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ANTIBACTERIAL POTENTIAL OF BANGKAL LEAVES (NAUCLEA SUBDITA (KORTH.) STEUD.) METHANOL EXTRACT AGAINST AEROMONAS HYDROPHILA

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ABSTRACT
The content of bioactive compounds in the leaves of the bangkal plant (Nauclea subdita (Korth.) Steud.) such as flavonoids and phenols can cause this bangkal plant to work as an antibacterial. The potential of bangkal leaf extract as a therapeutic agent in treating bacterial infections in fish, as well as providing a new direction in developing a more effective and sustainable management strategy for Aeromonas Hydrophila disease. The research aims to analyze the antibacterial potential of methanol extract of bangkal leaves (Nauclea subdita (Korth.) Steud.) against Aeromonas Hydrophila. The research was carried out at the Basic Laboratory of the Faculty of Fisheries and Maritime Affairs, Bagkal leaf extraction activities at the Mathematics and Natural Sciences Laboratory at Lambung Mangkurat University, Banjarbaru. The type of active compound contained in the methanol extract of bangkal leaves (Nauclea subdita (Korth.) Steud.) which has antibacterial properties against Aeromonas Hydrophila bacteria is that the methanol extract from bangkal leaves has the potential as an antibacterial agent against Aeromonas Hydrophila bacteria. Phytochemical analysis found the presence of alkaloids, flavonoids, tannins and saponins in the extract. Inhibitory power tests using the disk method and Minimum Inhibitory Concentration (MIC) test as well as the coculture method showed that the concentration of the extract significantly influenced its ability to inhibit bacterial growth.

KEY WORDS
Bangkal leaves, antibacterial effect, aeromonas hydrophila, bacteria.

The problem that farmers generally face in raising fish is that there are frequent attacks of disease caused by bacteria, one of which is the Aeromonas hydrophilla bacteria. Infection from Aeromonas Hydrophila bacteria will cause the disease Motile Aeromonas Septicemia (MAS). These Gram-negative bacteria can cause high mortality in several farmed freshwater fish species and can cause serious gastric disease epidemics, causing large economic losses in farmed fish (Angka et al., 1995; Zhou et al., 2018). Apart from causing disease in several types of freshwater fish, this bacterium is also pathogenic to amphibians, reptiles, birds, mammals and humans (Angka et al., 2004).

Fish that are attacked by this disease will experience bleeding on parts of the body, especially on the chest, stomach and base of the fins. It turns out that fish infected with bacterial disease will easily infect other fish, so fish that are infected and whose condition is serious must be treated immediately or immediately destroyed. Treatment and control of Aeromonas Hydrophila bacterial disease generally still uses chemicals such as antibiotics and natural ingredients. The use of antibiotics can cause residues in organisms and bacteria will become resistant (Badesso, 2017). Uncontrolled use of antibiotics can have a negative impact on fish because it can cause residues and can pose a health hazard to consumers who consume fish with antibiotic residues (Wahjuningrum, et al., 2012). An alternative for the treatment and control of Aeromonas Hydrophila bacterial infections that does not have a negative impact.

Herbal plant extracts or medicinal plants with natural ingredients which have anti-microbial substances and are able to cure Aeromonas Hydrophila bacterial infections such as the leaves of the bangkal plant (Nauclea subdita (Korth.) Steud.). The use of herbal or medicinal plant extracts has the advantage of not causing residue, being easy to obtain at a

relatively cheap price, being environmentally friendly and also not having a negative effect on cultivated organisms (Hardi et al., 2017). The bangkal plant (Nauclea subdita (Korth.) Steud.) is one of the medicinal plants that can be used for treatment. This bangkal plant is often found on riverbanks or swamps in South Kalimantan. This plant is widely used by the people of South Kalimantan because it contains antioxidants. The ethanol extract of bangkal leaves (Nauclea subdita (Korth.) Steud.) contains secondary metabolite compounds such as alkaloids, flavonoids, phenolics, saponins, steroids and tannins (Fadlilaturrahmah et al., 2023).

The research results of Wardhani and Akhyar (2018) stated that the ethanol extract of the bark of the bangkal plant (Nauclea subdita (Korth.) Steud.) contains bioactive compounds such as alkaloids, flavonoids, polyphenols and saponins, while the ethanol extract of the leaves of the bangkal plant contains alkaloid, flavonoid, polyphenols and quinones. Bioactive compounds in the leaves of bangkal plants (Nauclea subdita (Korth.) Steud.) are known to inhibit the activity of pathogenic bacteria such as Staphylococcus aureus at concentrations of 100 ppm, 300 ppm, and 500 ppm with respective inhibition zones of 6.8 mm; 9.3mm and 9.8mm.

