Antibacterial Activity Test of Nipa Palm Leaf Extract (Nypa fruticans) Against Staphylococcus epidermidis

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Skin disorders are a common health problem and can be caused by pathogenic bacterial infections. Staphylococcus epidermidis bacteria are known to play a role in various infections that can affect the skin surface. The use of natural ingredients as alternative antibacterial agents is crucial, one of which is nipah leaves (Nypa fruticans), which contain various secondary metabolites with potential antibacterial activity. This study aimed to determine the antibacterial activity of nipah leaf ethanol extract against two test bacteria and to determine the most effective concentration. Antibacterial testing used the disc diffusion method at concentrations of 200 µg/disc, 300 µg/disc, and 400 µg/disc, with chloramphenicol as a positive control and DMSO as a negative control. The results showed that nipah leaf extract formed a zone of inhibition at all tested concentrations. The largest zone of inhibition was found at a concentration of 400 µg/disc, measuring 8,2 mm against Staphylococcus epidermidis, which is classified as having medium inhibitory activity.

Keywords: Nypa fruticans, antibacterial, Staphylococcus epidermidis

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1. Introduction

Skin disorders are a common health problem and can be caused by pathogenic bacterial infections. Staphylococcus epidermidis and Pseudomonas aeruginosa are two bacteria frequently found on human skin and have the potential to cause infections, especially when the skin's protective layer is damaged [1,2]. Skin infections that are not properly treated can develop into serious conditions, especially in patients with weakened immune systems [3].

Data on the prevalence of infectious skin disorders in Indonesian healthcare facilities also shows an increasing trend, indicating that this problem requires greater attention [4]. Antibiotics are the primary therapy for treating bacterial skin infections. However, uncontrolled use can lead to bacterial resistance, which has now become a global problem and reduces the effectiveness of various antibiotics [3]. Therefore, the use of natural ingredients as alternative antibacterials is one solution that is starting to be widely developed [5,6]. Indonesia boasts rich biodiversity, including mangrove plants that have potential as traditional medicines. One example is the nipah palm (Nypa fruticans), a mangrove plant utilized by coastal communities for various traditional purposes, including medicinal purposes [6].

The leaves are known to contain secondary metabolites such as flavonoids, tannins, alkaloids, saponins, and terpenoids, which have antibacterial activity [7,8]. Several studies have shown that nipah palm leaf extract can inhibit various pathogenic bacteria, such as Pseudomonas aeruginosa, E. coli, and Staphylococcus aureus [9,10].

However, research on the antibacterial activity of nipah palm leaf ethanol extract against bacteria that cause skin disorders, particularly Staphylococcus epidermidis, is still very limited [11,12]. Based on this,

this study was conducted to evaluate the antibacterial activity of nipah palm leaf ethanol extract and determine the most effective concentration in inhibiting these two bacteria.

2. Literature Review and Problem Statement

The Nipah palm (Nypa fruticans Wurmb) is a member of the Arecaceae palm family and typically grows along riverbanks affected by sea level fluctuations. It is also a member of the mangrove habitat [6]. Nipah palms grow naturally and have significant ecological and economic value. Ecologically, they protect coastlines from wave erosion and provide habitat for fish, birds, and various other organisms in brackish waters. Furthermore, almost all parts of the Nipah palm can be utilized by the community, including for making palm fronds, roofing, firewood, animal feed, and even as a source of sugar from its sap [6].

Nipah palm (Nypa fruticans) contains various chemical substances such as alkaloids, flavonoids, polyphenols, steroids or triterpenoids, saponins, and tannins.The leaves are rich in alkaloids, flavonoids, steroids/triterpenoids, and tannins. The roots of the plant contain various chemical compounds such as alkaloids, steroids, triterpenoids, flavonoids, and tannins [7,8]. The Nipah palm (Nypa fruticans Wurmb) is a member of the Arecaceae palm family and typically Nipah palms grow naturally and have significant ecological and economic value. Ecologically, they protect coastlines from wave erosion and provide habitat for fish, birds, and various other organisms in brackish waters. Furthermore, almost all parts of the Nipah palm can be utilized by the community, including for making palm fronds, roofing, firewood, animal feed, and even as a source of sugar from its sap [6].

