

Antibacterial Activity of Keben (*Barringtonia Asiatica L.*) Leaf Extract on the Growth of Bacteria *Staphylococcus Epidermis* and *Pseudomonas Aureginosa*

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ABSTRACT

The use of antibiotics is very high. Antibiotics or antimicrobial drugs can be sourced from synthetic or natural / herbal ingredients. Keben leaves are one of the plants that have antibacterial properties because of their chemical content. This study aimed to determine the antibacterial activity of methanol, ethyl acetate, and n-hexane extracts from keben leaves (*Barringtonia asiatica*) against *Staphylococcus epidermal* and *Pseudomonas aureginosa* bacteria. The method used is the paper disc diffusion method with a concentration of 20%, 40%, and 60% keben leaf extract. The results of the antibacterial activity test of methanol, ethyl acetate, and n-hexane extracts of keben leaves against *Staphylococcus epidermal* bacteria with concentrations of 20%, 40%, and 60% resulted in an inhibition zone diameter of 7.65 mm - 17.48 mm, while in *Pseudomonas aureginosa* bacteria produces an inhibition zone diameter of 7.65 mm - 12.73 mm. the 20% and 40% n-Hexan extract had no inhibition zone and the 20% methanol extract had no inhibition zone. Based on the results of research, keben leaves have the potential as an antibacterial.

INTRODUCTION

In Indonesia, the use of antibiotics is very high. The prevalence of antibiotic use in Indonesia is quite high (40-60%) (Rahman et al., 2014). This figure is followed by inappropriate use of antibiotics. Inappropriate use of antibiotics can trigger antibiotic resistance. Antibiotic resistance has negative impacts including increased morbidity and mortality, costs and duration of treatment, and side effects (Ministry of Health of the Republic of Indonesia., 2015).

Antibiotics or antimicrobial drugs can come from synthetic or natural / herbal ingredients. Many antibiotics are resistant to bacteria. As an alternative, there have been many herbal medicines used to treat various diseases. Examples of plants that are often used as traditional medicine are keben leaves used as medicine for stomach aches and rheumatism (Kayser, 2005).

In the Riau Islands, there are many herbal plants whose benefits have not yet been widely discovered, including the keben plant (*Barringtonia asiatica*) which is located in Tanjung Pulau Pengapit, Batam City. The keben plant grows abundantly in coastal areas and has not been widely utilized.

According to research conducted by Bustanussalam & Simanjuntak, (2012) Keben leaves contain saponin, terpene, alkaloid, triterpenoid, phenolic and tannin compounds. Tannin and saponin can act as antibacterials. The antibacterial effects of tannins include through reactions with cell membranes, tannins will denature and coagulate proteins and damage cell wall membranes.

Antibacterial activity testing with keben leaf samples has previously been carried out with gram-positive bacteria *Staphylococcus aureus* and gram-negative bacteria *Escherichia coli* with extract concentrations used of 2%, 4%, and 6%. The results obtained for the antimicrobial test of keben leaf extracts and fractions can inhibit bacteria but are weak with an average clear zone of <5mm (Silvani, 2020).

Based on the background above, researchers are interested in conducting research on the antibacterial activity of keben extract (*Barringtonia asiatica*) using different bacteria, namely on the growth of *Staphylococcus epidermis* and *Pseudomonas Aureginosa* bacteria.

THEORETICAL REVIEW

Antibacterials are compounds that have the ability to inhibit the growth or kill bacteria. One potential natural source of antibacterial is medicinal plants, one of which is keben leaves (*Barringtonia asiatica*). This plant is known to contain various bioactive compounds which have the potential to act as antibacterials against various types of pathogenic bacteria, including *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*.

The keben plant is known to contain various active compounds that have antibacterial potential, including: Saponins, which are known to have antibacterial effects by damaging bacterial cell membranes. Flavonoids, which act as antioxidants and antibacterials by interfering with microbial metabolic processes. Tannins, which can inhibit bacterial growth by interfering with enzymes that play a role in bacterial metabolism. Alkaloids, which work by inhibiting bacterial protein synthesis thereby inhibiting the growth of

