

EFFECT OF ETHANOL EXTRACT OF *RHIZOPHORA MUCRONATA* LEAVES ON THE GROWTH OF *STAPHYLOCOCCUS EPIDERMIDIS*

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ABSTRACT

Background : *Staphylococcus epidermidis* is one of the bacteria that cause skin infections. Indonesian communities use treatments such as ointments for skin diseases; however, this approach often fails to provide healing. Therefore, alternatives are needed by utilizing antimicrobial active compounds from plants containing secondary metabolites that function as antibacterial agents, one of which is *Rhizophora mucronata*. This study aimed to determine the effect of ethanol extract of *Rhizophora mucronata* leaves on the growth of *Staphylococcus epidermidis*.

Methods : The inhibition test of *Staphylococcus epidermidis* growth was carried out on Mueller Hinton agar using the disk diffusion method. There were six treatment groups: a positive control with Ciprofloxacin 5 mcg, a negative control with sterile distilled water, and extract concentrations of 25%, 50%, 75%, and 100%, each performed in four repetitions.

Results : The results showed that the *Rhizophora mucronata* leaf extract in the positive control had an average inhibition zone of 30.85 mm; the 100% extract concentration was 11.24 mm; the 75% concentration was 9.45 mm; the 50% concentration was 8.09 mm; and the 25% concentration and negative control were 6.00 mm (no inhibition zone formed).

Conclusion : This study demonstrates that *Rhizophora mucronata* leaf extract has antibacterial effects in inhibiting the growth of *Staphylococcus epidermidis* at concentrations of 50%, 75%, and 100%.

Keywords : Antibacterial, bakau leaves, *Staphylococcus epidermidis*, *Rhizophora mucronata*

INTRODUCTION

Skin diseases are known to be common conditions in tropical countries such as Indonesia, affecting anyone and appearing on various parts of the body.¹ Overall, the World Health Organization (WHO) estimates that around 900 million people worldwide experience health disorders related to skin diseases.² Each skin disease presents different symptoms and levels of severity, ranging from barely noticeable manifestations to life-threatening conditions.³

The prevalence of skin diseases in Indonesia ranges from 4.60% to 12.95%, making them the third most common among the top ten diseases. Skin diseases are also among the top ten most common diseases in West Sumatra, with approximately 5,995 cases or about 5.20% recorded in

2019.⁴ Bacterial related diseases often arise in the surrounding environment. *Staphylococcus epidermidis* is a bacterium that commonly causes swelling (abscesses) such as acne, as well as skin infections, kidney infections, and urinary tract infections³ and it is capable of causing other diseases such as osteomyelitis and endocarditis. Approximately 75% of infections caused by coagulase-negative *Staphylococcus* (CoNS) are attributed to *S. epidermidis*. CoNS is a normal human microbiota but can sometimes cause infections, especially those associated with implanted devices such as joint prostheses, shunts, and intravascular catheters.⁵

In general, Indonesian communities use modern treatments such as ointments for skin diseases; however, these approaches often fail to provide healing. This results

in frustration and depression among patients, leading them to seek various alternative treatments considered more scientific but based on traditional methods.⁶

Rhizophora mucronata has been used to control pathogenic bacteria as an antibacterial agent. Antibacterial agents are compounds capable of inhibiting bacterial growth. They act by damaging bacterial cell structures without harming the host, thus exhibiting selective toxicity. *Rhizophora mucronata* has been reported to inhibit the growth of *Staphylococcus aureus* and *Vibrio harveyi*.⁷

This plant contains various secondary metabolites such as tannins, phenolic compounds, chlorophyll, carotenoids, and alkaloids. Its fruits can be used as food and beverages, young leaves as vegetables, and the wood and bark as tanning (tannin) materials and dyes. Extracts from boiled wood can be used as slimming agents, anti-diarrheal, and antiemetic remedies. *Rhizophora mucronata* leaves contain 2-(2-ethoxy ethanol), kau-16-ene, benzophenone, as well as phenolic compounds such as flavonoids, phenolic acids, and dihydroflavonol tannins. Additionally, they contain caffeic acid, vanillic acid, and p-hydroxybenzoic acid. Other components include alkaloids, coumarins, phenols, polyphenols, quinones, resins, saponins, phytosterols, xanthoproteins, pigments such as chlorophyll and carotenoids, and sugars.⁸

The purpose of this study was to determine whether the administration of ethanol extract of *Rhizophora mucronata* leaves at concentrations of 25%, 50%, 75%, and 100% affects the inhibition of *Staphylococcus epidermidis* bacterial growth.

MATERIALS AND METHODS

The experimental method used in this study was a *posttest-only control group design*. Six treatment groups were employed, each requiring four samples, resulting in a total of 24 samples. A *random sampling* method was used for sample selection.

