Antibacterial Activity Test of Ethanol Extract of Lemongrass Leaves (*Cymbopogon nardus* (L.) Rendle) Against *Staphylococcus epidermidis*

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Abstract. Bacterial infection is one of many people's most common health problems, especially in developing countries, including Indonesia. Staphylococcus epidermidis is one of the bacteria that causes infection. Lemongrass is a plant with several active compounds that can be use for treatment, one of which is an antibacterial. This research aimed to determine the antibacterial activity of lemongrass leaves ethanolic extract against Staphylococcus epidermidis. This type of research is a laboratory experiment using the disc diffusion method. The research stage includes making lemongrass leaves extract with variations concentrations of 5%, 10%, 20%, 40%, and 80%, phytochemical screening, and antibacterial activity testing. Data from the research were analyzed using One Way ANOVA. The results showed that the ethanol extract of Lemongrass leaves positively contained flavonoids, tannins, saponins, and essential oils. The results showed that lemongrass leaves extract had an inhibitory effect on the growth of Staphylococcus epidermidis at concentrations of 10%, 20%, 40%, and 80% with an inhibition zone diameter of 8.15 mm (moderate), 13.25 mm (substantial), 16.9 mm (concrete) and 20.6 mm (powerful), but at a concentration of 5% does not show antibacterial activity. Based on the One Way ANOVA testing results, the significance value was ($p < 0.05$), and the results showed a significant difference between the concentration variations of ethanolic extract lemongrass leaves against Staphylococcus epidermidis.

Keywords: Antibacterial activity · Ethanol extract of lemongrass leaves · Staphylococcus epidermidis

1 Introduction

Bacteria is one type of microbe that can cause disease for today's society, especially in developing countries such as Indonesia [1]. In our body we have normal microorganisms or normal flora. This normal flora is generally harmless, but under certain conditions it can be pathogenic and endanger health because it can cause diseases such as infections [2]. One of the microorganisms that can cause infection is Staphylococcus epidermidis bacteria. Several types of diseases that are often caused by these bacteria are swelling (abscesses) such as acne, urinary tract infections, skin infections and kidney infections [3].

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Indonesia is a country that has various types of plants that can be used as herbal medicine ingredients. One of the plants that is often used in herbal medicine is lemongrass. Empirically this plant is often used by the public to treat digestive disorders such as constipation and stomach pain, it is also used in making deodorants because it can control sweat and the oil from the fragrant citronella plant is often used as aroma therapy [4]. Based on the results of phytochemical screening research conducted by Zamzani [5] using thin layer chromatography, it showed that the secondary metabolite compounds contained in the ethanolic extract of the citronella plant were saponins, flavonoids, polyphenols and essential oils. The content of these secondary metabolites indicates that citronella has a fairly large antibacterial activity.

According to previous research conducted by Fitria and Febrianti (2020) [6], showed that 70% ethanol extract of fragrant lemongrass leaves had antibacterial activity against Staphylococcus aureus bacteria. The results of research by Winato et al. (2019) [7], stated that 96% ethanol extract of fragrant lemongrass leaves had an inhibitory effect on the growth of Propionibacterium acnes which causes acne. Another study by Mayasari and Sapitri (2019) [8] found that at concentrations of 20%, 30%, 40% and 50% lemongrass leaf juice was able to inhibit Streptococcus mutans bacteria. Based on this background, it is necessary to test the antibacterial activity of citronella leaves against other gram-positive bacteria, namely Staphylococcus epidermidis.

2 Method

A. *Equipments sterilization*

Glass-based tools were washed using detergent and rinsed with clean water, then dried in the open air and sterilized in an autoclave at 121℃ for 15 min. All equipment is packaged to prevent recontamination after exiting the autoclave. For tools such as tweezers and needles, they are sterilized by incandescent with direct fire.

B. *Sample Preparation*

The sample was taken from the Gayam area, Yogyakarta. Harvesting is done in the morning at 09.00 am. And the age of harvest is 7 months. A total of 500 g of citronella leaf powder samples were macerated in 5 L of 96% ethanol solvent for 3 days. The extraction results are filtered, concentrated and the percentage yield is calculated using the following formula:

$$\%\ \text{Yield} = \frac{\textit{thick extract weight (gram)}}{\textit{initial simplicia weight (gram)}} \times 100\%$$

C. *Phytochemical Screening*

Phytochemical screening consisted of identification of flavonoid compounds using concentrated Mg and HCl powder, alkaloid compounds with 2N HCl and Mayer reagent, tannin compounds with 1% FeCl3, saponin compounds using white foam formation

method, triterpenoid compounds using acetic anhydride and concentrated H2SO4 and volatile oil compounds with evaporation method over the water bath.