microorganisms. *Staphylococcus epidermidis* is a gram-positive bacterium that is generally found on human skin as normal flora. Although usually harmless, this bacteria can cause infections in individuals with weakened immune systems or in patients with implanted medical devices. Infections caused by *S. epidermidis* are often difficult to treat because of their ability to form antibiotic-resistant biofilms. *Pseudomonas aeruginosa* is a gram-negative bacterium that is opportunistic and often found in humid environments. This bacteria is the main cause of nosocomial infections, especially in patients with weak immune systems. *P. aeruginosa* has high resistance to various types of antibiotics, so the discovery of natural antibacterial agents is important in overcoming infections caused by this bacteria. Several studies show that keben leaf extract has antibacterial activity against *S. epidermidis* and *P. aeruginosa*. The mechanism of action is thought to involve: Damage to cell membranes, especially by saponin compounds which can cause bacterial cell lysis. Inhibition of metabolic enzymes, where flavonoids and tannins interfere with bacterial enzyme activity. Inhibition of protein synthesis, caused by alkaloids that disrupt translation processes in bacterial cells. Several methods used to test antibacterial activity include the disk diffusion method, liquid dilution, and spectrophotometry to measure the inhibition zone for bacterial growth.

METHODOLOGY

Tools and Materials

The tools used in this study were analytical scales, filter paper, petri dishes, autoclaves, laminar air flow (LAF), magnetic stirrers, disc paper, hot plates, incubators, rotary evaporators, micropipettes, calipers, rulers. The materials used in this study were samples of keben leaf extract, *Staphylococcus epidermis* bacteria, and *Pseudomonas aeruginosa*, methanol (CH₃OH), n-hexane (C₆H₁₄), ethyl acetate (C₄H₈O₂), distilled water (H₂O), paper discs, 2N hydrogen chloride (HCl), dimethyl sulfoxide, chloramphenicol discs, Nutrient agar (NA), Sulfuric acid (H₂SO₄), Barium chloride (BaCl₂).2H₂O.

Extract Preparation

Keben leaf powder was weighed as much as 2.5 kg and put into a container then added 1500 mL (1:3 w/v) n-hexane solvent (non-polar) macerated for 3x24 hours or until the color of the filtrate was almost the same as the color of the solvent. During maceration, stirring was done occasionally. After that, it was filtered and the filtrate and residue were produced. The n-hexane filtrate results were stored at room temperature and the residue was re-macerated using ethyl acetate solvent (semi-polar) as much as 1500 mL for 3x24 hours or until the color of the filtrate was almost the same as the color of the solvent. The extraction results with ethyl acetate were then filtered to obtain the filtrate and residue, the ethyl acetate filtrate was stored at room temperature. The residue was re-extracted using methanol solvent (polar) as much as 1500 mL for 3x24 hours or until the color of the filtrate was almost the same as the color of the solvent. Filtering was carried out and a methanol filtrate was produced. The three filtrates produced were evaporated using a rotary vacuum evaporator at a temperature

of 40°C until the methanol had completely evaporated, leaving only a thick extract (Markham, 1988).

After that, calculate the % yield based on the weight percentage (b/b) using the following equation :

$$\% \text{ yield} = \frac{\text{Extract weight}}{\text{Initial weight of sample}} \times 100\%$$

Determination of Drying Loss

Weigh 2 grams of thick extract and then put it into a porcelain crucible with a lid that has been previously heated at a temperature of 105°C for 30 minutes and has been tared. After that, the porcelain crucible is put into the oven with the lid open and then dried at a temperature of 105°C for 30 minutes, then removed and cooled in a desiccator for 15 minutes and reweighed. Repeat the method above until a constant weight is obtained (Permadi et al. 2015)

$$\% \text{ drying shrinkage} = \frac{(B - A)(C - A)}{(B - A)} \times 100\%$$

Description:

A = weight of empty porcelain crucible (grams)

B = weight of porcelain crucible + sample before heating (grams)

C = weight of porcelain crucible + sample that has been heated (grams)

Determination of Total Ash Content

Weigh 2 grams of thick extract and then put it into a porcelain crucible that has been heated at 105°C for 30 minutes and has been tared. After that, the porcelain crucible is put into a furnace and then heated at 600°C for 7 hours, then removed in a desiccator for 15 minutes and reweighed (Febriyanti et al. 2018).

$$\text{Ash content} = \frac{(C - A)}{(B - A)} \times 100\%$$

Description:

