

The activity of *Pometia pinnata* leaf extract against pathogenic bacteria in fish

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Abstract. The use of antibiotics to cure bacterial diseases has a negative impact on fish and the environment, so it is necessary to look for natural products that are safe for treating fish diseases. This study aims to determine the activity of *P. pinnata* extract as an antibacterial towards *V. alginolyticus*, *P. aeruginosa*, *A. salmonicida*, *E. ictaluri*, *A. hydrophila*, and *E. tarda* bacteria. *P. pinnata* extract was obtained by maceration using ethanol solvent. This extract was tested for its phytochemical content, characterized by FT-IR and tested for its antibacterial activity by the agar diffusion method. The results of the phytochemical test of *P. pinnata* extract produced terpenoids compounds flavonoids, saponins and phenolic. The results of the FT-IR characterization of *P. pinnata* extract contained O-H, C-H, C=O and C-O groups. The results of the inhibition test showed that *P. pinnata* extract was able to inhibit the growth of *V. alginolyticus* bacteria by 13.0 mm to 14.7 mm. *P. aeruginosa* 14.5 mm to 17.1 mm. *A. salmonicida* by 12.4 mm to 14.9 mm. *E. ictaluri* by 13.5 mm to 15.5 mm. *A. hydrophila* 13.9 mm to 17.1 mm. *E. tarda* 13.6 mm to 15.5 mm. In conclusion, *P. pinnata* extract can be used to inhibit the growth of pathogenic bacteria in fish.

1 Introduction

Bacteria *Vibrio alginolyticus*, *Pseudomonas aeruginosa*, *Aeromonas salmonicida*, *Edwardsiella ictaluri* *Aeromonas hydrophila*, and *Edwardsiella tarda* are infect many farmed fish. This pathogenic bacterial infection can cause fish death. *V. alginolyticus* is an opportunistic bacterium, including pathogenic bacteria that can threaten the development of aquaculture globally [1]. In fish, this bacteria can lead to several diseases such as ulcers, septicemia and exophthalmia [2]. *P. aeruginosa* bacteria cause many diseases in aquaculture fish [3]. *P. aeruginosa* causes gill necrosis, hemorrhagic septicemia, abdominal distension, congested kidney dan friable liver [4].

A. salmonicida bacteria cause many diseases in salmon [5]. *A. salmonicida* bacteria can also cause disease in various types of fish [6]. It can cause bleeding, ulcer, hemorrhage,

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thereby causing death in the fish [7]. *E. ictaluri* bacteria, is a pathogenic bacteria that causes many diseases in catfish [8].

E. tarda is a pathogenic bacteria that attacks many freshwater and marine fish. The consequences of this bacterial attack cause significant losses in aquaculture activity [9, 10]. *E. tarda* bacteria is a pathogen that causes many diseases in fish. Large deaths in *P. olivaceus* fish cultivation are caused by the bacteria *E. tarda* [11]. *A. hydrophila* is a pathogenic bacterium and is a Gram-negative bacterium. This bacteria can cause MAS (motile aeromonas septicemia) disease [12, 13]. The result of *A. hydrophila* infection is bleeding in the fish's body, especially in the chest, fins and stomach [14].

Antibiotics are often used to fight fish diseases that caused by bacterial. However, frequent use of antibiotics can inflict bacterial resistance [15] and can harm fish, especially consumption fish [16]. Natural products made from antibacterial plants such as the *Pometia pinnata* plant are sought after as an alternative to antibiotics.

P. pinnata is a plant that has a tap root and many branches, a shady plant with a tree height reaching 20-40 m. The leaves are green and have a pointed tip. This plant is often found in Indonesia and usually called matoa [17]. The *P. pinnata* plant has efficacy in the pharmaceutical and cosmetic fields [18]. It can be used as a medicinal ingredient, because it contains chemical compounds. *P. pinnata* leaf extract contains glycosides, alkaloids, steroids, flavonoids, saponins, tannins [19, 20] and terpenoid group compounds [21]. Other studies have proven the activity of α -glucosidase inhibitors from the ethanol extract of matoa stem bark as an antihyperglycemic agent [22, 23]. Methanol, ethylacetate and n-hexane extracts of *P. pinnata* seeds are antidiabetic [24].