D. *Preparation of Stock Solutions*

The test stock solution using a concentration of 100% w/v was prepared by dissolving 10 g of ethanolic extract of citronella leaves in 10 ml of 10% DMSO (negative control). The concentrations used in this study were 5%, 10%, 20%, 40% and 80%. The concentration solution of ethanol extract of citronella leaves was made by taking the test stock solution according to the calculation of the dilution of each concentration and then adding 10% DMSO until the volume was 5 ml.

E. *Media Preparation*

Weighed as much as 1.7 g of NA media powder, then put into an erlenmeyer and added 60 ml of distilled water, then heated on a magnetic stirrer while stirring until the NA medium dissolved completely. The NA media solution was covered with aluminum foil and sterilized in an autoclave for 15 min at 121 °C [7].

F. *Antibacterial Activity Testing*

Prepared petri dishes and NA media that have been sterilized. 10 ml of NA medium was taken and poured into each sterile petri dish and allowed to solidify. 1 ml of the test bacterial suspension was taken and spread over the solidified NA medium in a petri dish, then flattened using a drigalsky rod. Prepared 5 paper discs and then dipped into each erlenmeyer containing extracts whose concentrations had been determined, namely 5%, 10% 20%, 40%, 80% and 10% DMSO negative control. Place the disc paper on the surface of the NA medium and press it slightly. The petri dish was then incubated at 37 °C 9 for 1 × 24 h [9].

G. *Measurement of Dieameter Inhibition Zone*

The diameter of the inhibition zone or the clear zone formed around the paper disc was measured using a caliper.

3 Result and Discussion

A. *The results of the determination of lemongrass*

The determination of the sample was carried out at the Plant Systematics Laboratory, Faculty of Biology UGM, Yogyakarta. Determination aims to prove that the plant used in this study is the correct plant, namely Cymbopogon nardus (L.) Rendle. This is done to determine the correctness of the plant species studied. The results of the determination stated that the plant used was true lemongrass from the family Poaceae with the species Cymbopogon nardus.

B. *The results of the extraction of fragrant lemongrass leaves*

The process of extracting lemongrass leaves was carried out using the maceration method. This method was chosen because it is easy, simple and inexpensive, that is,

it does not require special tools to carry out the extraction process. In this study, maceration was carried out using 96% ethanol as solvent because this solvent can extract almost all simplicia content, both non-polar, semi-polar and polar. This solvent is selective, non-toxic and universal which is suitable for extracting all classes of secondary metabolites [10]. The maceration was carried out for 3x24 hours, then the maceration results were filtered and concentrated over a water bath to obtain a thick extract as shown in Fig. 1 with a weight of 39 g, blackish green in color, with a typical simplicia aromatic smell with an extract yield value of 7.8% (Table 1).

C. *Phytochemical Screening Results*

Phytochemical screening aims to determine the content of secondary metabolites contained in the ethanol extract of lemongrass leaves. Based on Table 2, the results of the phytochemical screening test in this study showed that the ethanolic extract of fragrant lemongrass leaves contained secondary metabolites, namely flavonoids, tannins, saponins and essential oils, while triterpenoids and alkaloids were not detected. Undetectable alkaloid compounds are possible because alkaloids are present in relatively low amounts and the phytochemical screening method used is less sensitive to detect these compounds. Triterpenoid compounds could not be detected in this study because a good solvent used to extract triterpenoids is ether or chloroform [11].

The mechanism of action of flavonoids as antibacterial is by the interaction between flavonoids and bacterial DNA, it can damage the permeability of the bacterial cell wall and the hydroxyl group contained in the structure of flavonoid compounds is able to make changes in organic components and nutrient transport so that it can cause toxic effects on bacteria [12] Tannin compounds are polymers of phenolic compounds that

Fig. 1. The extract thick lemongrass leaves (private doc.)

Table 1. The results of the organoleptic test of the extract thick fragrant lemongrass leaves

Organoleptic	**Observations**
Color	Dark green
Form	Thick Extract
Scent	Scented typical simplicia

Table 2. Phytochemical screening test results

Test Compounds	Testing	Result	Description
Flavonoid	Mg powder +	Formed a	+ +
	concentrated	blackish red	
	HCl	color	
Alkaloid	2N HCl +	Formed a	-
	Mayer's	green or blue	
	reagent	color	
Tanin	1% FeCl3	Formed a	+ +
	reagent	blackish	
		green color	
Saponin	distilled water	Formed a	+
	+ shaken	stable foam	
	vigorously		
Triterpenoid	acetic	A white	-
	anhydride +	precipitate	
	concentrated	was formed	
	H2SO4		
Essential Oil	The results of		+ +
	the maceration	There is a	
	are evaporated	distinctive	
	over the	smell	
	waterbath		

have the ability to inactivate bacterial cell adhesion, inactivate enzymes and interfere with protein transport in the inner layer of cells. Tannin compounds also attack cell wall polypeptides so that the formation of cell walls becomes less than perfect. This causes the bacterial cell to lyse due to osmotic pressure and physical pressure so that the bacterial cell will die [13].