3. Method

The equipment used in this study included a rotary evaporator (Heidolp made in Germany), an autoclave (Nesco), a laminar air flow apparatus (Magnehelic), an incubator (Memmert), a hot plate (Velp), a magnetic stirrer, a vortex mixer, an analytical balance, a Bunsen burner, an Erlenmeyer flask, a Pyrex beaker, a graduated cylinder, test tubes, a test tube rack, a Petri dish, a porcelain dish, a glass jar, a blender (Miyako), a micropipette, a dropper, a stirring rod, tweezers, a vernier caliper, an eyepiece needle, a paper disc, a funnel, and aluminum foil.

The materials used in this study included nipah leaves (Nypa fruticans), 96% ethanol (C2H5OH), Staphylococcus epidermidis, nutrient agar (NA), sodium chloride (NaCl), barium chloride dihydrate (BaCl2.2H2O), distilled water (H2O), chloramphenicol 30 µg/disk, 10% dimethyl sulfoxide (DMSO), Mayer's reagent, Dragendorff's reagent, magnesium (Mg) powder, 2N hydrochloric acid (HCl), iron (III) chloride (FeCl3), chloroform, acetic acid (CH3COOH), ammonia (NH3), and concentrated sulfuric acid (H2SO4).

Nipah Leaf Extraction Method

Nipah leaf extraction was obtained through maceration with 96% ethanol. 600 g of powdered nipah leaves were soaked in 96% ethanol for 24 hours. The filtrate was filtered and remacerated three times. The filtrate was then placed in a rotary evaporator at 40-50°C until a thick extract was obtained [13].

Phytochemical Screening

Nipah leaves were subjected to a phytochemical screening test to identify the presence of secondary metabolites such as alkaloids, flavonoids, steroids, terpenoids, saponins, and tannins [14].

Antibacterial Activity Test

An antibacterial activity test was conducted on two test bacteria, Staphylococcus epidermidis and Pseudomonas aeruginosa, using the disc diffusion method. The test was divided into five groups: a negative control group (DMSO), a positive control group (chloramphenicol 30 µg/disk), and Three treatment groups were each given ethanol extract of nipah leaves at concentrations of 200 µg/disk, 300 µg/disk, and 400 µg/disk. Bacterial suspensions were prepared with a specific turbidity standard to ensure a homogeneous colony count, then inoculated evenly onto the surface of Nutrient Agar media. Sterile paper discs impregnated with nipah leaf extract at each concentration were placed on top of the inoculated media, and all plates were incubated at 37°C for 24 hours. The diameter of the inhibition zone was measured using a vernier caliper [14,16]. Before the testing process, all equipment was sterilized and the media was prepared according to procedures to avoid contamination. After the incubation period, the diameter of the inhibition zone was measured using a vernier caliper.

The measurement was made by measuring the diameter of the clear circle around the test disc and comparing it between the treatment group, the positive control, and the negative control. The data obtained was then recorded and analyzed to determine whether antibacterial activity increased with increasing extract concentration. The results are presented in a table to illustrate the effectiveness of nipah leaf ethanol extract in inhibiting the growth of *Staphylococcus epidermidis.*

4. Results and Discussion

Phytochemical Screening Results

The results of this study showed an extract yield of 15.0%. Phytochemical screening tests on nipah leaves revealed the presence of alkaloids, flavonoids, saponins, phenols, tannins, steroids, and terpenoids, as shown in Table 1.

