



Comparison of Antibacterial Activity of Ethanolic Extract from Immature and Mature Nipa Leaves (*Nypa fruticans*, Wurmb) against *Staphylococcus aureus* and *Escherichia coli*

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Abstract : Background: Nipa (*Nypa fruticans*, Wurmb) is a species of Arecaceae, has been used to treat stomach ache, diabetes mellitus and febrifuge traditionally and give satisfactory results.

Objective: The aim of this research was intended to know the antibacterial activity of ethanolic extract of immature and mature Nipa leaves against *Staphylococcus aureus* and *Escherichia coli*.

Methods: Simplex were characterized, phytochemical screening and extracted by a maceration method using ethanol 96% as solvent. An ethanolic extract of immature and mature Nipa leaves was tested the antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* which was conducted with in vitro agar diffusion method using the paper disk.

Results: The characterization of simplex of immature and mature Nipa leaves (*Nypa fruticans*, Wurmb) obtained water content respectively 5.64% and 5.81%. The content of water soluble respectively 19,27% and 22.30%. Content of ethanol soluble respectively 16.32% and 18.05%. Total ash content, respectively 6.36% and 6.49%. Content of acid insoluble ash, respectively 1.58% and 1.61%. Phytochemical screening of simplex and ethanolic extract immature and mature Nipa leaves showed the presence of steroids/triterpenoids, flavonoid, glycosides, saponins and tannins. The result of antibacterial activity of ethanolic extract of immature and mature Nipa leaves can inhibit the growth of the bacteria *Staphylococcus aureus* at concentration 100 mg/mL; 75mg/mL with the effective diameter of the inhibition area respectively 14,27 mm and 14,10 mm; bacteria *Escherichia coli* at the same concentration 75 mg/mL, with the effective diameter of the inhibition area respectively 14,10 mm and 14,80 mm.

Conclusion: Based on the results, ethanolic extract of mature Nipa leaves are more effective to inhibit the growth of the bacteria *Staphylococcus aureus* and *Escherichia coli* than the ethanolic extract of immature Nipa leaves.

Key words : *Nipa leaves, ethanolic extract, antibacterial, Staphylococcus aureus, Escherichia coli.*

Introduction

Since a long time ago Indonesian society has been recognized and used medicinal plants as part efforts to cure the health problems. The utilization of medicinal plants in Indonesia is traditionally more preferred because less side effects than synthetic drugs and synthetic drugs much more expensive. Indonesia has the potential of Nipa forests is the widest in the world with an area of 700,000 hectares. Nipa plants (*Nypa fruticans*

Wurmb) have been used by society as a traditional medicine to treat stomach ache, diabetes, diarrhea and febrifuge were also used by the community coastal Banyuasin, South Sumatra. The nipa plants extract could inhibit tuberculosis, liver disease as well as a carminative¹.

The people generally used the leaves of the mature and immature who still rolls and used as a cough medicine, diarrhea by crushing and then squeezed or boiled, then juice or cooking water mixed with honey and drink. Based on previous research, the ethanolic extract of mature Nipa leaves that have been formulated into soap has antibacterial activity against *Staphylococcus aureus*².

Previous research was extract n-hexane, ethyl acetate, acetone and methanol of Nipa leaves are extracted by soxhletation has antibacterial activity against *Escherichia coli*, *Agrobacterium tumefaciens*, *Streptococcus mutans* and *Staphylococcus aureus*. Nipah (*Nypa fruticans* Wurmb) contains a variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids have been studied in vitro to have antimicrobial properties^{3,4}.

Flavonoids can be efficacious as antidiabetic⁵, antimalarial⁶, anticancer^{7,8} and antibacterial⁹. *Staphylococcus aureus* can produce enterotoxin, which can cause food poisoning to cause a variety of symptoms, such as nausea, vomiting, diarrhea, cramps and abdominal cramps and headache. Except bacteria *Staphylococcus aureus*, *Escherichia coli* can cause diarrhea and urinary tract infections¹⁰.

Based on the description, then this research want to compare the antibacterial activity of ethanolic extract from immature and mature Nipa leaves (*Nypa fruticans* Wurmb) against *Staphylococcus aureus* and *Escherichia coli*.

This research includes characterization simplex, extracted by a maceration method using ethanol 96%, phytochemical screening at simplex and ethanolic extract of immature and mature Nipa leaves, manufacturing test solution with varying concentrations and test the antibacterial activity of ethanolic extract from immature and mature Nipa leaves against gram-positive bacteria such as *Staphylococcus aureus* and gram-negative bacteria is *Escherichia coli*.

Methods

This research was done by experimental methods.

Preparation of ethanolic extract

Simplex powder of Nipa immature and mature leaves each as much as 300 g soaked with 75 parts 96% ethanol for 5 days protected from light while stirring often, then strained the liquid off or pressed. The pulp is washed with 96% ethanol sufficient to obtain 100 parts. Left in a cool, protected from light for 2 days, decant or filtered¹¹. Macerate obtained was concentrated by using a rotary evaporator at a temperature of ± 40 ° C to obtain a thick extract.

