

COMPARATIVE ANTIBACTERIAL EFFICACY OF ETHANOL AND AQUEOUS EXTRACTS OF BANGKA LOCAL LEAF EXTRACT AGAINST *Aeromonas hydrophila*

Perbandingan Aktivitas Antibakteri Ekstrak Etanol dan Akuades Daun Lokal Bangka
Terhadap *Aeromonas hydrophila*

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ABSTRACT

Motiled *Aeromonas* Septicemia (MAS) is a disease caused *Aeromonas hydrophila*. It is opportunistic pathogen that can cause fish mortality up to 90%. The use antibiotics to control MAS has led resistance on bacteria, therefore the use herbal for alternative treatments. The aim is to determine the effect of different solvents on the antibacterial activity of leaf extracts of bini simpur, kedebik, and mengkirai against *Aeromonas hydrophila*. The methods was diffusion with two types of solvents (ethanol and distilled water). Phytochemical screening included alkaloids, tanins, saponins, triterpenoids, and flavonoids. The results of phytochemical screening showed that ethanol extract contain tanins, saponins, triterpenoids, and flavonoids. The highest inhibition zone was bini simpur extract with ethanol, and the lowest was mengkirai extract with distilled water.

Keywords: *Aeromonas hydrophila*, aquades, ethanol, local leaf extract

ABSTRAK

Penyakit *Motile Aeromonas Septicemia* disebabkan bakteri *Aeromonas hydrophila*, yang merupakan bakteri patogen oportunistik sehingga mengakibatkan kematian ikan hingga 90%. Penggunaan antibiotik untuk menanggulangi penyakit MAS mengakibatkan resistensi, oleh karena perlu adanya Solusi penggunaan bahan herbal sebagai pengobatan. Tujuan penelitian adalah mengetahui pengaruh perbedaan aktivitas antibakteri dari ekstrak daun bini simpur, kedebik, dan mengkirai. Metode yang digunakan dalam penelitian ini adalah dengan difusi cakram dengan dua jenis pelarut, yaitu etanol dan akuades. Skrining fitokimia meliputi alkaloid, flavonoid, saponin, tanin, dan triterpenoid. Hasil skrining fitokimia menunjukkan bahwa ekstrak etanol mengandung alkaloid, flavonoid, tanin, saponin, dan triterpenoid. Sedangkan pelarut akuades mengandung saponin, tanin dan flavonoid. Perbedaan ini

disebabkan karena sifat dari pelarut. Hasil zona hambat yang terbaik adalah ekstrak bini simpur dengan pelarut etanol, sedangkan yang paling rendah zona hambat adalah mengkirai dengan pelarut akuades.

Kata Kunci : *Aeromonas hydrophila*, akuades, etanol, tanaman lokal bangka

INTRODUCTION

Motile *Aeromonas* Septicemia (MAS) is a disease that frequently affects freshwater aquaculture. It is caused by **Aeromonas hydrophila**, a Gram-negative, short-rod-shaped, motile, and non-spore-forming pathogenic bacterium (Azhar et al., 2020). MAS causes significant fish mortality: 33% in **Pangasius hypophthalmus** (Ulandari et al., 2025), 13.33% in catfish (Cerlina et al., 2021), and 37% in tilapia (Anggi et al., 2024). Such high mortality rates result in financial losses for fish farmers. Currently, the solution often employed to address mass mortality is the indiscriminate use of antibiotics.

Antibiotics are compounds used to inhibit or kill pathogenic microorganisms. They are frequently used to treat or prevent disease in fish susceptible to infection (Kusumaningrum et al., 2022). Common antibiotics used in aquaculture include chloramphenicol, nitrofurans, and dimetridazole-group drugs. Unregulated use—regarding dosage, timing, and type—can lead to antibiotic resistance within the fish, necessitating higher doses and increased usage during subsequent infections. Misuse leads to the accumulation of antibiotic residues in fish tissues, posing risks to human health (Najah et al., 2024). Another consequence is the rejection of aquaculture products for export. Therefore, there is a need for an environmentally friendly solution to combat fish diseases that utilizes materials abundantly available in nature.

