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Observation of the Antibacterial Potential of *Aloe vera* Extract against *Staphylococcus aureus*

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Abstract

This study aims to determine the antibacterial potential of ethanol extract of *Aloe vera leaf bark* against the growth of *Staphylococcus aureus*. The research used a laboratory experimental method with a disc diffusion test (Kirby–Bauer). *Aloe vera* leaf bark simplicia powder is extracted using the maceration method with 96% ethanol solvent, then made in four treatment concentrations, namely 25%, 50%, 75%, and 100%. The positive control used 1% chloramphenicol, while the negative control used 96% ethanol. The *Staphylococcus aureus bacterial suspension* was adjusted to the McFarland standard of 0.5 and inoculated into the Nutrient Agar medium. The measurement of the diameter of the inhibition zone was carried out after 24 hours of incubation at 37°C, and each treatment was repeated three times. The results showed that *Aloe vera extract* at concentrations of 25%, 50%, and 75% produced an inhibitor diameter of 1.98 mm; 1.77 mm; and 3.72 mm respectively which were in the very weak category. The 100% concentration results in an inhibition zone of 5.10 mm and belongs to the moderate category based on the criteria of Davis and Stout (1971). Positive control results in an inhibition zone of 27.90 mm, while negative control does not exhibit significant inhibition activity. These results suggest that the higher the concentration of the extract, the greater the inhibition produced, although its antibacterial effectiveness is still limited. Thus, ethanol extract *of Aloe vera* leaf bark has the potential as an antibacterial against *Staphylococcus aureus*, but it cannot match the effectiveness of standard antibacterial, so it is necessary to optimize the extraction method or purification of the active compound to increase its activity.

Keywords: *Aloe vera*; Antibacterial; Ethanol extract; *Staphylococcus aureus*; Obstruction Zone.

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INTRODUCTION

Bacterial infections are still one of the major health problems that contribute greatly to the number of illnesses and deaths worldwide. Pathogenic bacteria can infect various tissues of the human body and cause a wide range of clinical conditions, ranging from mild to severe. One of the bacteria that has an important role in various cases of infection is *Staphylococcus aureus*, which is a cocus-shaped Gram-positive bacterium arranged in groups resembling grapes. These bacteria can live as normal flora on human skin, hair, and mucosa, but have the ability to transform into pathogens when the body's environmental conditions change or when the immune system weakens (Permatasari, 2020). Its ability to infect various organs makes *Staphylococcus aureus* one of the most commonly found bacteria in clinical cases.

Staphylococcus aureus It is known to be able to produce various virulence factors such as coagulase, hemolysin, leukocydine, enterotoxins, and enzymes that support the colonization of host tissues. The existence of these factors allows these bacteria to cause a variety of infections, including furuncle, carbuncle, impetigo, folliculitis, surgical wound infections, to more serious diseases such as pneumonia, osteomyelitis, endocarditis, to food poisoning due to the production of heat-resistant enterotoxins. Not only that, *Staphylococcus aureus* It can also form biofilms, a complex structure that is able to protect bacteria from host antibodies and antibiotic mechanisms, making these bacteria more difficult to eradicate (Yusitta, 2018).

Another challenge that exacerbates the control of this bacterial infection is the increasing resistance to antibiotics. The irrational use of antibiotics, both in clinical contexts and in general public use, has triggered the development of strains of *Staphylococcus aureus* that are resistant to various antibiotics. One of the most well-known strains of resistance is *Methicillin-Resistant Staphylococcus aureus* (MRSA), which exhibits resistance to β-lactam antibiotics. The emergence of MRSA increases the risk of infection complications, prolongs the length of treatment, increases treatment costs, and limits effective therapy options. Therefore, the search for alternative antibacterial agents that are safer, more affordable, and have a lower risk of resistance is important to be developed as a mitigation measure for the increasing global antibiotic resistance.

In an effort to reduce dependence on synthetic antibiotics, the use of natural ingredients as antibacterial agents began to be widely researched and empowered. Indonesia with its rich biodiversity has many traditional medicinal plants that have been used for a long time by the community. One of the plants that is often used is *Aloe vera*, which is widely known for its properties in various fields, ranging from health, cosmetics, to traditional medicine. This plant contains various secondary metabolites such as flavonoids, saponins, tannins, alkaloids, sterols, as well as anthraquinone compounds that are known to have pharmacological activity, including as antibacterial, anti-inflammatory, and antioxidant (Surjushe et al., 2008).

