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Original Article

In Vitro Testing of the Antibacterial Activity of Ethanol Extract of Lontar Leaves *(Borassus flabellifer)* Against *Staphylococcus aureus*

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Abstract

Background : *Staphylococcus aureus* is a bacterium that frequently causes infections and often develops resistance to antibiotics. Efforts to identify alternative treatments using herbal remedies are increasing. In Indonesia, a country rich in biodiversity, lontar leaves *(Borassus flabellifer)* have been traditionally used and show potential antibacterial activity. The aims of this study was to evaluate the antibacterial activity of ethanol extract of lontar leaves against *Staphylococcus aureus* in vitro.
Methods : This experimental study employed a post-test-only control group design. Antibacterial activity was tested using the well diffusion method. Extracts were obtained through maceration with 96% ethanol and tested at 75%, 50%, and 25% concentrations. Ciprofloxacin was used as the positive control, and 10% DMSO as the negative control.
Results : The inhibition zone diameters were 21.86 mm (75%), 19.64 mm (50%), and 18.37 mm (25%). The positive control (ciprofloxacin) measured 24.43 mm, while the negative control (DMSO) showed 0 mm.
Conclusion : The 96% ethanol extract of lontar leaves demonstrated antibacterial activity against *Staphylococcus aureus*, with higher concentrations yielding stronger inhibition.

Keywords : *Borassus flabellifer*, antibacterial activity, *Staphylococcus aureus*, herbal extract, in vitro study

INTRODUCTION

Staphylococcus aureus is one of the most commonly ncountered bacteria of clinical significance due to its diverse clinical manifestations.[1] It is part of the normal human microbiota, with 60% of healthy individuals having *S. aureus* colonized on their skin, particularly in moist areas.[2–4] Although *S. aureus* is a normal component of human microbiota, it can become pathogenic and infect humans.[4] Transmission of *S. aureus* primarily occurs via direct skin-to-skin contact rather than through the air, leading to various conditions ranging from minor skin infections to life-threatening diseases such as meningitis.[2,4] Local skin infections caused by *S. aureus* include impetigo, folliculitis, abscesses, and cellulitis.[5]

Treatment of *Staphylococcus aureus* infections presents a challenging issue due to the bacterium's ability to rapidly adapt to antibiotic treatments, leading to the emergence of resistant strains such as MRSA *(methicillin-resistant Staphylococcus aureus)*. According to the WHO, MRSA is a pathogen that requires special attention in its treatment.[6] Epidemiological studies over the past two decades have shown a significant increase in MRSA prevalence in the United States, reaching up to 40%. In Asia, MRSA incidence is reported to be the highest in the world. Data from the Asian Network for Surveillance of Resistant Pathogens (ANSORP) indicate that in Southeast Asia, particularly Indonesia, MRSA prevalence is approximately 28%.[7–9]

Due to this bacterium's increasing prevalence and antibiotic resistance, researchers are investigating alternative antibiotics. This necessitates the exploration of other substances that could serve as alternative antibiotics to inhibit or eliminate the growth of *Staphylococcus aureus*, such as herbal plants.[10]

In Indonesia, particularly in South Sulawesi, there is a plant that serves as a botanical symbol of the region: the lontar palm. Every part of the lontar plant from its roots to its leaves is utilized for various purposes. It is used in daily activities such as construction, traditional ceremonies, and herbal remedies. The lontar plant is utilized as a herbal medicine due to its pharmacological activities in its flowers, fruit, leaves, roots, and seeds, including antioxidant, antibacterial, antifungal, anti-arthritis, anti-infland immunomodulatory effects.[11]

Lontar fruit extract demonstrated antibacterial effects against *Staphylococcus aureus*. This is attributed to compounds in the fruit that exhibit antibacterial properties, including alkaloids, flavonoids, tannins, triterpenoids, and saponins.[12] Additionally, phytochemical analysis of lontar leaves revealed a similar profile of compounds, including flavonoids, glycosides, tannins, proteins, steroids, triterpenoids, carbohydrates, fats, and oils.[10] These compounds in the lontar leaves have potential antibacterial activity. Supporting this, one study demonstrated the broad-spectrum antimicrobial potential of *Borassus flabellifer* extract, including activity against several Gram-negative bacteria. Although the study focused on multiple pathogens, the findings support the plant's potential role in inhibiting *Vibrio cholerae* growth in vitro due to its rich phytochemical profile.[11]

Unfortunately, studies investigating the antibacterial properties of *Borassus flabellifer* (lontar) leaf extract remain limited. To date, no substantial research has been conducted to explore its activity against *Staphylococcus aureus*, a Gram-positive bacterium commonly associated with antibiotic resistance. Further investigation using resistant bacterial strains is needed to uncover the potential of plant-based therapeutics in addressing antimicrobial resistance. Based on this gap, the current study aims to evaluate whether ethanol extract of lontar leaves exhibits inhibitory effects on the growth of *Staphylococcus aureus*.