Bangka Belitung is an island boasting extraordinary plant biodiversity. Several local plants found on Bangka Belitung Island include akar lunding (*Baccaurea lanceolata*), lelambek (*Coelogyne* sp.), bini simpur (*Dillenia suffruticosa*), kedebik (*Melastoma malabathricum*), mengkirai (*Trema orientalis*), and akar sepiding (*Anisophyllea disticha*), among others (Afriyansyah et al., 2022). These plants can be utilized for medicinal purposes as well as for daily needs. Local plants from Bangka are known to contain metabolites—such as alkaloids, flavonoids, tannins, and saponins—capable of killing or inhibiting bacteria (Anjani et al., 2023). The utilization of local plants not only reduces antibiotic usage but also decreases reliance on imported materials.

Therefore, this study aims to conduct phytochemical screening and evaluate the antimicrobial activity of local Bangka plants using alcohol and distilled water as solvents. The findings are expected to contribute scientifically to the advancement of healthcare and the sustainable utilization of the region's biological resources.

RESEARCH METHODS

Place and Time

This study was conducted from September to November 2025. It began with the collection of leaf samples, which were then air-dried and pulverized. Following pulverization, the leaves underwent soaking and evaporation at the Integrated Laboratory of Bangka Belitung University. Antibacterial testing was performed at the Microbiology Laboratory of the Department of Fisheries, Bangka Belitung University.

Tools and Materials

The materials used in this study were **bini simpur**, **mengkirai**, and **kedebik** leaves; 70% ethanol; distilled water; TSA medium; and Rimler-Shotts medium. The **bini simpur**, **mengkirai**, and **kedebik** leaves were obtained from the vicinity of Bangka Belitung

University (Figure 1). The equipment used included an L-shaped spreader, Petri dishes, Erlenmeyer flasks, beakers, a hot plate, an autoclave, and a digital balance.



Figure 1. Kedebik (a), Mengkirai (b), and Bini simpur (c) plants

Preparation of Extract

Extracts were prepared using ethanol and distilled water using 100 grams of dried leaves dissolved in 400 ml of solvent. Ethanol extracts were prepared by washing the leaves and then air-drying them. The leaves were then dried and ground using a blender until smooth. The extract was macerated for 3-4 days, stirring twice a day. The maceration results were filtered using a cloth to obtain a filtrate and then evaporated. Extracts were prepared using aquadest using fresh leaves, then blended until smooth. The solution was filtered using a cloth to obtain a filtrate (Anjani, et al., 2023).

Media preparation

TSA media was prepared by weighing 3 grams of media and dissolving it in 100 ml of distilled water. The media was homogenized using a hot plate stirrer until boiling. The TSA media was sterilized using an autoclave at 121°C for 15 minutes (Anjani et al., 2023).

Phytochemical Screening

The phytochemical tests conducted in this study covered flavonoids, saponins, triterpenoids, alkaloids, and tannins. The testing procedures were based on the studies by Luringunusa et al. (2023) and Anjani et al. (2023).

- Flavonoids

A 1-gram sample was dissolved in 10 mL of distilled water, and 5 drops of HCl and magnesium powder were added. The sample was boiled for 5 minutes. A positive result was indicated by a reddish-brown color.

- Saponin.

A 100 mg sample was dissolved in 1 mL of distilled water, then boiled and filtered. 0.5 mL was added to the filtrate, and the mixture was shaken vigorously for 5 minutes. A positive result was indicated by the formation of foam.

- Triterpenoid.

A 1-gram sample is dissolved in 5 mL of 50% ethanol and boiled for 3 minutes. To the filtered solution, 1 mL of ethyl ether is added, and the mixture is shaken for 5 minutes. Then, 10 mL of chloroform is added and stirred for 5 minutes; 0.5 mg of anhydrous sodium sulfate is added, and the mixture is shaken and filtered. The filtrate is tested using the Liebermann-Burchard reagent by adding 1 mL of sulfuric acid. A positive result is indicated by the appearance of a reddish-brown and yellowish-green ring.

- Alkaloid

A 0.5-gram sample was weighed, treated with two drops of ammonia, and dissolved in 5 mL of CHCl₃. Three drops of H₂SO₄ were added to the filtered sample, and the mixture was shaken until two layers formed. Dragendorff's, Wagner's, and Mayer's reagents were then added. A positive result was indicated by the appearance of purple (Dragendorff's), brown (Wagner's), and creamy-white (Mayer's) colors.

- Tannin

A 1-gram sample was weighed and dissolved in 20 mL of distilled water. The sample was boiled for 5 minutes and filtered. One milliliter of the filtrate was diluted with 5 mL of distilled water, and 3 drops of 10% ferric chloride were added. The mixture was observed for changes; a positive result was indicated by the formation of a green or greenish-brown color.