A = weight of empty porcelain crucible that has been incandescent

B = weight of porcelain crucible + sample before incandescence

C = weight of porcelain crucible + sample after incandescence

Antibacterial Activity

15 mL of Nutrient Agar was put into a petri dish, then the bacterial suspension solution was evenly rubbed on the surface of the solidified agar media using a sterile cotton swab and left for 5 minutes. Furthermore, the disc that had been dripped with 10 µL of n-hexane, ethyl acetate and methanol extracts at various concentrations, namely 20%, 40% and 60%. The disc was placed on the surface of the petri dish containing the bacterial suspension. The disc paper that had been dripped as a positive suspension used Chloramphenicol

disc (35 µg) and as a negative control used 10% DMSO. The disc was placed on the surface of the Nutrient Agar petri dish then closed and incubated in an incubator at a temperature of 37°C for 24-48 hours. Furthermore, the observation and measurement of the diameter of the clear zone around the disc using a caliper (Putri et al., 2019).

Data Analysis

Data analysis was carried out descriptively in the form of a table by observing the measurement of the diameter of the inhibition zone of the keben leaf extract (*Barringtonia Asiatica*).

RESULTS AND DISCUSSIONS

The weight of the keben leaf sample in this study was 2.5 kg. The results of the keben leaf extract yield can be seen in Table 1. The results of the drying shrinkage determination test for methanol, ethyl acetate, n-Hexane extracts were respectively 0.91%; 4.9%; 4.34% and the total ash content determination test for methanol, ethyl acetate, n-Hexane extracts were respectively 2.49%; 2.49%; 4.35%.

Table 1. Results of Keben Leaf Extract (*Barringtonia asiatica*)

No.	Plant Samples	Hasil		Yield (%)
		initial weight	extract weight	
1	Ekstrakt n-Heksana	2.500 gram	78,92 gram	3,15 %
2	Ekstrakt Etil acetat	2.500 gram	74,60 gram	2,98 %
3	Ekstrakt Methanol	2.500 gram	90,35 gram	3,61 %

Extraction in this study used a multilevel maceration method because it is classified as a simple method, namely by dissolving active substances based on the level of polarity in a solvent (Marjoni, 2016). The macerate results obtained from the three solvents n-Hexane, ethyl acetate, and methanol in a rotary evaporator so that a thick extract of keben leaves was obtained. This study produced 78.92 grams of n-Hexane extract and a yield value of 3.15%, 74.60 grams of ethyl acetate with a yield value of 2.98%, and 90.35 grams of methanol with a yield value of 3.61%.

The results of the drying shrinkage test of methanol, ethyl acetate, and n-Hexane extracts were 0.91%; 4.9%; and 4.34%, respectively. The drying shrinkage aims to determine the maximum limit of the number of compounds lost during the drying process in the extract. And the results of this study indicate that the n-Hexane, Ethyl acetate, and methanol extracts of keben leaves meet the requirements, which are no more than 10% (Ministry of Health of the Republic of Indonesia., 1989)

The results of the ash content test of methanol, ethyl acetate, and n-Hexane extracts were 2.49%; 2.49%, 4.35%, respectively. The ash content test aims to

determine the description of the mineral content originating from the initial process until the extract is formed. The results of this study indicate that the n-Hexane, ethyl acetate, and methanol extracts of keben leaves meet the requirements, which are no more than 5.5% (Ministry of Health of the Republic of Indonesia., 2000).

The antibacterial activity test was conducted to determine the ability to inhibit the bacteria tested on the three keben leaf extracts. The magnitude of the bacterial inhibition power is indicated by the formation of a clear zone around the disc paper. Testing of antibacterial activity with keben leaf samples has previously been carried out with gram-positive bacteria *Staphylococcus aureus* and gram-negative bacteria *Escherichia coli* with extract concentrations used of 2%, 4%, and 6%. In testing the antibacterial activity of methanol extract of keben leaves on *Staphylococcus aureus* bacteria with a concentration of 2%, the average diameter of the inhibition power was 2.61 mm (categorized as weak), a concentration of 4% obtained an inhibition zone of 3.43 mm (categorized as weak), and a concentration of 6% obtained an inhibition zone of 5.23 mm (categorized as moderate) and In testing antibacterial activity of methanol extract of keben leaves on *Escherichia Coli* bacteria with a concentration of 2% obtained an average diameter of inhibition of 1.75 mm (categorized as weak), a concentration of 4% obtained an inhibition zone of 1.75 mm (categorized as weak), a concentration of 6% obtained an inhibition zone of 2.85 mm (categorized as weak) (Silvani, 2020).