Saponin compounds have the ability to damage the cytoplasmic membrane, resulting in a decrease in the permeability of the bacterial cell membrane and substances present in the cell such as enzymes, organic ions, amino acids and nutrients leaving the cell. When cells release enzymes along with substances such as water and nutrients, metabolism is hampered and causes a decrease in ATP needed for cell growth and proliferation, causing bacterial cell death [13]. Essential oils are active as antibacterial because they contain hydroxyl (-OH) and carbonyl functional groups which are phenol derivatives that can interact with bacterial cells through an adsorption process involving hydrogen bonds. At low levels, phenol protein complexes are formed with weak bonds and immediately

Table 3. Diameter of inhibition zone of *Stahpylococcus epidermidis* using ethanol extract of lemongrass leaves. Inhibition zone diameter (mm).

Extract Concentration	**R1**	**R2**	**R3**	$\overline{\mathbf{X}} \pm$ **SD**	**Antibacterial Potential**
5%	0	0	0	0,00 ± 0,00	None
10%	8,36	7,21	8,90	8,16 ± 0,86	Moderate
20%	13,50	12,30	13,95	13,25 0,85	Strong
40%	16,40	17,50	16,90	16,93 ± 0,55	Strong
80%	20, 60	19, 80	21, 50	20, 63 ± 0,85	Very Strong
Negative control	0	0	0	0,00 ± 0,00	None

Information:
Control (-): DMSO (Dimethyl Sulfoxide) 10%
R1: First Replication
R2: Second Replication
R3: Third Replication

undergo decomposition, followed by phenol penetration into cells and cause precipitation and protein denaturation. At high levels it can cause protein coagulation and cell membranes will undergo lysis [14].

D. *Antibacterial activity test results*

Based on Table 3, the results of the antibacterial activity test of lemongrass leaf extract against Stahpylococcus epidermidis at a concentration of 5% showed no response to the bacterial inhibition zone, at a concentration of 10% indicated a moderate response to the bacterial inhibitory zone (8.15 mm), at a concentration of 20% indicates a strong bacterial inhibition zone of (13.25 mm), at a concentration of 40% indicates a strong bacterial inhibition zone of 16.9 mm, at a concentration of 80% indicates a very strong bacterial inhibition zone of (20.6 mm). The negative control did not show the inhibition zone formed. This shows that DMSO does not have antibacterial activity, so it can be ascertained that the antibacterial activity produced is not influenced by DMSO as an extract solvent. According to Nugroho et al. (2022) [15], the higher the concentration of the extract, the more compounds the extract has so that the antibacterial activity is greater

and the diameter of the inhibition zone formed is also wider. This is in accordance with the results in this study which showed that citronella leaf extract at a concentration of 80% produced the largest inhibition zone. The choice of DMSO as the extract solvent in this study was because this solvent was able to dissolve both polar and non-polar compounds [16]. The concentration of DMSO used was 10% because DMSO 10% is an organic solvent that does not have bactericidal properties (Fristiohady et al., 2020).

One Way ANOVA test is a way to determine whether or not there are significant differences between the treatment groups tested for the growth of Stahpylococcus epidermidis bacteria. Based on the test, the probability value (p) = 0.000 is < 0.05. This value indicates that the ethanolic extract of fragrant lemongrass leaves has an antibacterial effect against Staphylococcus epidermidis bacteria and there are significant differences in at least 2 treatment groups tested against Staphylococcus epidermidis, it is necessary to carry out the next test, namely the Post Hoc test.

The Post Hoc test was conducted with the aim of knowing which treatment groups had a significant or significant difference with a 95% confidence level. Based on the test results showed that each group of ethanolic extract concentration of citronella leaf, namely 5%, 10%, 20%, 40% and 80% had a significant difference in inhibition against Stahpylococcus epidermidis bacteria, except the negative control did not have a significant difference to 5% concentration. This is because at a concentration of 5% does not have the ability to inhibit the bacterium Stahpylococcus epidermidis.