The content of active compounds in *Aloe vera* plays a role in antibacterial mechanisms through various ways, including damaging the structure of bacterial cell walls, increasing membrane permeability so as to cause cell content leakage, inhibiting protein and DNA synthesis, and inactivating important enzymes that bacteria need to survive (Sari et al., 2024). Most previous studies have focused on Aloe vera leaf gel or flesh, whereas *Aloe vera* leaf bark also contains active metabolites, especially anthraxinone, which have significant antibacterial potential (Fujiana et al., 2022). The bark of the leaves is often discarded, so that its use as a source of active compounds can provide added value economically and ecologically.

The extraction method is an important factor in the utilization of the active metabolites of medicinal plants. Extraction with a 96% ethanol solvent is known to be effective in attracting polar and semipolar compounds such as flavonoids and anthraxinone. Ethanol is also safe to use in health-related products, is easy to vaporize, and does not leave harmful residues (Permatasari et al., 2020). However, the results of studies related to the antibacterial activity *of Aloe vera* show considerable variation. These variations can be caused by differences in the type of solvent, extraction time, the part of the plant being extracted, the environmental conditions of the plant growing, and the concentration of the extract used in the test. Therefore, more specific and systematic research is needed to evaluate the effectiveness of *Aloe vera* extract, especially the leaf bark, in inhibiting the growth of *Staphylococcus aureus*.

In addition, research on the variation in the concentration of *Aloe vera extract* using 96% ethanol solvent against S. aureus is still not widely done. In fact, determining the concentration range is very important to find out the relationship between the dose of the extract and the amount of inhibition produced. This information can be the basis for the development of Aloe vera-based antibacterial product formulations in the future.

Based on this background, this study was conducted to evaluate the antibacterial activity of ethanol extract of *Aloe vera* leaf bark against the growth *of Staphylococcus aureus* at various concentrations. This research is expected to make a scientific contribution to the potential of *Aloe vera leaf bark* as a natural antibacterial source and provide an initial overview of the effectiveness of ethanol extract against Gram-positive bacteria.

MATERIALS AND METHODS

This study is a laboratory experimental study that aims to test the antibacterial activity of *Aloe vera* ethanol extract against *Staphylococcus aureus* bacteria using the disc diffusion method (Kirby-Bauer). The research was carried out on November 20, 2025 at the Microbiology Laboratory, Department of Biology, State University of Medan. The sample used was in the form of *fresh Aloe vera* leaf bark , while the test bacteria used were pure *Staphylococcus aureus* cultures.

The tools used include analytical scales, blenders, maceration containers, autoclaves, laminar air flow (LAF), water baths, rotary evaporators, petri dishes, test tubes, micropipettes, vortex mixers, incubators, calipers, and sterile cotton. The ingredients used include Aloe vera leaf bark simplicia, 96% ethanol, Nutrient Agar (NA) media, 0.9% physiological NaCl solution, McFarland standard 0.5, 6 mm diameter sterile paper disc, 1% chloramphenicol as positive control, and 96% ethanol as negative control.

The sample preparation begins with the separation of the leaf bark from *the Aloe vera* gel, then washed, dried in the sun, and mashed until simplicia powder is obtained. A total of 25 grams of simplicia were macerated in 96% ethanol at a ratio of 1:10 (b/v) for 5 × 24 hours at room temperature. The macerated filtrate is filtered and evaporated using a rotary evaporator, followed by heating in a 50°C water bath until a solvent-free viscous extract is obtained. The concentrated extract is then made in concentration variations of 25%, 50%, 75%, and 100% through dilution using 96% ethanol.

All glassware is sterilized using an autoclave at a temperature of 121°C. *Staphylococcus aureus bacterial suspensions* are made by taking a few bacterial colonies and suspending them in a 0.9% NaCl solution, then adjusting the turbidity level to be equivalent to the McFarland standard of 0.5 ($\pm 1.5 \times 10^8$ CFU/mL). The bacterial suspension is inoculated evenly on the surface of the NA media using a sterile cotton swab.