Table 2. Average Results of the Inhibition Zone Diameter of N-Hexane Extract of Keben Leaves Against *Staphylococcus epidermis* Bacteria.

Treatment of <i>Staphylococcus epidermis</i> bacteria	Repetition			Average (mm)	Inhibition zone category
	I	II	III		
Control (+) kloramfenikol	25,7	39,05	40,05	34,93	Very Strong
Control (-) DMSO 10%	0	0	0	0	-
Concentration 20 %	9,3	9,6	5,85	8,25	Currently
Concentration 40 %	11,05	12,95	10,7	11,56	Strong
Concentration 60%	18,2	14,1	12	14,76	Strong

The results of the antibacterial activity test of n-Hexane extract of keben leaves on *Staphylococcus epidermis* bacteria with a concentration of 20% obtained an average diameter of inhibition of 8.25 mm (categorized as moderate), 40% concentration of 11.56 mm (categorized as strong), 60% concentration of 14.76 mm (categorized as strong), positive control of 34.93 mm (categorized as

very strong), and negative control of 0 mm. In the antibacterial test of n-Hexane extract on *Staphylococcus epidermis* bacteria, the results obtained were the largest at a concentration of 60% with an average inhibition zone of 14.76 mm (categorized as strong). In the antibacterial test of ethyl acetate extract on *Staphylococcus epidermis* bacteria, the results obtained were the largest at a concentration of 60% with an average inhibition zone of 13.95 mm (categorized as strong). In the antibacterial test of methanol extract against *Staphylococcus epidermis* bacteria, the highest results were obtained at a concentration of 60% with an average inhibition zone of 17.48 mm (categorized as strong).

In the antibacterial test of n-Hexane extract against *Pseudomonas aureginosa* bacteria, the results obtained were greatest at a concentration of 60% with an average inhibition zone of 9.63 mm (categorized as moderate). In the antibacterial test of ethyl acetate extract against *Pseudomonas aureginosa* bacteria, the results obtained were greatest at a concentration of 60% with an average inhibition zone of 12.73 mm (categorized as strong). In the antibacterial test of methanol extract against *Pseudomonas aureginosa* bacteria, the results obtained were greatest at a concentration of 60% with an average inhibition zone of 10.73 mm (categorized as strong). Gram-positive *Staphylococcus epidermidis* bacteria are more sensitive to keben leaf extract compared to gram-negative *Pseudomonas aeruginosa* bacteria. This can be seen at a concentration of 60%, n-Hexane, ethyl acetate, and methanol extracts of keben leaves are able to inhibit the growth of *Staphylococcus epidermidis* with a strong category while for *Pseudomonas aeruginosa* bacteria it is still classified as a moderate category in n-Hexane extract and is classified as a strong category in ethyl acetate and methanol. According to Ajizah (2004) the concentration of antibacterial compounds is very influential in inhibiting the growth of the microorganisms tested. The size of the inhibition zone in the media is the size of the response of keben leaf extract to the sensitivity of bacteria at a certain concentration.

Table 3. Average Results of the Inhibition Zone Diameter of Ethyl Acetate Extract of Keben Leaves Against *Staphylococcus epidermis* Bacteria

Treatment of <i>Staphylococcus epidermis</i> bacteria	Repitition (mm)			Average (mm)	Inhibition zone category
	I	II	III		
Control (+) Chloramphenicol	31,95	39,83	40,7	37,49	Very Strong
Control (-) DMSO 10%	0	0	0	0	-
Consentration 20 %	8,2	10,35	9,45	9,3	Currently
Consentration 40 %	9,5	12,35	11,9	11,25	Strong
Consentration 60%	16,8	12,4	12,65	13,95	Strong

The results of the antibacterial activity test of ethyl acetate extract of keben leaves on *Staphylococcus epidermis* bacteria with a concentration of 20% obtained an average diameter of inhibition power of 9.3 mm (categorized as moderate), a concentration of 40% of 11.25 mm (categorized as strong), a concentration of 60% of 13.95 mm (categorized as strong), a positive control of 37.49 mm (categorized as very strong), and a negative control of 0 mm.