Staphylococcus epidermidis was obtained from the Microbiology Laboratory, Faculty of Medicine, Hang Tuah University, Surabaya. The bacteria were cultured in liquid MHA (*Mueller Hinton Agar*) and adjusted to match the turbidity of a 0.5 McFarland standard. A 0.5 McFarland standard corresponds to approximately 1.5×10^8 CFU/mL of bacterial density.⁹

The antibacterial activity test was conducted using the disk diffusion method. Mueller Hinton Agar (MHA) was poured into six Petri dishes, with 10 mL allocated to each dish. A suspension of *Staphylococcus epidermidis*, adjusted to the 0.5 McFarland standard, was uniformly inoculated onto the surface of the medium. Filter paper disks that had been immersed in *Rhizophora mucronata* leaf extract at concentrations of 25%, 50%, 75%, and 100% were then placed onto the inoculated plates. Ciprofloxacin served as the positive control, while sterile distilled water was used as the negative control. All plates were incubated at 37 °C for

24 hours. Following incubation, the diameter of the inhibition zones was measured using a caliper to evaluate the antibacterial activity of the extract.

PREPARATION OF THE EXTRACT

The mangrove leaf extract was prepared using the maceration method. A total of 500 g of *Rhizophora mucronata* leaves were cleaned, dried, and pulverized into *simplicia*. The powdered material was macerated in 96% ethanol for 72 hours. The macerate was subsequently filtered, and the residue was remacerated under the same conditions. All filtrates were combined and concentrated using a vacuum rotary evaporator to obtain 40 g of semisolid extract. The extraction rendement was calculated by dividing the final extract weight by the initial weight of plant material and multiplying by 100%.¹⁰

PREPARATION OF EXTRACT CONCENTRATIONS

The extract concentrations were prepared by diluting *Rhizophora mucronata* leaf extract in sterile aquadest to obtain final volumes of 1 mL. A 100% concentration was prepared by dissolving 1 mg of the extract in 1 mL of sterile aquadest. A 75% concentration was prepared by dissolving 0.75 mg of the extract in 0.25 mL of sterile aquadest. A 50% concentration was obtained by diluting 0.50 mg of the extract with 0.50 mL of sterile aquadest, while a 25% concentration was prepared by dissolving 0.25 mg of the extract in 0.75 mL of sterile aquadest.

RESULT

The culture of *Staphylococcus epidermidis* was subjected to six treatments consisting of a positive control, extract concentrations of 100%, 75%, 50%, and 25%, as well as a negative control.

In **table 1**, presents the inhibition zone measurements obtained from each concentration in the respective treatment groups.

The paper disc used in this study had a diameter of 6,00 mm. Therefore, if an inhibition zone measuring 6,00 mm appears in the table, it indicates that the treatment did not produce any inhibitory effect.

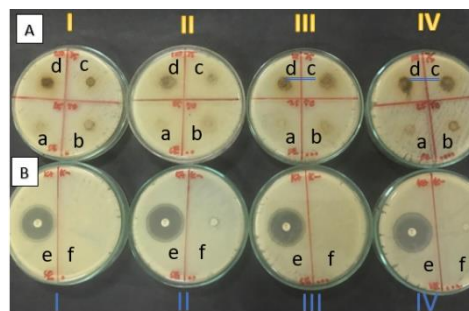


Figure 1 Inhibition zone diameter

(A) Four petri dishes containing the extract group ;
(B) Four petri dishes containing the control group ; (I) first
replication; (II) second replication; (III) third replication;
(IV) fourth replication;

(a) 25% extract group; (b) 50% extract group; (c) 75%
extract group; (d) 100% extract group;
(e) positive control; (f) negative control

Table 1 Inhibition zone diameter measurement results

Perlakuan	Diameter Zona Hambat (mm)			
	Pengulangan I	Pengulangan II	Pengulangan III	Pengulangan IV
Kontrol positif	30,27	31,55	30,01	31,57
Konsentrasi 25%	6,00	6,00	6,00	6,00
Konsentrasi 50%	8,42	8,09	7,32	8,56
Konsentrasi 75%	8,92	9,55	9,30	10,05
Konsentrasi 100%	10,85	11,41	11,14	11,58
Kontrol negatif	6,00	6,00	6,00	6,00

DISCUSSION

This study showed that the administration of mangrove leaf extract (*R. mucronata*) had an effect on the growth of *Staphylococcus epidermidis*. This was demonstrated by the formation of a clear zone around the sterile paper disk, which indicated an inhibition zone. In this study, the positive control used ciprofloxacin, while the negative control used sterile distilled water. The mangrove leaf extract (*R. mucronata*) was prepared in four concentrations: 25%, 50%, 75%, and 100%.

Ciprofloxacin was able to produce an inhibitory effect on *Staphylococcus epidermidis*. This was indicated by the formation of an inhibition zone in the positive control group, with an average inhibition zone diameter of 30,85 mm. Furthermore, the presence of an inhibition zone on Mueller Hinton agar demonstrated that the *Staphylococcus epidermidis* used in this study did not exhibit resistance.