Characterization and phytochemical screening

Characterization of simplex includes determination of water content, water-soluble content, ethanol soluble content, total ash content, and ash-not dissolve in acid content. Determining the phytochemical screening carried out on simplex and ethanolic extract of immature and mature Nipa leaves.

Preparation of ethanolic extract of test solution

An ethanolic extract of Nipa leaves was weighed as much as 5 g and reconstituted with the solvent dimethyl sulfoxide (DMSO) up to 10 ml. The concentration of ethanolic extract of the test solution is 500 mg/ml. Created dilution to obtain a test solution with a concentration of ethanolic extract 400 mg/ml, 300 mg/ml, 200 mg/ml, 100 mg/ml, 75 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml.

Antibacterial activity test

0.1 ml inoculum was put in a sterile petri dish, then poured the agar media as much as 15 ml with temperatures 45-50°C. Furthermore homogenized so that the media and the bacterial suspension well mixed and allowed to solidify. Testing the antibacterial activity was used paper disc diffusion method which was the paper disc that has been soaked in some concentration of ethanolic extract of the test solution put above the solid media were inoculated bacteria. Left 15 minutes, then incubated in an incubator at 37°C for 18-24 hours, then measured the diameter of the area barrier (clear zone) growth around the disc by using a caliper.

Result and Discussion

Characteristic result of simplex power dan extract from immature and mature Nipa leaves can be seen in Table 1.

Table 1: Characteristic of immature and mature Nipa leaves

Parameter	Result (%)	
	Immature	Mature
Water content	5,64	4,98
Content of water soluble	19,27	22,32
Content of ethanol soluble	16,20	18,02
Total ash contant	6,36	7,97
Content of acid insoluble ash	1,59	2,02

Determination of water content in simplex conducted to determine the amount of water contained in the bulbs. The results obtained from the determination of moisture content, less than 10% are immature and mature leaves respectively 5.64% and 4.98%. The water content exceeding 10% can be a good medium for mold growth. Assay of the water soluble extract was conducted to determine the number of compounds that are polar solvents that may be taken in water. Levels of a water soluble extract obtained is immature and mature leaves respectively 19.27% and 22.32%. The ethanol soluble extract assay was conducted to determine the number of compounds that are polar or non-polar solvents that may be taken in ethanol. Levels of soluble extract derived ethanol is immature and mature leaves respectively 16.20% and 18.02%. Determination of total ash conducted to determine the amount of minerals contained in the sample. Total ash obtained are immature and mature leaves respectively 6.36% and 7.97%. Determination of acid insoluble ash content was conducted to determine the amount of minerals that do not dissolve in acid, such as silicates. Acid insoluble ash content obtained are immature and mature leaves respectively 1.59% and 2.02%.

The phytochemical screening result of simplex power and extract from immature and mature Nipa leaves can be seen in Table 2 and Table 3.

Table 2: Results of phytochemicals screening of simplex from immature and mature Nipa leaves

Compounds	Simplex	
	Immature	Mature
Alkaloids	-	-
Flavonoids	+	+
Gycosides	+	+
Steroids	+	+
Saponins	+	+
Tannins	+	+

Table 3: Results of phytochemicals screening of extract from immature and mature Nipa leaves

Compounds	Simplex	
	Immature	Mature
Alkaloids	-	-
Flavonoids	+	+
Gycosides	+	+
Steroids	+	+
Saponins	+	+
Tannins	+	+

Phytochemical screening results showed that ethanolic extracts of Nipa leaves contain a class of chemical compounds such as steroids / triterpenoids, flavonoids, saponins, glycosides and tannins. Secondary metabolites that possess antibacterial activity with the mechanism of action differ. Phenolic compounds and polyphenols are a group of secondary metabolites have antibacterial activity, have hydroxyl groups attached to the aromatic compound. The location and number of hydroxyl groups of phenolic compounds affect the toxicity of micro-organisms¹². The combination of phenolic compounds may provide a synergistic effective and add antibacterial reaction is better than single compounds. Phenol compounds at low concentrations can affect the activity of the enzyme, whereas at high concentrations causes the protein denaturation¹³. Flavonoids and tannins are chemical compounds that have potential as an antibacterial. Flavonoids are a group of phenolic compounds have a tendency to bind to proteins, thereby disrupting the bacterial metabolism, but it also acts as an antibacterial flavonoids by forming complex compounds against extracellular proteins that disrupt the integrity of bacterial cell membranes¹⁴. Terpenoids as antibacterial mechanism is reacted with porin (transmembrane protein) on the outer membrane of the bacterial cell wall, forming a strong bond polymer that cause damage to porin. Terpenoids compound soluble in lipids, which causes the properties of these compounds readily penetrate the bacterial cell wall of gram-positive and gram-negative. Steroid as an antibacterial mechanism associated with sensitivity of membrane lipid and steroid compounds can cause leaks in liposomes¹⁵.

Extraction results

The extraction of 300 g of powder simplex from immature and mature Nipa leaves by maceration method with 96% ethanol was concentrated by rotary evaporator and freeze dryer became viscous extract obtained from immature and mature Nipa leaves respectively 53.20 g and 55.32 g.