Table 4. Average Results of the Inhibition Zone Diameter of Methanol Extract of Keben Leaves Against *Staphylococcus epidermis* Bacteria

Treatment of <i>Pseudomonas aeruginosa</i> Bacteria	Repetition (mm)			Average (mm)	Inhibition zone category
	I	II	III		
Control (+) Chloramphenicol	19,6	40,55	41,2	33,78	Very Strong
Control (-) DMSO 10%	0	0	0	0	-
Concentration 20 %	9,5	9,75	9,65	9,63	Currently
Concentration 40 %	9,9	11,5	10,85	10,75	Strong
Concentration 60%	23,85	13,65	14,95	17,48	Strong

The results of the antibacterial activity test of ethyl acetate extract of keben leaves on *Pseudomonas Aureginosa* bacteria with a concentration of 20% obtained an average diameter of inhibition power of 12 mm (categorized as strong), a concentration of 40% of 10.96 mm (categorized as strong), a concentration of 60% of 12.73 mm (categorized as strong), a positive control of 39.11 mm (very strong), and a negative control of 0 mm.

Table 5. Average Results of the Inhibition Zone Diameter of Ethyl Acetate Extract of Keben Leaves Against *Pseudomonas aureginosa* Bacteria.

Treatment of <i>Pseudomonas aeruginosa</i> Bacteria	Repetition (mm)			Average (mm)	Inhibition zone category
	I	II	III		
Control (+) Chloramphenicol	28,8	46,85	41,7	39,11	Very Strong
Control (-) DMSO 10%	0	0	0	0	-

Concentration 20 %	11,55	11,85	12,6	12,0	Strong
Concentration 40 %	9,5	9,6	13,8	10,96	Strong
Concentration 60%	10,7	12,5	15,05	12,73	Strong

Table 6. Average Results of the Inhibition Zone Diameter of Methanol Extract of Keben Leaves Against Pseudomonas aureginosa Bacteria

Treatment of Pseudomonas aeruginosa Bacteria	Repitition (mm)			Average (mm)	Inhibition zone category
	I	II	III		
Control (+) Chloramphenicol	39,75	50,7	40,6	43,68	Very strong
Control (-) DMSO 10%	0	0	0	0	-
Concentration 20 %	-	-	-	-	-
Concentration 40 %	6,7	6,3	9,95	7,65	Weak
Concentration 60%	12,4	11,0	8,8	10,73	Strong

In antibacterial activity, there are several factors that can influence the size of the inhibition zone, namely extract concentration, antibacterial compound content, extract diffusion power and the type of bacteria inhibited (Marselia et al., 2015).

From the results it can be seen that the largest inhibition zone is found in Staphylococcus epidermis bacteria. Staphylococcus epidermis bacteria are gram-positive bacteria while in gram-negative bacteria Pseudomonas aureginosa the inhibition zone formed is not so large because Gram-negative bacteria have a multi-layered and very complex cell wall structure, containing three layers of polymers located outside the peptidoglycan layer, namely lipoproteins, the outer membrane consists of phospholipids and liposaccharides, the outer membrane is phospholipid. This condition can also be stated that the lipids that are abundant in the cell walls of Gram-negative bacteria can affect the activity of thymohydroquinone, thereby reducing the inhibitory power produced (Jawetz et al., 1987).

This study is also in line with Widyana W. et al, 2014, where the difference in inhibitory responses is due to the two bacteria having different cell wall structures. Staphylococcus epidermidis bacteria and Pseudomonas aeruginosa bacteria have different levels of cell wall sensitivity to physical, enzyme, and antibiotic treatments. Pelezar and Chan (2005) stated that the cell wall of Staphylococcus epidermidis bacteria is simpler so that it can facilitate

antibacterial compounds to enter the cell. *Pseudomonas aeruginosa* has a more complex cell wall structure and contains more lipid component compounds compared to *Staphylococcus epidermidis* bacteria.

CONCLUSION AND RECOMMENDATIONS

Based on the results of the study, it can be concluded that

1. Keben leaf extract has antibacterial activity against *Staphylococcus epidermis* as an antibacterial with a strong inhibition zone.
2. Keben leaf extract with Ethyl acetate solvent and methanol solvent has antibacterial activity against *Pseudomonas aureginosa* has antibacterial inhibition with a strong inhibition zone.

FURTHER STUDY

Future research is expected to further explore combination studies with antibiotics, formulation development, and safety testing.

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