In light of this evidence gap, the present study aimed to evaluate the in vitro antibacterial activity of ethanol extract of lontar leaves against the Gram-positive bacterium *Staphylococcus aureus*.

METHODS

This study employed a true experimental design using a post-test-only control group approach to evaluate the antibacterial activity of lontar leaf extract *(Borassus flabellifer)* against *Staphylococcus aureus*. The research was conducted from September to November 2023 at the Microbiology Laboratory and the Natural Pharmaceutical Materials Laboratory of the Pharmacy Undergraduate Program, Universitas Muslim Indonesia. Ethical approval was obtained from the Health Research Ethics Committee of the Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Makassar, under the number 378/UM.PKE/VIIII/45/2023.

The samples used in this study consisted of lontar leaf extract and *Staphylococcus aureus* ATCC 25923, which were cultured on Nutrient Agar and incubated at 37°C for 24 hours. The inclusion criteria required the use of uncontaminated and viable *S. aureus* colonies, while the exclusion criteria involved the removal of non-growing bacterial cultures. Dried lontar leaves were subjected to maceration using 96% ethanol for approximately three days to obtain the crude extract. The filtrate was then evaporated to remove the solvent, and the resulting extract was analyzed through phytochemical screening to identify active compounds such as tannins, steroids, flavonoids, and saponins.

Following extraction, the crude extract was diluted using 10% DMSO to obtain the concentrations of 25%, 50%, and 75%. Ciprofloxacin was used as the positive control, and 10% DMSO served as the negative control. The antibacterial activity was evaluated using the well diffusion method. A standardized suspension of

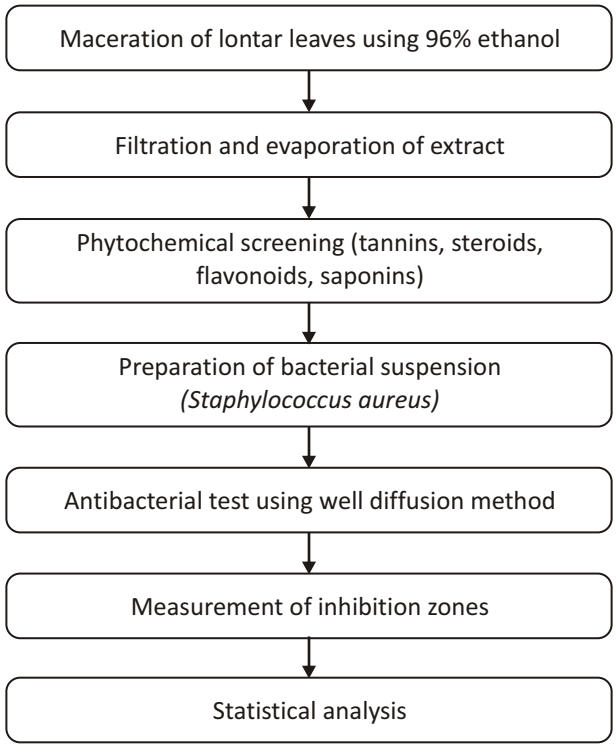

Figure 1. The Flowchart Of The Data Collection

Staphylococcus aureus was evenly spread on Nutrient Agar plates, and wells were created using a sterile cylinder. Each well was filled with 100 µL of the respective extract concentrations, positive control, and negative control. The plates were then incubated at 37°C for 24 hours. Zones of inhibition were measured using a digital caliper, and their diameters were interpreted based on the Greenwood classification: inhibition zones greater than 20 mm were considered strong, 16–20 mm moderate, 10–15 mm weak, and less than 10 mm indicated no inhibition.

Data obtained from the five groups was analyzed using SPSS software. The normality of the data distribution was tested using the Shapiro-Wilk test due to the sample size being fewer than 50. Homogeneity of variances was tested using Levene's test. Since the data was normally distributed and homogenous, parametric testing was conducted using one-way ANOVA, followed by the LSD post hoc test to determine specific group differences. A p-value of less than 0.05 was considered statistically significant at a 95% confidence interval.

The instruments used in this study included a rotary evaporator, incubator, autoclave, digital caliper, analytical balance, micropipettes, petri dishes, and sterile cotton swabs. The materials used consisted of lontar leaves, 96% ethanol, Nutrient Agar (Oxoid), *Staphylococcus aureus* ATCC 25923, ciprofloxacin, and 10% DMSO.