The content of bioactive compounds in the leaves of the bangkal plant (Nauclea subdita (Korth.) Steud.) such as flavonoids and phenols can cause this bangkal plant to work as an antibacterial. This is because flavonoid compounds can denature proteins which will stimulate damage to the bacterial cell nucleus of bacterial cells so that it can cause bacterial lysis (Simbala, 2009). The results of Aisiah's research (2020) concluded that the methanol extract of Bangkal leaves contains the antibacterial bioactive Aeromonas Hydrophila. Research on the effectiveness of methanol extract of bangkal leaves (Nauclea subdita (Korth.) Steud.) on the health status of fish infected with Aeromonas Hydrophila bacteria needs to be carried out. A deeper understanding of the potential use of bangkal leaf methanol extract as an antibacterial agent in the context of treating infections in fish can be obtained. This step will make a significant contribution to the development of health and disease management strategies in farmed fish populations.

The potential of bangkal leaf extract as a therapeutic agent in treating bacterial infections in fish, as well as providing a new direction in developing a more effective and sustainable management strategy for Aeromonas Hydrophila disease. The research aims to analyze the antibacterial potential of methanol extract of bangkal leaves (Nauclea subdita (Korth.) Steud.) against Aeromonas Hydrophila.

MATERIALS AND METHODS OF RESEARCH

The research was carried out at the Basic Laboratory of the Faculty of Fisheries and Marine science, Bagkal leaf extraction activities at the Mathematics and Natural Sciences Laboratory, Lambung Mangkurat University, Banjarbaru.

Aeromonas Hydrophila Antibacterial Activity Test:

1. Antibacterial Test of Bangkal Leaf Extract Using the Disc Method. The antibacterial activity test against Aeromonas Hydrophila using the disc method is an antimicrobial testing technique that involves measuring the diameter of the resistance area formed around the paper disc that has been impregnated with the test substance, namely bangkal leaves. This method is to quantitatively evaluate the effectiveness of certain compounds or materials in inhibiting the growth of Aeromonas Hydrophila bacteria. The size of the obstacle area can provide useful information about the potential of the test substance as an antibacterial agent. The antibacterial test technique using paper discs is one of the approaches commonly used in antimicrobial research because it is simple, fast, and can provide clearly measurable results to evaluate antimicrobial activity;
2. Test the Phytochemical Content of Bangkal Leaf Extract:
   - Identification of alkaloids from bangkal leaf extract;
   - Gram is moistened with 30% ammonia, the solution inside is added with Dragendroff's and Mayer's reagents respectively. The formation of a brick red

precipitate with Dragendroff's reagent or white with Mayer's reagent indicates the presence of alkaloid compounds;

- Flavonoid Identification A total of 5 ml of bangkal leaf extract solution, add Mg powder or plates and 1 ml of concentrated HCl and then add amylalcohol, shake vigorously and leave to separate. When a red color forms in the amyl alcohol, shake vigorously and allow it to separate. The formation of a red color in amyl alcohol indicates the presence of flavonoid compounds;
- Identify Saponins. A total of 10 ml of bangkal leaf extract solution was put into a test tube and shaken vertically for 10 seconds. Then leave it for 10 minutes. The formation of stable foam in the tube indicates the presence of saponin group compounds. Add 1 drop of 1% HCl, the foam remains stable;
- Tannin Identification Bangkal leaf extract solution is put into a test tube. The extract was saturated with sodium acetate, a few drops of 1% iron (III) chloride were added. The formation of a blue ink color indicates the presence of tannins.

3. MIC test (Minimum Inhibitory Concentration) The MIC test is carried out to determine the minimum inhibitory concentration of an antimicrobial substance contained in bangkal leaf extract as an inhibitor of the growth of Aeromonas Hydrophila bacteria. This method allows determining the lowest concentration level of bangkal leaf extract which is still able to inhibit the growth of Aeromonas Hydrophila bacteria. Determination of the MIC is important in this research because it provides specific and quantitative information about the effectiveness of bangkal leaf extract as an antimicrobial agent against Aeromonas Hydrophila bacteria. MIC, researchers can evaluate the potential of bangkal leaf extract to be used as an alternative in controlling bacterial infections in fish, as well as assisting in the development of more effective therapies in the field of aquaculture health.

