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POTENTIAL OF VARIOUS INDONESIAN MEDICINAL PLANTS TO INHIBIT THE GROWTH OF *Aeromonas hydrophila* BACTERIA

Ronal Kurniawan[1*], Windarti[2], Irwan Effendi[3], Mega Novia Putri[1], Tomi Syahputra[1], Dimas Gusriansyah[1], M. Riswan[4]
[1]Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Riau, Pekanbaru, 28293 Indonesia
[2]Department of Aquatic Resources Management, Faculty of Fisheries and Marine, Universitas Riau, Pekanbaru, 28293 Indonesia
[3]Department of Marine Science, Faculty of Fisheries and Marine, Universitas Riau, Pekanbaru, 28293 Indonesia
[4]Department of Marine Science, Faculty of Marine and Fisheries, Universitas Syiah Kuala, Banda Aceh, 23111 Indonesia
*kurniawanronal09@gmail.com

ABSTRACT

Aeromonas hydrophila is a gram-negative bacterium that can infect fish and is zoonotic to humans. Efforts to prevent this bacterial infection can be made using herbal plants due to the content of secondary metabolite compounds. This study aims to explore plants that have the potential as antibacterials, especially *A. hydrophila* bacteria. This research was conducted in February 2024 at the Marine Biotechnology Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Riau. The experimental method used included the stages of preparation of plant leaf samples and antibacterial tests using Kirby-Bauer discs. The plants used were the leaves of senduduk (*Melastoma malabathricum*), kantong semar (*Nepenthes* sp), lime (*Citrus aurantifolia)*, belimbing bukit (*Acetosella barreligeri),* sirsak (*Annona muricata*), mucuna (*Mucuna bracteata),* Israel grass (*Asystasia gangetica*), and miana (*Coleus scutellariodes*). The results showed that the leaves of kantong semar, senduduk, and belimbing bukit could inhibit bacterial growth in a strong category. In contrast, the leaves of sirsak, mucuna, Israel grass, miana, and lime were classified as moderately inhibiting the growth of *A. hydrophila* bacteria. In conclusion, these herbal plants can potentially prevent *A. hydrophila* bacterial infection.

Keywords: Herbal plant, *Motile Aeromonas Septicemia*, Kriby-Bauer, Grass, Leaves

1. INTRODUCTION

The transition from extensive to intensive farming systems causes environmental changes. It increases stress levels in fish, leading to decreased growth and appetite and weakened immunity, making them more susceptible to infection[1]. *Aeromonas hydrophila* is a gram-negative bacterium found in freshwater, brackish water, and seawater[2]. It poses a significant threat to freshwater fish species, such as striped catfish, tilapia, catfish, and carp[3]. This bacterium causes the disease Motile Aeromonas Septicemia (MAS). Clinical symptoms include wounds, eye opacity, immobility, skin darkening, gill tissue damage[4], and hemorrhagic and gastric swelling[5].

According to Pal[6], *A. hydrophila* infections in humans can cause gastroenteritis, wound infections, and septicemia, with symptoms such as abdominal pain, nausea, vomiting, diarrhoea, and local wound infections such as cellulitis and necrotizing fascitis, especially in immunocompromised individuals. Using synthesized antibiotics as a preventive measure against these bacterial

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infections has led to the emergence of antibacterial-resistant bacteria bio-accumulation in organisms and the aquatic environment[7,8]. This requires developing and preventing alternative disease strategies to reduce the negative effects, such as using herbal plants as antibacterials.

Herbal plants are plant species that contain active components with therapeutic properties used to treat various diseases[9,10] The utilization of herbal plants has several advantages, such as being easily available, cost-effective, and environmentally friendly. In addition, herbal plants contain different bioactive compounds such as alkaloids, flavonoids, tannins, saponins, steroids, phenols, terpenoids, carbohydrates, glycosides, coumarins, and scopoletin, which exhibit antimicrobial, immuno-stimulatory, antiparasitic properties[11], enhance fish growth and immune response, and act as antibiotics and antioxidants[12].

Various studies have shown the effectiveness of different herbal plants against *A. hydrophila* bacteria. such as, essential oil from *Cymbopogon flexuosus*[13], *Psidium guajava*, *Azadirachta indica*, and *Terminalia cattapa*[14]. Therefore, the exploration and utilization of herbs as antibacterials in fish is necessary, as well as a promising and sustainable approach for fish health management and ensuring the safety of aquaculture products.

This study aims to explore plants that have the potential to inhibit the growth of *A. hydrophila* bacteria to improve fish health and productivity.

2. RESEARCH METHOD

Time and Place

This research was conducted in February 2024 at the Marine Biotechnology Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Riau.

Method

The experimental research method includes several stages: preparing plant leaf samples and antibacterial tests using Kirby-Bauer discs. To reduce the level of error, replication was carried out three times. The concentrations used were pure extracts from plant leaves and oxytetracycline as a comparison (control).