RESULTS

In the antibacterial activity test, ethanol extract of lontar leaves *(Borassus flabellifer L.)* was used at 25%, 50%, and 75%. Ciprofloxacin was the positive control, while 10% DMSO was the negative control. The results are presented in the Tabel 1.

To conduct the One-Way ANOVA hypothesis test, normality testing using the Shapiro-Wilk test and homogeneity testing using Levene's test were performed, with *p*-values >0.05. Since the results of both normality and homogeneity tests met the criteria, parametric testing using One-Way ANOVA was conducted. The ANOVA test yielded a *p*-value <0.001, indicating significant differences among the five treatment groups. To further determine if there are significant differences between specific treatment groups, a post-hoc LSD test will be conducted.

DISCUSSION

In this study, the ethanol extract of lontar leaves was prepared using the maceration method with 96% ethanol as the solvent. Maceration is a simple extraction technique that does not involve heating, thus preserving the secondary metabolites in the plant. Ethanol is considered a universal solvent capable of extracting both polar and non-polar compounds. It is less toxic compared to other solvents, such as methanol and water, allowing

TABLE 1
Results of the Diameter Measurement of Inhibition Zones for Various Concentrations of 96% Ethanol Extract of Lontar Leaves Against Staphylococcus aureus Growth

Extract Concentration	Diameter of inhibition zone (mm)					Average	Description	*P* Value**
	1*	2*	3*	4*	5*			
K1	18.35	18.35	18.33	18.63	18.21	18.37	Moderate	<0.001
K2	19.65	19.89	19.33	19.85	19.51	19.64	Moderate	
K3	21.95	21.68	21.96	22.01	21.73	21.86	Strong	
K (+)	24.28	23.99	24.45	24.69	24.76	24.43	Strong	
K (-)	0	0	0	0	0	0	Not Inhibit	

Note :
K1 : Group with 25% concentration of ethanol extract of lontar leaves
K2 : Group with 50% concentration of ethanol extract of lontar leaves
K3 : Group with 75% concentration of ethanol extract of lontar leaves
K(+) : Positive control (Ciprofloxacin)
K(-) : Negative control (10% DMSO)
* : Replicate
** : One-Way ANOVA test (significant if $p < 0.05$)

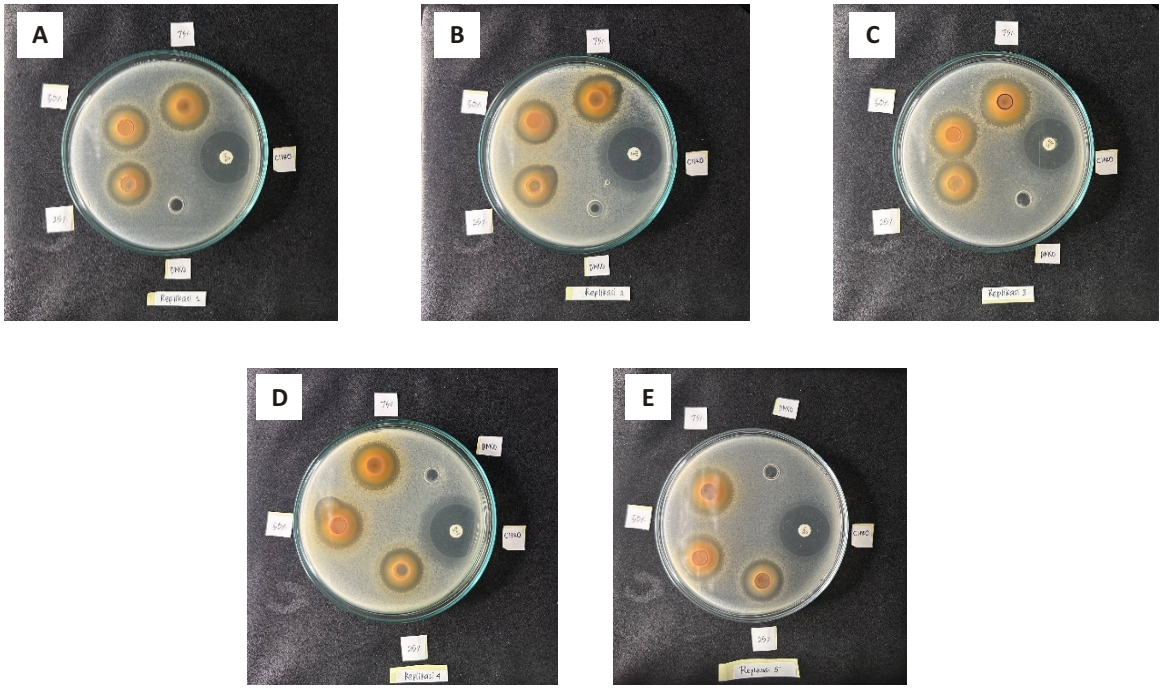

Figure 2. Replication (A. Replication 1, B. Replication 2, C. Replication 3, D. Replication 4, E. Replication 5)

for a higher yield of metabolites.[13]