Antibacterial Test

Antibacterial testing began with the revitalization of *Aeromonas hydrophila* bacteria. The bacteria were cultured on Rimler-Shotts medium using the streak plate method and incubated at 37°C for 24 hours. Subsequently, the bacteria were cultured in Tryptic Soy Broth (TSB) and incubated for 24 hours while being agitated on a shaker. The incubated bacterial culture (1 mL) was then spread onto Tryptic Soy Agar (TSA) plates using an L-shaped glass spreader. After allowing the inoculum to settle for 5 minutes, paper discs—previously soaked in extracts of **Simpur**, **Mengkirai**, and **Kedebik**—were placed on the agar surface. Following incubation at 37°C for 24 hours, the clear zones were measured using a vernier caliper (Anjani *et al.*, 2023)

Data Analysis

Data from the antibacterial activity tests were analyzed using One-Way ANOVA at a 95% confidence level, followed by Duncan's test if significant differences were found. Data on metabolite compounds were analyzed descriptively. The data were subsequently processed and presented in the form of tables and graphs.

RESULT

Phytochemical screening of bini simpur, kedebik, and mengkirai plants using ethanol and distilled water as solvents revealed that ethanol was more effective at extracting metabolites than distilled water (Table 1). Ethanol extracted five compounds, whereas the other solvent extracted only three.

Table 1. Phytochemical Screening of Local Bangka Leaves Using Different Solvents

Types of Qualitative Tests	Binisimpur		Kedebik		Mengkirai		Observation results if (+)
	Distilled water	Ethanol	Distilled water	Ethanol	Distilled water	Ethanol	
Alkaloids	-	+	-	+	-	+	chocolate
Flavonoids	+	+	+	+	+	+	yellow, orange, red
Tanin	+	+	+	+	+	+	blue-green
saponin	+	+	+	+	+	+	foam forms
triterpenoid	-	+	-	+	-	+	formation of a red/brown ring

The results of secondary metabolite compounds showed a trend in line with antibacterial activity. The extract with ethanol solvent produced higher antibacterial activity compared to the extract with aquadest solvent. The ethanol extract of bini simpur leaves produced the highest antibacterial activity (17.8 mm), while the extract with aquadest solvent showed the lowest antibacterial activity (10.88 mm) (Figure 2).

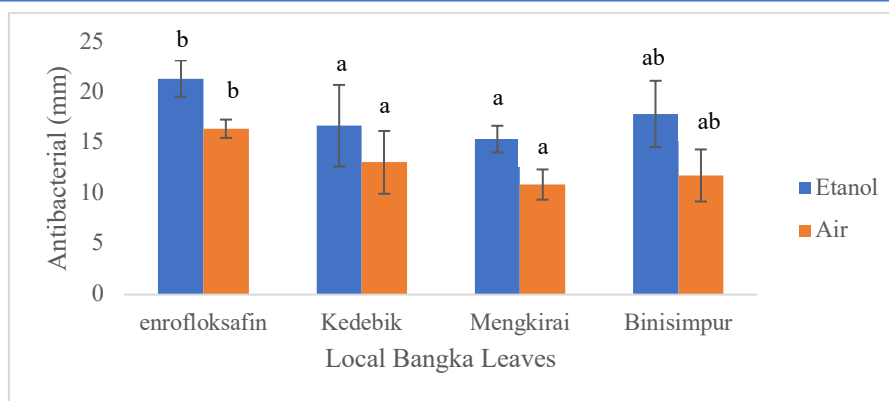


Figure 2. Antibacterial test of local Bangka plants against *Aeromonas hydrophila*

DISCUSSION

Native plants of Bangka are those that grow on Bangka Island. Some of these local plants serve medicinal purposes. Plants frequently utilized by the people of Bangka Belitung include *kedebik*, *mengkirai*, *binisimpur*, *pelawan*, *mensirak*, and others (Swandi et al., 2021; Anjani et al., 2023). These local plants contain secondary metabolites such as saponins, alkaloids, triterpenoids, anthraquinones, flavonoids, and tannins (Sofyana et al., 2024).

Phytochemical analyses using ethanol and distilled water extracts of *kedebik*, *mengkirai*, and *binisimpur* revealed significant differences. Variations in the resulting metabolites were attributed to the polarity of the solvents used. According to Pangisian et al. (2022), solvents influence extraction yields and the secondary metabolites obtained; extraction success depends on the type and ratio of solvents. The principle of extraction relies on polarity compatibility, where polar compounds dissolve more readily than non-polar ones (Riskiana & Vifta, 2021; Putri et al., 2023; Satriawan & Wijaya, 2023).