This study showed that the administration of aquadest did not affect the growth of *Staphylococcus epidermidis*, as indicated by the absence of an inhibition zone on Mueller Hinton agar media.

The average inhibition zones formed at extract concentrations of 100% were 11,24 mm, 75% were 9,45 mm, 50% were 8,09 mm, while at 25% concentration no inhibition zone was formed. The results of this study demonstrate that the mangrove leaf extract *Rhizophora mucronata* at concentrations of 100%, 75%, and 50% has antimicrobial activity against *Staphylococcus epidermidis*.

The results of this study indicate that the *R. mucronata* mangrove leaf extract at a 25% concentration did not form an inhibition zone, which may be influenced by several factors. Afifi and Erlin reported that the concentration of antimicrobial agents affects the growth of microorganisms, where higher concentrations increase microbial mortality. Therefore, differences in concentration will result in varying inhibition zones in microbial growth.¹¹ The higher the concentration of a substance, the greater the content of its active antibacterial compounds, thereby increasing its ability

to kill bacteria.¹² In accordance with this, the inhibitory effect on the growth of *Staphylococcus epidermidis* in this study was caused by the administration of different concentrations of *R. mucronata* mangrove leaf extract.

Additionally, this outcome may be influenced by the sensitivity and cellular response of *Staphylococcus epidermidis* to the antibacterial compounds present in the *R. mucronata* leaf extract. Based on the observations, the inhibition zones consistently increased with rising extract concentrations, ranging from the lowest to the highest levels. This pattern is attributed to the greater abundance of antibacterial compounds at higher concentrations, which facilitates more effective penetration of these compounds into bacterial cells through their respective mechanisms.¹³

A study conducted by Amirullah *et al.* examining the sensitivity of mangrove leaves (*Rhizophora stylosa*) against *Escherichia coli* using the Kirby–Bauer disk diffusion method was performed with three concentrations: 100%, 150%, and 200%. The 100% and 150% concentrations did not produce inhibition zones because the extract content at these concentrations was insufficient to generate measurable antibacterial activity. Another study by Ernawati (2016, cited in Amirullah, 2022, p. 302) on the inhibitory effect of ethanol extracts of *Rhizophora mucronata* mangrove leaves against *Staphylococcus aureus* and *Escherichia coli*, as well as its antidiabetic effect in alloxan-induced mice, also showed no antibacterial activity. This was presumed to be due to the flavonoid concentration in the extract being inadequate to damage the bacterial cell membrane, allowing the bacteria to continue proliferating.¹⁴ This is consistent with the increase in antibacterial compounds as the concentration rises, which facilitates the penetration of these compounds into bacterial cells and aligns with the findings of the present study.

Another biological material was studied by Pertiwi *et al.*, who evaluated the antibacterial activity of ethanol extract of butterfly pea flowers (*Clitoria ternatea* L.) against *Staphylococcus epidermidis*. The study employed three concentrations—10%, 15%, and 20%—along with two

control groups, namely a positive control using chloramphenicol and a negative control using DMSO. The results showed inhibition zones of 2.31 mm at the 10% concentration, 3.05 mm at the 15% concentration, and 6.20 mm at the 20% concentration. The positive control (chloramphenicol) produced an average inhibition zone diameter of 8.41 mm, whereas the negative control (DMSO) produced no inhibition zone.¹⁵ It is possible that the butterfly pea flower extract contains a higher level of secondary metabolites capable of inhibiting *S. epidermidis* compared to the secondary metabolites present in *R. mucronata* leaf extract, allowing even low concentrations of the butterfly pea extract to inhibit *S. epidermidis*, although the effect remains in the weak category.

From this study, it was found that *Staphylococcus epidermidis* was successfully inhibited by the mangrove leaf extract (*R. mucronata*) at concentrations of 50%, 75%, and 100%. This study demonstrates that the *R. mucronata* leaf extract possesses antibacterial properties against *Staphylococcus epidermidis*, as evidenced by the formation of inhibition zones on Mueller Hinton agar.

1. CONCLUSIONS AND SUGGESTIONS

The mangrove leaf extract of *Rhizophora mucronata* at a concentration of 100% was the most effective treatment group in inhibiting the growth of *Staphylococcus epidermidis*, with an inhibition zone diameter of 11.24 mm. However, it was still less effective compared to the positive control group, Ciprofloxacin 5 µg, which produced an inhibition zone diameter of 30.85 mm. At a concentration of 75%, the inhibition zone formed was 9.45 mm, at 50% the inhibition zone was 8.09 mm, and at 25% no inhibition zone was formed.

The recommendations for this study are as follows:

Further research is needed to determine whether the inhibited bacteria experience only growth suppression or eventually die.

Additional studies are required to determine the optimal concentration and maximum dosage of *R. mucronata* leaves against *S. epidermidis*.

In vivo studies are necessary to ensure the effectiveness and safety of the extract more comprehensively.

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