Paper discs with a diameter of 6 mm are dripped with each concentration of *Aloe vera* extract, then placed on the surface of the inoculated NA media. 1% chloramphenicol is used as a positive control, while 96% ethanol is used as a negative control. The entire incubation cup is stored at 37°C for 24 hours. After incubation, the barrier zone formed was measured using calipers in millimeters. Each treatment is carried out three times.

Data were obtained from the measurement of the diameter of the barrier zone and analyzed descriptively by calculating the mean value and standard deviation. The antibacterial activity of the extract is determined based on the size of the inhibition zone compared between concentrations as well as by positive and negative controls.

RESULTS AND DISCUSSION

Extraction Using Ethanol Solvent Aloe vera simplisia is crushed in a ratio of 1:10, namely 25 grams of simplicia powder and 250 ml extracted using maceration and remaceration methods for a total of 7 days with 96% ethanol solvent used as much as 500 mL and concentrated so as to obtain a thick extract. The condensed extract obtained has a yellowish-brown color and has a distinctive smell. The antibacterial test was carried out with the aim of finding out whether aloe vera has antibacterial activity against *Staphylococcus aureus bacteria*. The results of the antibacterial activity test can be seen in the table.

a. Diameter of the inhibition zone (D)

$$D = \frac{D_{vertikal} + D_{horizontal}}{2}$$

b. Net diameter (minus 6 mm disc)

$$D_{\text{bersih}} = D - 6$$

c. Average between 3 replications

$$\bar{D} = \frac{D_1 + D_2 + D_3}{3}$$

$$\bar{D}_{\text{bersih}} = \bar{D} - 6$$

Concentration	Height (mm)	Width (mm)	Average diameter (mm)	Net diameter (mm)
25%	6.87	7.28	7.08	1.08
50%	6.01	5.83	5.92	−0.08
75%	5.77	5.18	5.47	−0.53
100%	6.94	7.38	7.16	1.16

Table 1. Replication 1

Concentration	Height (mm)	Width (mm)	Average diameter (mm)	Net diameter (mm)
25%	8.14	8.92	8.53	2.53
50%	10.97	11.46	11.21	5.21
75%	10.26	11.63	10.95	4.95
100%	11.35	11.91	11.63	5.63

Table 2.Replication 2

Concentration	Height (mm)	Width (mm)	Average diameter (mm)	Net diameter (mm)
25%	6.93	9.76	8.34	2.34
50%	6.10	6.28	6.19	0.19
75%	13.40	12.06	12.73	6.73
100%	15.18	13.82	14.50	8.50

Table 3. Replication 3

A. B. C.

Figure 1. A.

Replication 1, B. Replication 2. C. Replication 3

Control	Height (mm)	Width (mm)	Average diameter (mm)	Net diameter (mm)
Negative	6.32	7.64	6.96	0.96
Positive	34.34	33.45	33.90	27.90

Table 4. Control (Negative & Positive)

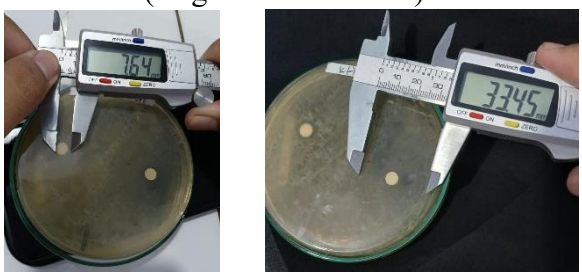

Figure 2. Control (Negative & Positive)

Group	Concentration	Average Diameter (mm)	Net Diameter (mm)	Mean Net Diameter (mm)	SD (mm)
Control (-)	Ethanol 96%	6.96	0.96	0.96	0.00
Aloe Extract	25%	7.08, 8.53, 8.34	1.08, 2.53, 2.34	1.98	0.75
Aloe Extract	50%	5.92, 11.21, 6.19	- 0.08 5.21 0.19	1.77	2.80
Aloe Extract	75%	5.47, 10.95, 12.73	- 0.53, 4.95, 6.73	3.72	3.82
Aloe Extract	100%	7.16, 11.63, 14.50	1.16, 5.63, 8.50	5.10	3.70
(+) Controls	Chloramphenicol 30 µg	33.90	27.90	27.90	0,00

Table 5. Jamming Zone Diameter

The antibacterial activity of plant extracts is largely determined by the content of secondary metabolites, especially flavonoids, tannins, saponins, and alkaloids that work to damage the cell wall, disrupt membrane permeability, and inactivate the bacteria's essential enzymes. Lestari and Permatasari (2022) stated that the higher the concentration of the extract, the greater the number of bioactive compounds that diffuse into the agar media and the stronger the inhibition formed. The stability of antibacterial activity will also be seen if the inhibition zone pattern is consistent between replications.