Procedures

Preparation of Leaf Solution

The process involved in making leaf juice is as follows: The first step is taking leaf samples from several types of plants, such as senduduk leaves (*Melastoma malabathricum*), kantong semar (*Nepenthes* sp), lime (*Citrus aurantifolia),* belimbing bukit (*Acetosella barreligeri*), sirsak (*Annona muricata),* mucuna (*Mucuna bracteata),* Israel grass (*Asystasia gangetica),* and miana (*Coleus scutellariodes),* which were obtained around the Faculty of Fisheries and Marine Sciences, Universitas Riau and then weighed as much as 20 g, cleaned using running water and dried. Next, the leaves were pounded and filtered to produce extracts for antibacterial tests.

Rejuvenation of *A. hydrophila* Bacterial Isolate

The *A. hydrophila* bacteria used in this study came from the collection of the Marine Biotechnology Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Riau. This isolate was then cultured on agar media (Nutrient Agar) and incubated in an incubator for 18-24 hours at a temperature of 31^{o}C. Preparation of bacterial suspensions by taking the culture using an ose, inserting it into liquid media (Nutrient Broth), and incubating it in an incubator for 24 hours at 31^{o}C. The bacterial suspension was first equalized with Mc Farland 0.5-1 standard solution, whose bacterial density equals 10^8 CFU/mL[15].

Inhibition Zone Observation

The inhibition zone of extracts of several plant species against *A. hydrophila* bacteria was observed based on the Kirby-Bauer disc method with a blank disk with a diameter of 6 mm. The initial stage of the agar medium was dripped with 50 µL of *A.*

hydrophila bacterial inoculant with a bacterial density of 10^8 CFU/mL. After that, a blank disk that had previously been given a solution of extracts from several types of plants as much as 50 µL and Oxytetracycline as a control was placed on agar media (NA), which already contained inoculants of *A. hydrophila* bacteria and incubated in an incubator for 24 hours at a temperature of 31°C. After 24 hours, the inhibition zone was measured by measuring the diameter of the clear zone formed using a caliper[15,16].

3. RESULT AND DISCUSSION

The results of the inhibition test on several types of plants produced different diameters of inhibition against the growth of *A. hydrophila* bacteria, which ranged from 8.60 to 14.9 mm. More clearly can be seen in Table 1.

Table 1. Inhibition zone of some plants against *A. hydrophila* bacteria

Plant name	Diameter of inhibition (mm)			Mean ± Std. deviation
	I	II	III	
Kantong semar (*Nepenthes* sp)	14.9	14.6	14.7	14.73±0.15
Senduduk (*Melastoma malabathricum*)	13.65	13.78	13.98	13.80±0.17
Belimbing bukit (*Acetosella barreliaeri*)	13.8	13.75	13.78	13.78±0.03
Sirsak (*Annona muricata*)	8.8	8.95	8.98	8.91±0.10
Mucuna (*Mucuna bracteata*)	8.98	8.94	8.91	8.94±0.04
Israeli grass (*Asystasia gangetica*)	8.66	8.65	8.78	8.70±0.07
Miana (*Coleus scutellariodes*)	8.6	8.70	8.65	8.65±0.05
Lime (*Citrus aurantifolia*)	8.4	8.35	8.5	8.42±0.08
Oxytetracycline (control)	27.5	28.00	27.75	27.75±0.25