Several previous studies have assessed the effect of ethanol concentration on the extraction of bioactive compounds. One study found that 96% ethanol extracted a higher flavonoid content from red dragon fruit peel than 70% ethanol.[14] While previous studies reported better results using 70% ethanol for flavonoid extraction from *Centella asiatica* and *Sargassum polycystum*.[13,15] In contrast, 96% ethanol has also been shown to be effective in extracting terpenoids and tannins from red ginger

TABLE 2
Results of the Post-Hoc Least Significant Difference (LSD) Test

Treatment		Differences Average	Confidence Interval 95%		*P* Value**
I	II		Min	Max	
K1	K2	-1.27200*	-1.6703	-0.8737	<0.001
	K3	-3.49200*	-3.8903	-3.0937	<0.001
	K+	-6.06000*	-6.4583	-5.6617	<0.001
	K-	18.37400*	17.9757	18.7723	<0.001
K2	75%	-2.22000*	-2.6183	-1.8217	<0.001
	K+	-4.78800*	-5.1863	-4.3897	<0.001
	K-	19.64600*	19.2477	20.0443	<0.001
K+	K+	-2.56800*	-2.9663	-2.1697	<0.001
	K-	21.86600*	21.4677	22.2643	<0.001
K (+)	K (-)	24.43400*	24.0357	24.8323	<0.001

Note :
K1 : Group with 25% ethanol extract of lontar leaves
K2 : Group with 50% ethanol extract of lontar leaves
K3 : Group with 75% ethanol extract of lontar leaves
K (+) : Positive control (Ciprofloxacin)
K (-) : Negative control (10% DMSO)
P : Significant if $p < 0.05$

residue.[16] These findings highlight that optimal ethanol concentration may vary depending on the polarity of the target compounds and the plant matrix.

Phytochemical screening of the ethanol extract of lontar leaves was performed to identify the presence of compounds both qualitatively and quantitatively. The results indicated that the extract contained tannins and steroids, but saponins and flavonoids were undetected. This differs from previous studies that reported flavonoid presence,[10] which could be attributed to environmental or methodological variations.

The antibacterial activity of the lontar leaf extract *(Borassus flabellifer)* against *Staphylococcus aureus* was assessed using the well diffusion method. The extract exhibited moderate to strong antibacterial activity, as evidenced by the formation of inhibition zones around the wells in the medium after a 24-hour incubation period. The inhibition zone diameter was directly proportional to extract concentration, confirming a dose-response relationship.

This finding is consistent with a previous study, which demonstrated that 80% ethanol extract of lontar leaves produced an inhibition zone of 18.37 mm against *Vibrio cholerae*.[13] Although *Vibrio cholerae* is Gram-negative, the comparable results support the extract's potential as a broad-spectrum antibacterial.

Furthermore, lontar fruit extract has also been shown to exhibit antibacterial activity against *Staphylococcus aureus*, suggesting that various parts of the plant contain bioactive compounds with antibacterial effects.[12]

The mechanism of action is believed to be due to the presence of tannins and steroids. Tannins interfere with polypeptide synthesis required for bacterial cell wall construction, while steroids reduce membrane integrity, leading to leakage of cell contents and eventual bacterial cell death.

These results underline the potential of lontar leaf extract as a natural antibacterial agent, particularly in response to the growing resistance of *Staphylococcus aureus* to conventional antibiotics such as MRSA strains. While ciprofloxacin showed higher inhibition, the plant-based extract demonstrated promising activity and could be explored further in topical formulations or as an adjunct treatment.

Future research should aim at isolating specific bioactive compounds, standardizing extraction processes, and testing broader bacterial strains. In vivo studies and toxicity profiling would also be necessary before clinical application.

CONCLUSION

The 96% ethanol extract of lontar leaves contains bioactive compounds, specifically tannins and steroids, that exhibit antibacterial potential. This study demonstrated that the extract produced measurable antibacterial activity against Staphylococcus aureus, with inhibition zone diameters increasing proportionally to the concentration of the extract.

These findings suggest that lontar leaf extract could serve as a promising alternative antibacterial agent, especially in the context of rising antibiotic resistance such as MRSA. While its efficacy did not surpass that of ciprofloxacin, the extract's natural origin and local availability provide valuable prospects for future development.

Further research is recommended to explore its application in pharmaceutical formulations, conduct in vivo studies, and evaluate the toxicity to assess its safety and clinical potential.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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