 70% of lemongrass leaves (*Cymbopogon citratus* (DC.) Stapf)[17].

Moreover, each tube containing the extract, positive control, negative control, and bacterial suspension was then vortexed. Former tubes were then added with 0.2 mL suspension of *Staphylococcus epidermidis* which was equivalent to a standard McFarland

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turbidity 0.5. Later tube with a bacterial suspension was incubated at 37^{o}C for 24 hours. Afterward, the tube was removed and then observed for its turbidity visual appearance. The tube which is clearer than the positive control tube is stated as MIC.

Likewise, the minimum bactericidal concentration (MBC) determined using sub-cultured each tube on MHA media in petri dishes over anaerobic conditions. Further, the subcultures were incubated at 37^{o}C for 24 hours. Bacterial growth was seen on MHA media after 24 hours. Petri dishe that do not contain bacterial growth is stated as MBC[14].

Statistical Data Analysis

The statistical data analysis was processed in Microsoft Excel. Further, the normality test analyzed by the Shapiro-Wilk test, it's followed by the One-Way ANOVA test or the Kruskal-Wallis test depending on the normality result. Moreover, the data analyzed by homogeneity of variance test by Levene's test.

RESULT AND DISCUSSION

Extraction of the Lemongrass Leaf

Lemongrass leaf samples were obtained from Tigaraksa, Tangerang. The water content in simplicia is 8%. Hence, it has met the stated requirements based on the literature[18], where the percentage of water content contained in simplicia should not be more than 10%.

Table 1. Phytochemical Result

Secondary Metabolites	Results
Saponins	+
Flavonoids	+
Alkaloids	+
Tanins	+
Steroids dan Triterpenoids	+
Phenol	+
Quinone	+

Notes: (+) = detected; (-) = not detected

The results of the filtrate and the percentage yield of the extract are shown in Table 1. The percentage yield obtained is less than 17.2% compared to the literatur[18]. In addition, the percent yield provides information of the ability on the solvent to attract compounds in the extract. On the other hand, phytochemical test results are shown in Table 2.

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Antibacterial Assay

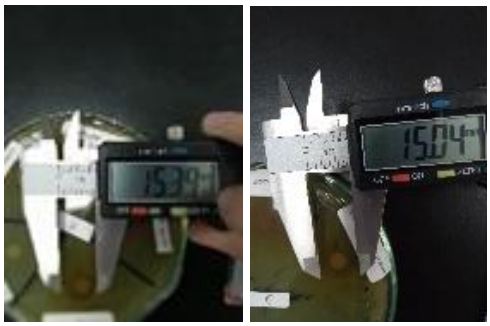

Figure 1. Antibacterial assay

Antibacterial activity the extract 70% ethanol of lemongrass leaves against the *Staphylococcus epidermidis* growth tested in concentrations of 200,000 ppm, 400,000 ppm, 600,000 ppm, 800,000 ppm, and 1,000,000 ppm. The inhibition zone diameter of the extract at a concentration of 1,000,000 ppm was greatest than that of others. Hence, the results indicated enhanced antibacterial activity at higher concentrations. The results of the antibacterial activity assay are shown in Table 3 and Figure 1.

The research on extract 96% ethanol of lemongrass against *Staphylococcus aureus* through the lotion formula at a concentration of 1% (w/v) or 10,000 ppm lemongrass extract showed an average inhibition zone diameter of 10 mm, while at a concentration of 5% (w/v) or 50,000 ppm showed 18.6 mm[19]. Further, another study on the antibacterial properties of extract 70% ethanol of lemongrass leaves (*Cymbopogon citratus*) against *Staphylococcus aureus* found that at a concentration of 25 mg/mL or 25,000 ppm showed an inhibitory diameter of 14 mm, while at a concentration of 50,000 ppm showed 15 mm, and lastly at a concentration of 100,000 ppm showed 17 mm. Compared to this study, all previous studies showed a larger diameter of the inhibition zone even in at lower concentrations[20]. On the other hand, the antibacterial assay of

Table 2. Antibacterial Assay

Repetition of Measure-ment	DMSO	Inhibition zone diameter (mm) Extract Concentration (ppm)					Clinda-mycin
		200.000	400.000	600.000	800.000	1.000.000	
I	0	7,995	8,745	8,82	12,435	15,06	15,79
II		8,285	8,665	9,19	11,11	15,235	
III		8,345	8,745	9,69	10,81	15,07	
IV		8,32	8,785	9,52	12,74	15,17	
Mean ±SD	0	8,234	8,735	9,305	11,774	15,134	15,79
Category	*R*	*R*	*R*	*R*	*R*	*I*	*I*