RESULTS AND DISCUSSION

Antibacterial activity was analyzed using a disc test to determine the inhibitory strength of the fraction and to determine the best fraction. Testing using the disc method is seen based on the diameter of the inhibition zone (clear zone) that is formed, where the diameter of the zone indicates that bacterial growth is inhibited. Bacterial growth categories based on the diameter of the inhibition zone formed are divided into 4, namely weak (diameter <5 mm), medium (diameter 5 – 10 mm), strong (diameter 10 – 20 mm) and very strong (diameter > 20 mm).

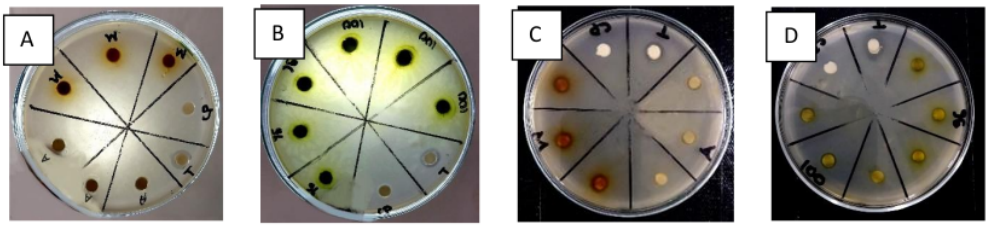


Figure 1 – Documentation of antibacterial test results using the disc diffusion method
Information: (A) Antibacterial test results of fresh Bangkal leaves with distilled water and methanol; (B) Antibacterial test results of fresh bangkal leaves with 100% ethanol and 96% ethanol; (C) Antibacterial test results of dried Bangkal leaves with distilled water and methanol; (D) Antibacterial test results of dried bangkal leaves with 100% ethanol and 96% ethanol (Source: Personal Documents, 2023)

Table 2 – Inhibition Zone Diameter of Bangkal Leaf Extract Fraction Disc Test

No.	Solvent	Fresh Bangkal (cidal)	Fresh Bangkal (static)	Dry Bangkal (cidal)	Dry Bangkal (static)
1.	Methanol	4,9	6,9	5,3	9,3
2.	Aquadest	2,6	-	2,4	-
3.	Ethanol 100%	-	6	2,4	-
4.	Ethanol 96%	4,7	-	2,3	5,1

Based on the inhibition zone, it was found that the Bangkal (Nauclea subdita (Korth.) Steud.) leaf extract fraction with methanol (PA) solvent inhibited the growth of Aeromonas Hydrophila bacteria with values of 4.9 mm, 6.9 mm, 5.3 mm respectively. and 9.3mm.

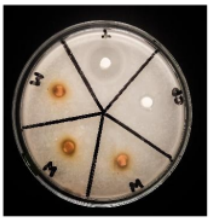


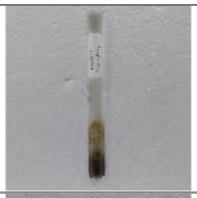
Figure 2 – Second resistance test

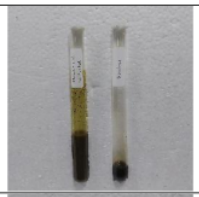
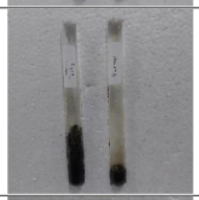
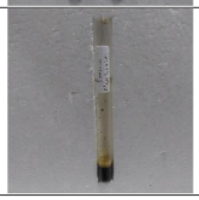
Table 2 – Second Methanol Inhibitory Test

Tetracycline	16,9 (cidal)	28,9 (static)
Cepadroxyle	9 (cidal)	19 (static)
Methanol 1	11,3	
Methanol 2	11,7	
Methanol 3	10,6	

Phytochemical analysis of bangkal leaf extract (Nauclea subdita (Korth.) Steud.) using methanol solvent can be seen in Table 4.2 based on parameter tests for alkaloid, flavonoid, tannin and saponin compounds showing positive results.

Table 3 – Results of Phytochemical Screening of bangkal leaf extract

Compound identification	Testing	Result	Picture	Information
Alkaloid	Dragendoff	-		No red color is formed
	Mayer	+		Formation of yellow/white color
	Wagner	+		Formation of red/brick red color
Flavonoid	Mg+HCl 1%	+		A red/yellow orange color is formed

Flavonoid	Pb Acetate 10%	+		A yellow color forms
Tanin	Iron (III) Chlorida 1%	+		A blackish blue color is formed
Saponin	Vortd ex	+		Forms stable foam

The results obtained are based on the table above, in accordance with previous research, namely Aisiah (2020), where bangkal leaf extract (Nauclea subdita (Korth.) Steud.) with methanol solvent showed positive results for alkaloid, flavonoid, tannin and saponin compounds.