Result of antibacterial activity test of extract ethanol immature and mature Nipa leaves

The test results of antibacterial activity of ethanolic extract of immature Nipa leaves can be seen in Table 4 and Table 5.

Table 4: Results of the measurement diameter zone of growth inhibition of *S. aureus*

Concentration (mg/ml)	Diameter inhibition area (mm)*
	<i>Staphylococcus aureus</i>
500	17,77
400	16,67
300	16,23
200	15,37
100	14,27
75	13,83
50	12,60
25	10,57
Blank	-

Table 5: Results of the measurement diameter zone of growth inhibition of *E. Coli*

Concentration (mg/ml)	Diameter inhibition area (mm)*
	<i>Escherichia coli</i>
500	18,67
400	17,17
300	16,40
200	15,63
100	15,03
75	14,10
50	12,73
25	11,43
Blank	-

The antibacterial activity of ethanolic extract of mature Nipa leaves can be seen in Table 6 and Table 7.

Table 6: Results of the measurement diameter zone of growth inhibition of *S. aureus*

Concentration (mg/ml)	Diameter inhibition area (mm)*
	<i>Staphylococcus aureus</i>
500	18,67
400	17,57
300	16,57
200	16,30
100	15,40
75	14,37
50	13,00
25	12,76
Blank	-

Table 7: Results of the measurement diameter zone of growth inhibition of *E.coli*

Concentration (mg/ml)	Diameter inhibition area (mm)*
	<i>Escherichia coli</i>
500	18,67
400	17,17
300	16,40
200	15,63
100	15,03
75	14,10
50	12,73
25	11,43
Blank	-

Based on the measurement results of growth inhibition area diameter. *Staphylococcus aureus* and *Escherichia coli*, showed that the ethanolic extract of Nipa leaves was effectively in inhibiting the growth of both bacteria. Antibacterial inhibition area diameter is most effective against antibacterial tests was 14 to 16 mm. The ethanolic extract of immature Nipa leaves begin to inhibit the growth of *Staphylococcus aureus* at a concentration of 100 mg/ml with an inhibition area diameter of 14.27 mm and *Escherichia coli* at a concentration of 75 mg/ml with an inhibition area diameter of 14.10 mm. The ethanolic extract of mature Nipa

leaves begin to inhibit the growth of *Staphylococcus aureus* at a concentration of 75 mg/ml with an inhibition area diameter of 14.37 mm and *Escherichia coli* at a concentration of 75 mg / ml with an inhibition area diameter of 14.80 mm. The comparison between the test results of antibacterial activity of ethanolic extract from immature and mature Nipa leaves can be illustrated in the Figure 1.

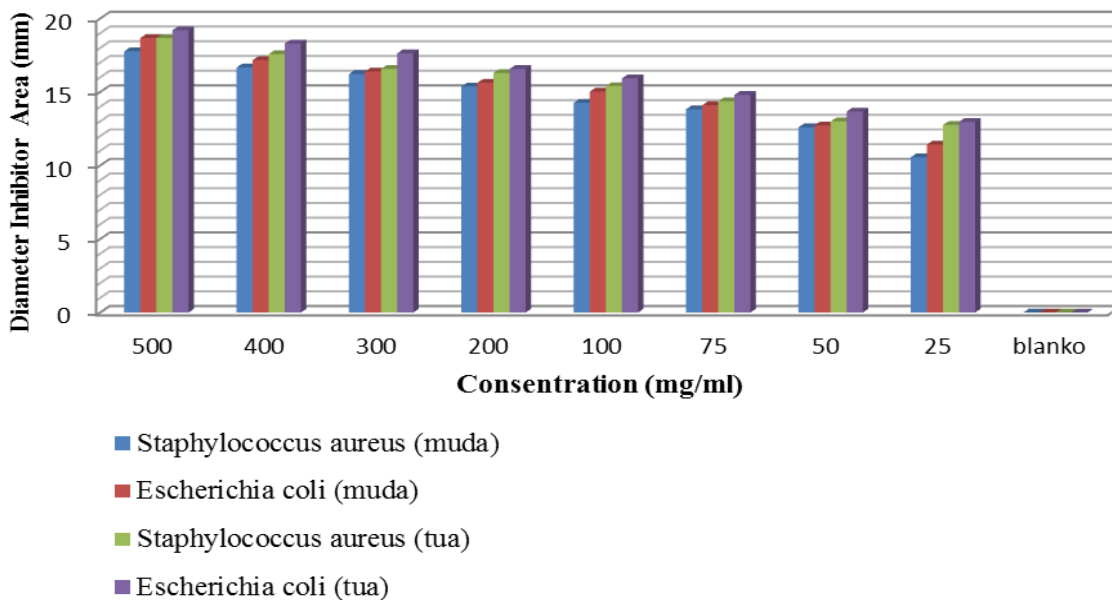


Figure 1: Comparison of test results of antibacterial activity of immature and mature ethanolic extract Nipa leaves

Conclusion

The ethanolic extract of mature Nipa leaves are more effective to inhibit the growth of the bacteria *Staphylococcus aureus* and *Escherichia coli* than ethanolic extract of immature Nipa leaves

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