Note: *Resistant (R), Intermediate (I),* (CSLI, 2020)

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extract 96% ethanol avocado leaf shown almost similar inhibitory zone, 8,50±0,28 mm at 10%[21]. This is presumably due to the different types of bacteria used. Although the bacteria used both belong to the genus *Staphylococcus*, *Staphylococcus epidermidis* can protect itself by forming a biofilm as a bacterial defence from the immune system to carry out phagocytosis. Further, the results of statistical analysis showed that there was no significant difference between the concentrations of 70% ethanol extract of lemongrass leaves (*Cymbopogon citratus* (DC.) Stapf) which had been used. Hence, the concentration of 200,000 ppm was the most appropriate concentration to be used as an antibacterial to against *Staphylococcus epidermidis*.

MIC and MBC Result

The concentration of 5000 ppm has chosen as the upper limit of measurement based on preliminary tests. Observations were carried out according to the previous method described. Further, a concentration of 25% or 1250 ppm showed clarity comparable to the blank. Hence, it is appointed as the minimum inhibitory concentration (MIC)[14] (Table 4).

The previous research on lemongrass stalks using 90% ethanol solvent found that the MIC against *Staphylococcus aureus* observed at a concentration of 4096 g/mL[5]. This concentration where the MIC had observed is greater than the result of MIC in this study. Another study showed that

Table 3. Minimum Inhibitory Concentration (MIC)

Measurement variations	Result	MIC Category (CSLI, 2020)
DMSO (Negative Control)	+	*resistant*
Clindamycin (Positive Control)	-	*intermediate*
Extract 5000 ppm	-	*resistant*
Extract 2500 ppm	-	*resistant*
Extract 1250 ppm	-	*resistant*
Extract 625 ppm	+	*resistant*
Extract 312.5 ppm	+	*resistant*
Extract 156.3 ppm	+	*resistant*
Extract 78.1 ppm	+	*resistant*
Extract 39 ppm	+	*resistant*
Extract 19.5 ppm	+	*resistant*

Notes: (+) Cloudy (there is bacterial growth); (-) Not cloudy (no bacterial growth)

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lemongrass leaf extract with 95% ethanol solvent had a MIC of 150,000 g/mL against *Staphylococcus aureus*[6]. Compared to both studies above, it is indicated that the secondary metabolites in the lemongrass plant limited be extracted in semi-polar solvents. Hence, that large amounts of extract are needed to achieve the MIC. This indication is aligned with the literature that discusses that 70% ethanol is effectively used as a solvent in the extraction process[22].

Further, the MBC determined at concentration of 100% or 5000 L/mL or 5000 ppm (Figure 1). This determination is based on appearance the petri dish that containing extract concentration of 5000 ppm which looks clear, that indicated there is no bacterial growth[14]. Previous research on lemongrass stems with 90% ethanol as a solvent found that the MBC concentration against *Staphylococcus aureus* was >4096 g/mL[6]. Thus, the data obtained in this study is aligned with previous study. MIC and MBC data are attached in Table 4 and Figure 1, respectively.

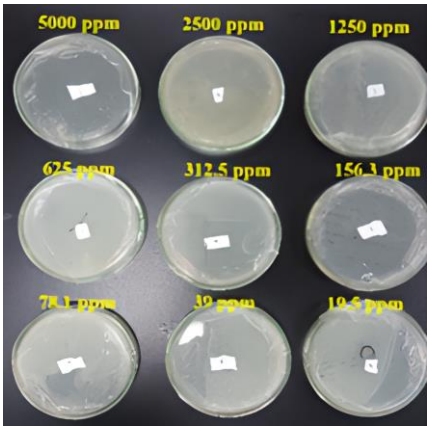

Figure 2. MBC Result

STATISTICAL ANALYSIS

The data distribution tested by Shapiro-Wilk, and it found that the data were not normally distributed. Moreover, the data homogeneity tested by Levene's-test, and it was found that the data were not homogeneous, hence, the statistical analysis was continued with the Kruskal-Wallis test, and it obtained the H value was 18.2857. The data analysis showed that between all tested concentrations antibacterial activity of the extract there was not significantly difference.

CONCLUSION

Overall, the extract 70% ethanol of lemongrass leaves (*Cymbopogon citratus* (DC.) Stapf) has antibacterial activity against *Staphylococcus epidermidis* in intermediate category (1.000.000 ppm). Furthermore, based on the statistical analysis, the concentration of 200,000

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ppm was the most appropriate concentration to be used as an antibacterial to against *Staphylococcus epidermidis*. The concentration of 25% or 1250 ppm determined as MIC. Then, the concentration of 100% or 5000 ppm determined as MBC.

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