The Minimum Inhibition Concentration (MIC) test is carried out and determined visually to determine the concentration of each fraction that can inhibit bacterial growth. This MIC test is used because it is a simple method, sensitivity and relatively low cost (Elisha et al., 2017). The MIC results of each fraction of bangkal leaf extract (Nauclea subdita (Korth.) Steud.) showed different results.

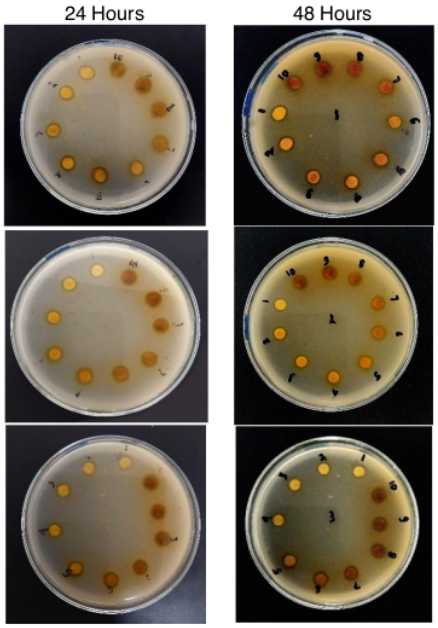


Figure 3 – MIC results of Bangkal Leaf Extract Fraction

Table 4 – MIC test against Aeromonas Hydrophila

No.	Bangkal Leaf Extract (ppm)	Average Inhibitory Power After 24 hours (mm) (cidal)	Average Inhibitory Power After 24 hours (mm) (static)	Average Inhibitory Power After 48 hours (mm) (cidal)	Average Inhibitory Power After 48 hours (mm) (static)
1.	100	3,9	3,6	3,6	3,5
2.	200	4,1	3,7	3,7	3,7
3.	300	4,3	4,2	3,9	4,9
4.	400	4,2	4,2	4,1	5,3
5.	500	4,1	5,3	3,7	6,4
6.	600	4,1	6	3,3	6,6
7.	700	3,9	7	3,6	8,8
8.	800	4,0	7,3	3,6	12,3
9.	900	4,2	8,3	3,7	13,7
10.	950	4,4	9,2	4,0	14,7

Effect of Bangkal leaf extract concentration on bacterial inhibitory power. Table 4.5 shows the results of the MIC (Minimum Inhibitory Concentration) test of Bangkal leaf extract on bacterial growth. The concentration of Bangkal leaf extract (expressed in ppm), the higher the average inhibitory power against Aeromonas Hydrophila bacteria, both after 24 hours and 48 hours of incubation. This shows that the concentration of Bangkal leaf extract significantly influences its ability to inhibit bacterial growth, with a significant increase in inhibitory power seen at extract concentrations above 500 ppm. The difference between the inhibitory power after 24 hours and 48 hours of incubation, which indicates the possible influence of time on the effectiveness of the extract in inhibiting the growth of Aeromonas Hydrophila bacteria.

The comparison of the cidal and static inhibitory power of Bangkal leaf extract in the table also shows the comparison between the cidal (which causes the death of bacteria) and static (which only inhibits bacterial growth) inhibitory power of Bangkal leaf extract. The average inhibitory power tends to be lower than the static inhibitory power at the same concentration. This shows that Bangkal leaf extract has more of an effect on inhibiting bacterial growth than killing it. However, there was a significant increase in cidal inhibition at extract concentrations above 500 ppm, indicating the potential of the extract in significantly reducing the number of bacteria and causing the death of Aeromonas Hydrophila bacteria at higher concentrations.

CONCLUSION

The type of active compound contained in the methanol extract of bangkal leaves (Nauclea subdita (Korth.) Steud.) which has antibacterial properties against Aeromonas Hydrophila bacteria is that the methanol extract from bangkal leaves has the potential as an antibacterial agent against Aeromonas Hydrophila bacteria. Phytochemical analysis found the presence of alkaloids, flavonoids, tannins and saponins in the extract. Inhibitory power tests using the disk method and Minimum Inhibitory Concentration (MIC) test as well as the coculture method showed that the concentration of the extract significantly influenced its ability to inhibit bacterial growth.

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