4 Conclusion

The ethanolic extract of citronella leaves had antibacterial activity against Staphylococcus epidermidis bacteria at concentrations of 10%, 20%, 40% and 80%, while at a concentration of 5% there was no antibacterial activity. The use of variations in the concentration of ethanolic extract of citronella leaves showed a significant difference in inhibiting the growth of Staphylococcus epidermidis bacteria. Secondary metabolite compounds contained in the ethanol extract of fragrant lemongrass leaves are flavonoids, tannins, saponins and essential oils.

References

1. Katrin, D., Idiawati, N., dan Sitorus, B. (2015), Uji Aktivitas Antibakteri Ekstrak Daun Malek (*Litsea graciae Vidal*) terhadap Bakteri *Stapylococcus* dan *Escherichia coli*, *Jkk : Jurnal Kimia Khatulistiwa*, 8:7-12
2. Pratiwi, S. T. (2008), *Mikrobiologi Farmasi*, Penerbit Erlangga, Jakarta.
3. Radji, M. (2011), Buku Ajar Mikrobiologi Panduan Mahaisiwa Farmasi dan Kedokteran, Penerbit EGC, Jakarta.
4. Suharman. (2020), Tanaman Potensial Berkhasiat Obat Cengkeh, Temulawak, Jahe, Kunyit, Kencur dan Serai, CV. Budi Utama, Yogyakarta.
5. Zamzani, M. C. (2011), Aktivitas Antibakteri Ekstrak Etanol Tanaman Serai *(Cymbopong nardus* (L.) Rendle*)* terhadap Pertumbuhan *Staphylococcus aureus* dan *Escherichia coli* Multiresisten serta Bioautografinya, *Skripsi*, Fakultas Farmasi, Universitas Muhammadiyah Surakarta.

6. Fitria, Y., dan Febrianti, Y. (2020), Antibacterial Activity Lemongrass Leaves of *Staphylococcus aureus* Inhibition One, *JPBIO : Jurnal Pendidikan Biologi*, 177:176-183.
7. Winato, B. M., Sanjaya, E., Siregar, L., Fau, S. K. Y. M. V., dan Mutia, D. M. S. (2019), Uji Aktivitas Antibakteri Ekstrak Daun Serai Wangi (*Cymbopogon Nardus*) terhadap Bakteri *Propionibacterium acnes*, *BioLink : Jurnal Biologi Lingkungan Industri Kesehatan*, 53:50-58.
8. Mayasari, U., dan Sapitri, A. (2019), Uji Aktivitas Antibakteri Daun Sereh Wangi (*Cymbopogon nardus*) Terhadap Pertumbuhan Bakteri Streptococcus mutans, *Jurnal Klorofil*, *3*(2), 15–19.
9. Kamal, S. E., dan Tiara, D. L. (2019), Uji Aktivitas Antibakteri Ekstrak Buah Pepino (*Solanum Muricatum* Ait) terhadap Pertumbuhan Bakteri *Escherichia coli*, *Jurnal Farmasi Sandi Karsa*, 16:15-18.
10. Kapondo, G. L., Fatimawali, F., dan Jayanti, M. (2020), Isolasi, Identifikasi Senyawa Alkaloid dan Uji Efektivitas Penghambatan dari Ekstrak Daun Sirih (*Piper betle* L.) terhadap Bakteri *Staphylococcus epidermidis*, *Jurnal e-Biomedik*, 185:180-186.
11. Harborne, J. B. (1987), *Metode Fitokimia* (Edisi II), Penerbit ITB, Bandung.
12. Zainab. (2022), Monograf Khasiat Kandungan dan Aktivitas Antibakteri pada Ekstrak Daun Kelambu Menjangan, Penerbit NEM, Jakarta.
13. Jafriati. (2022), Ekstraksi Senyawa pada Thalassia hemprichii dan Salmonella typhi, CV. Literasi Nusantara Abadi, Malang.
14. Parwata, O. A., dan Dewi, P. F. S. (2008), Isolasi dan Uji Aktivitas Antibakteri Minyak Atsiri dari Rimpang Lengkuas (*Alpinia galanga* L.), *Jurnal Kimia*, 102:100-104
15. Nugroho, H. P., Fauziah, P. N., dan Alislam, M. A. (2022), Aktivitas Antibakteri Ekstrak Etanol Daun Jambu Biji (*Psidium guajava* L.) pada Bakteri *Salmonella typhi* ATCC 14028, *Jurnal Ilmiah Analis Kesehatan*, 92:88-101.
16. Assidqi, K., Tjahjaningsih, W., dan Sigit, S. (2012), Potensi Ekstrak Daun Patikan Kebo (*Euphorbia hirta*) sebagai Antibakteri terhadap Aeromonas hydrophila secara In Vitro, *Journal of Marine and Coastal Science*, *1*(2), 113–124.