Flavonoids and phenols are polar; tannins and alkaloids are semi-polar; and saponins and terpenoids are non-polar. Alkaloids and triterpenoids range from semi-polar to predominantly non-polar (Hakim & Saputri, 2020). These two types of metabolites dissolve in semi-polar to non-polar solvents like ethanol but do not dissolve in distilled water. In contrast, flavonoids, tannins, and saponins were detected in both solvents (distilled water and ethanol), as these compounds are polar or water-soluble. Flavonoids possess unsubstituted hydroxyl groups, making them readily soluble in polar solvents such as water or ethanol (Putri et al., 2023). These findings align with Anjani et al. (2023), who reported that the use of distilled water as a solvent yielded positive detections for flavonoid, saponin, and tannin metabolites, whereas alkaloids tested negative. This is likely because alkaloids are basic in nature and require an acidic environment for precipitation when reacting with Dragendorff's and Mayer's reagents.

Aeromonas hydrophila is an opportunistic Gram-negative bacterium capable of causing up to 90% mortality in fish. Transmission occurs through direct contact with infected fish and via shared water sources (Sari et al., 2017). The bacterium produces toxins by forming lipopolysaccharide endotoxins within its cell wall (Anjani et al., 2024).

The best inhibition zone was observed in the positive control (using antibiotics), while the smallest inhibition zone was produced by the Mengkirai extract using distilled water. The Bini Simpung extract produced the largest inhibition zone compared to the others. This is attributed to the presence of alkaloids and triterpenoids, which can damage bacterial membranes, proteins, and metabolic processes. However, the use of distilled water as a solvent did not result in optimal bacterial inhibition. According to Anjani et al. (2023), Bini Simpung extract possesses antimicrobial properties capable of killing bacteria due to its content of

alkaloid and flavonoid metabolites. Alkaloids act as antibacterial agents by inhibiting peptidoglycan formation in the bacterial cell wall, leading to cell wall damage and lysis. Additionally, nitrogen groups within the compounds can interact with bacterial DNA, thereby disrupting replication processes.

Flavonoids are secondary metabolites that function as antiseptics and antibacterial agents. They act as antiseptics by denaturing bacterial proteins. Flavonoids damage the cell wall, causing the bacterial cell membrane to lose its permeability and resulting in the loss of nutrients and enzymes. The leakage of enzymes disrupts cellular metabolism and inhibits ATP production required for cell growth (Hong et al., 2018). As antibacterial agents, flavonoids function by inhibiting nucleic acid synthesis and disrupting bacterial cytoplasmic processes and metabolism (Sujana et al., 2024).

The inhibition zone results for kedebik extracts—using either ethanol or distilled water as solvents—showed consistency comparable to that of other plants. Anjani et al. (2023) identified flavonoids and tannins as the most potent active compounds in the kedebik plant. Tannins kill bacteria through toxicity that damages the bacterial cell membrane. Possessing astringent properties, tannins can shrink cell walls and disrupt cell permeability (Anjani & Hanifah, 2022). Tannins exert antibacterial effects by interfering with bacterial adhesion and enzyme activity, as well as disrupting intracellular protein transport, thereby inhibiting metabolic enzymes. When metabolic enzymes fail to function, energy (ATP) production ceases, halting DNA replication and protein synthesis, which leads to bacterial death (Susanti, 2019).

The mengkirai plant exhibited the smallest inhibition zones among the samples tested with ethanol and distilled water. However, when comparing the two solvents, the ethanol extract of mengkirai produced a larger inhibition zone. This is likely because ethanol is semi-polar, allowing it to extract secondary metabolites—such as alkaloids and flavonoids—that are effective at killing bacteria. Tannin and saponin compounds function to inhibit bacteria and disrupt cell wall permeability (Hakim & Saputri, 2020).

CONCLUSION

The conclusion of this study is that the solvent influencing the phytochemical profile is ethanol, which yielded five types of secondary metabolites (flavonoids, tannins, alkaloids, saponins, and triterpenoids). The strongest antibacterial activity was exhibited by the Simpura seed extract obtained using ethanol (17.9 mm). Among the extracts prepared with distilled water, the Kedebik extract showed the highest antibacterial activity.

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