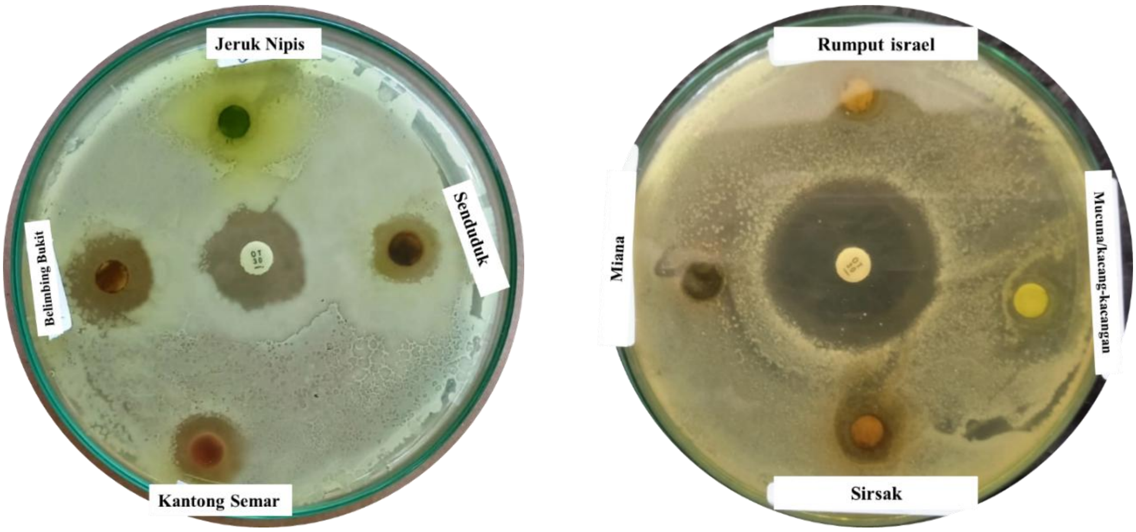

Figure 1. Measurement of the inhibition zone of some plants

The leaves of kantong semar, senduduk, and belimbing bukit can inhibit the growth of *A. hydrophila* bacteria which is classified as strong. Meanwhile, the ability of lime, sirsak, Mucuna, Israeli grass, and miana to inhibit bacteria is categorized as moderate (Figure 1). According to Davis & Stout[17], the criteria for the strength of an antibacterial material, namely the diameter of the inhibition zone of 5 mm or less, is categorized as weak. The inhibition zone of 5-10 mm is classified as moderate, while the diameter of the inhibition zone of 10-20 mm is classified as strong, and even more than 20 mm is categorized as very strong.

Several factors, including the concentration of natural ingredients and the content of secondary metabolite compounds from each plant, can influence the inhibition of an antibacterial substance. The content of phytochemicals such as flavonoids, saponins, tannins, alkaloids, and poly-phenols are antibacterial, antifungal, and antioxidant. Herbal plants generally contain phytochemical compounds, such as flavonoids, terpenoids, tannins, alkaloids, and steroids, that contribute to their diverse pharmacological activities, such as antibacterial, antifungal, antimalarial, antioxidant, antidiabetic, antiosteoporotic, anti-inflammatory, cytotoxic, and hypolipidemic effects[18]. In addition, essential oils, ferulic acid, chlorogenic acid, caffeic acid, coumaric acid, quercetin, and kaempferol exhibit potent antioxidant and antifungal properties[19].

According to Tiwari et al.[20], the presence of polyphenols, tannins, saponins, flavonoids, triterpenes, flavan-3-ol, anthocyanins, and steroids, which exhibit antibacterial, antiviral, antioxidant, anticoagulant, antiinflammatory, anti-hypertensive, and immunemodulating actions. The diverse phytochemicals of each plant, especially its phenolic and flavonoid compounds, contribute to its broad-spectrum antibacterial activity, including against *A. hydrophila*, making it a valuable plant in traditional and modern medicine.

Phytochemicals can directly affect the structure and function of bacterial cells, such as inhibiting peptidoglycan synthesis, disrupting microbial membrane integrity, and changing membrane hydrophobicity, which jeopardizes bacterial cell viability, interfering with bacterial metabolic processes, such as inhibiting glycolytic enzymes and preventing pH drop, which are critical for bacterial survival and proliferation[21]. Furthermore, phytochemical compounds can increase membrane permeability and decrease membrane integrity, leading to cell lysis and death. Generally, secondary metabolite compounds from plants are antibacterial and become a sustainable and effective strategy for preventing infection and bacterial resistance.

The control used in this study is oxytetracycline, which has a reasonably broad spectrum inhibiting the growth of *A. hydrophila* bacteria by 27.75 mm. Oxytetracycline antibiotics as control have an average inhibition zone of 27.83 mm, which shows that antibiotics are very influential in the growth of *A. hydrophila* bacteria[22]. The oxytetracycline system of action disrupts bacterial protein synthesis and is a broad-spectrum antibiotic that can inhibit Gram (+) and Gram (-) bacteria[16].

The zone of inhibition of oxytetracycline is greater than that of herbal plants, which is thought to be because the content and concentration of secondary metabolite compounds influence the antibacterial properties of herbal plants. Minden et al.[23] stated that plant performance can be affected by environmental factors such as soil conditions and the presence of antibiotics, which can alter their phytochemical composition and metabolic processes, thus impacting their antibacterial efficacy.

Meanwhile, the dosage and function of synthetic antibiotics have been developed to inhibit bacterial growth. According to Mathur[24]; Mitcheltree et al.[25], synthetic antibiotics have been developed to overcome the limitations of natural antibiotics, including those derived from herbal plants, by increasing their potency, stability, and spectrum of activity. The content of synthetic antibiotics often includes modifications to the structure of natural antibiotics or entirely new chemical scaffolds designed to overcome resistance mechanisms and improve efficacy. In general, the use of synthetic antibiotics in inhibiting bacterial growth cannot be entirely replaced, and herbal plants' use needs to be developed to be fully utilized as a substitute for synthetic antibiotics.

4. CONCLUSION

The results showed that the leaves of kantong semar, senduduk, and belimbing bukit can inhibit bacterial growth with a strong category so that it can be utilized to prevent *A. hydrophila* bacterial infection.

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