It was found that *Aloe vera* against *Staphylococcus aureus* showed a consistent pattern. In the first replication, the antibacterial activity was still low because the concentration of the active compound was not enough to produce stable inhibition. The second replication showed a more pronounced increase in the inhibition zone, indicating that the amount of active metabolites diffusing into agar began to achieve inhibitory effectiveness. Third replication strengthens the dose-response relationship, where increased concentrations result in larger, more consistent inhibitory zones, as per the dose-dependent mechanism described in theory. The validity of the method is also

seen from negative controls that do not produce resistance and positive controls that indicate optimal inhibition zones. The mean and low standard deviation indicate that the resistance pattern is stable, so the antibacterial effectiveness of Aloe vera can be categorized as weak to moderate, but the mechanism remains according to the theory of secondary metabolite activity against Gram-positive bacteria.

Concentration	Mean Net Diameter (mm)	Category	Interpretation
25%	1.98 mm	< 5 mm	Inactive / very weak
50%	1.77 mm	< 5 mm	Inactive / very weak
75%	3.71 mm	< 5 mm	Inactive / very weak
100%	5.10 mm	5–10 mm	Moderate activity

Table 6. Classification Table of Antibacterial Activity of Aloe vera Extract

The results of the measurement of the diameter of the inhibition zone show that the antibacterial power of *Aloe vera ethanol extract* can be assessed based on the size of the clear zone formed. According to the criteria of Davis and Stout (1971), the inhibition zone ≥20 mm is classified as very strong, 10–20 mm strong, 5–10 mm medium, and ≤5 mm weak. Based on this classification, *Aloe vera extract* at concentrations of 25%, 50%, and 75% produces an inhibition zone of ≤5 mm so that it is included in the weak category, while the concentration of 100% reaches around 5 mm which is in the medium category. Thus, *Aloe vera* extract has inhibition against *Staphylococcus aureus*, but its effectiveness is still limited and has not reached the strong category.

Based on the results of phytochemical screening reported by Kumala (2023), aloe vera extract (*Aloe vera*) tested with two types of solvents, namely 70% ethanol and ethyl acetate, showed the presence of several groups of secondary metabolites. In 70% ethanol solvents, saponins, anthracines, flavonoids, and alkaloids were detected, respectively characterized by the formation of foam, discoloration to red or orange, and the appearance of reddish-yellow deposits in the Dragendorff and Meyer tests. In contrast, in ethyl acetate solvents only alkaloid compounds give positive results, while saponins, anthraquinones and flavonoids show no change in reaction. This pattern shows that ethanol solvents are 70% more effective in extracting the active compounds *of Aloe vera* that are polar to semipolar, especially flavonoids and anthraquinones that are known to play a role in antibacterial activity.

CONCLUSION

Based on the results of the research that has been conducted, Aloe vera ethanol extract shows antibacterial activity against Staphylococcus aureus, but its effectiveness is still relatively low to moderate. The concentrations of 25%, 50%, and 75% result in an inhibition zone diameter of less than 5 mm so it is categorized as very weak antibacterial activity. The 100% concentration results in an inhibitory diameter of about 5.10 mm, which belongs to the moderate category according to the criteria of Davis and Stout (1971). This shows that increased concentration of extracts has an effect on increased inhibition, although it has not yet reached the strong category. Thus, Aloe vera extract has the potential to be an antibacterial, but it requires improved extraction methods, purification of active compounds, or more selective use of solvents to obtain more optimal effectiveness.

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CONFLICT OF INTEREST

The authors declare no conflict of interest and take full responsibility for the content of the article, including the implications of AI-generated content.

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