Table 1. Phytochemical Screening Results

Examination	Reagent	Test Result
Alkaloid	Mayer's Reagent	(+)
Alkaloid	Dragendorff's Reagent	(-)
Flavonoid	Concentrated HCl and Mg Powder	(+)
Tannin	1% $FeCl_3$	(-)
Saponin	Hot Distilled Water + Concentrated HCl	(-)
Steroid	Sulfuric Acid (H_2SO_4)	(+)
Terpenoid	Acetic Acid (CH_3COOH)	(+)

Table 2. Observation Results of Staphylococcus epidermidis Bacteria

Treatment (Staphylococcus epidermidis)	I (mm)	II (mm)	III (mm)	Average (mm)	Inhibition Strength
Concentration 200 µg/disk	6,1	6.4	6.2	6,2	medium
Concentration 300 µg/disk	7.5	6.5	7.2	7,0	medium
Concentration 400 µg/disk	8.2	8.1	8.4	8,2	medium
Positive Control	17.8	19.9	20.7	19.4	Strong

Treatment (Staphylococcus epidermidis)	I (mm)	II (mm)	III (mm)	Average (mm)	Inhibition Strength
(Chloramphenicol 30 µg/disk)					
Negative Control (DMSO)	0	0	0	0	None

Based on the results of the antibacterial activity test against Staphylococcus epidermidis, the tested sample at several concentrations showed the ability to inhibit bacterial growth, as indicated by the formation of a clear inhibition zone around the disc on agar media. This method is commonly used to evaluate antimicrobial activity by measuring the diameter of the inhibition zone produced around the tested substance [17]. At a concentration of 200 µg/disk, the average diameter of the inhibition zone was 6.2 mm, which is categorized as medium inhibition strength. This result indicates that at a relatively low concentration, the active compounds present in the sample have begun to exhibit antibacterial activity against *Staphylococcus epidermidis*. This bacterium is a Gram-positive microorganism that normally exists as part of the human skin microbiota but can act as an opportunistic pathogen causing skin infections and nosocomial infections under certain conditions [18]. At a concentration of 300 µg/disk, the average inhibition zone increased to 7.0 mm. This increase indicates that antibacterial activity improved with increasing concentration of the tested sample. This phenomenon is consistent with the general principle that higher concentrations of antibacterial compounds tend to produce greater inhibitory effects on bacterial growth [19]. Furthermore, at a concentration of 400 µg/disk, the average inhibition zone reached 8.2 mm, which is still categorized as medium inhibition strength, but it shows a noticeable increase compared to the lower concentrations. This finding suggests that the bioactive compounds present in the sample possess potential antibacterial properties. Secondary metabolites such as flavonoids, alkaloids, tannins, and terpenoids are known to exert antibacterial activity through several mechanisms, including disruption of bacterial cell membranes, inhibition of protein synthesis, and interference with bacterial metabolic processes [20].

As a comparison, the positive control using chloramphenicol (30 µg/disk) produced an average inhibition zone of 19.4 mm, which falls into the strong inhibition category. Chloramphenicol is a broad-spectrum antibiotic that works by inhibiting bacterial protein synthesis through binding to the 50S ribosomal subunit, thereby preventing peptide bond formation [21].Therefore, the inhibition zone produced by chloramphenicol is significantly larger than that produced by the tested sample. Meanwhile, the negative control using DMSO did not produce any inhibition zone (0 mm). This result indicates that the solvent used in the experiment does not possess antibacterial activity, confirming that the inhibition zones observed in the treatment groups were caused by the bioactive compounds contained in the tested sample. Overall, the results of this study indicate that the tested sample exhibits antibacterial activity against *Staphylococcus epidermidis* with moderate inhibition strength at concentrations ranging from 200–400 µg/disk. In addition, there is a clear trend showing that the inhibition zone increases with increasing sample concentration. Therefore, the tested sample has potential to be further developed as a natural antibacterial agent, although its effectiveness is still lower than that of standard antibiotics.

5. Conclusion

The conclusion of this study indicates that the ethanol extract of nipah (Nypa fruticans) leaves has been proven to have antibacterial activity against Staphylococcus epidermidis. The most effective concentration based on the inhibition zone was 400 µg/disk, with an inhibition zone of 8.2 mm, classified as moderate.

This antibacterial activity is supported by the content of secondary metabolites such as flavonoids, tannins, alkaloids, and terpenoids. Therefore, nipah leaves have the potential to be developed as an antibacterial agent.

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