The benefits of the *P. pinnata* plant include diarrhea, dysentery, antiviral, diabetes, antioxidant [25]. *P. pinnata* leaf extract contains saponin compounds which have antimicrobial activity against *Staphylococcus epidermidis* [26]. Ethanol extract of *P. pinnata* leaves has antifungal activity against *Trichophyton mentagrophytes* [27]. *P. pinnata* leaf extract contains flavonoid compounds which have strong antioxidant activity [28, 22]. Ethyl acetate extract of the endophytic fungus *P. pinnata* leaves has antimicrobial against *P. aeruginosa*, *Candida albicans* and *S. aureus* [29]. The purpose of this research is to find out the activity of *P. pinnata* extract as an antibacterial towards *E. tarda*, *V. alginolyticus*, *P. aeruginosa*, *A. salmonicida*, *E. ictaluri*, *A. hydrophila* bacteria.

2 Material and Method

2.1 Extraction of *Pometia pinnata* leaf

P. pinnata leaves were obtained from the experimental garden of the Faculty of Agriculture at the Universitas Islam Riau Pekanbaru, Indonesia. The research was conducted in May-June 2023 and 3 Kg of leaf samples were collected. These leaves were dried in an oven at 40°C for 48 hours. Then, they were finely ground in a blender. The obtained *P. pinnata* powder was macerated in 90% ethanol solvent for 36 hours. Subsequently, to obtain the extract, the macerated phytrate is evaporated at 50°C using a rotary evaporator [30].

2.2 Phytochemical test and Fourier-transform infrared spectroscopy (FT-IR)

Chemical analysis was performed on the *P. pinnata* extract to detect the presence of different compounds, which include alkaloids, flavonoids, phenolics, saponins, and terpenoids. In the alkaloid test, 250 μ L of Mayer reagent was added to 10 mg of the *P. pinnata* extract, a white precipitate was formed. When Dragendorff reagent was added, it turned orange, indicating a positive result. For the flavonoid test, 0.05 g of magnesium and

10 drops of 37% HCl were mixed with 10 mg of *P. pinnata* extract and stirred for 1 minute. A positive result was indicated by a colour change to red.

The phenolic test, 10 mg of *P. pinnata* extract was mixed with 500 µL of 5% FeCl₃, and a positive result was indicated by colour change to blue. To test for saponins, 10 mg of *P. pinnata* extract was shaken with 5 mL of distilled water for 1 minute, followed by the addition of 150 µL of 1N HCl and another 1-minute of shaking, resulting in a positive result due to the formation of the results have been shown foam. For the terpenoid test, mixing 10 mg of *P. pinnata* extract with 10 drops of CH₃COOH and 3 drops of H₂SO₄, gave a positive result indicated by the formation of a red colour [30].

To further determine the functional groups present in the *P. pinnata* extracts, FT-IR spectroscopy analysis was performed. 1 mg of each extract was ground, added to KBr, and mixed vigorously until homogeneous. The infrared absorption of the resulting mixture was then measured in the wavelength range of 4500–450 cm, allowing identification of the functional groups.

2.3 Inhibitory activity of *P. pinnata* extract

P. pinnata extract was tested on the bacteria *V. alginolyticus*, *E. ictalurid*, *P. aeruginosa*, *A. salmonicida*, *E. tarda*, *A. hydrophila* using the agar diffusion method. 1 mL of pathogenic bacterial inoculum (0.08-0.1 at 600nm) was mixed evenly in 15 mL of NA medium and poured into a petri dish. After the NA media had solidified, Oxytetracycline paper was used as a positive control, as a negative control a paper disk was used that was treated with 30 mL of methanol. The concentrations of *P. pinnata* extract tested were 100 mg mL⁻¹, 200 mg mL⁻¹, 300 mg mL⁻¹, 400 mg mL⁻¹, 500 mg mL⁻¹. From this concentration, 30 µL was taken and dropped on a paper disc, then incubated for 1 days at 30°C. Observation of the inhibitory power of the extract was the area around the paper disc that was not growing with pathogenic bacteria [30].

2.4 Data Analysis

The collected data was tabulated and descriptive analysis was carried out by evaluating the amount of inhibitory power formed as an antibacterial indicator.

3 Results and Discussion

3.1 Phytochemical test and functional groups

The purpose of this test is to find out the compounds that have an active role in antimicrobial activity. Compounds in the *P. pinnata* extract were identified by monitoring the colour changes that occurred after being given the reagent. This phytochemical test on the *P. pinnata* extract was carried out to confirm the chemical compounds in the *P. pinnata* extract, then identify the main chemical compound groups that have an active role for antibacterial (Table 1).

Table 1. Result Phytochemical Test

No.	Compound	Results	Test
1	Terpenoids	Formed colour-red	+
2	Flavonoids	Formed colour-pink	+
3	Saponins	Foamy	+

4	Phenolic	Formed colour blue-black	+
5	Alkaloids	No orange precipitate is formed	-

There was no noticeable color change or precipitate formation when *P. pinnata* extracts were exposed to Mayer's and Dragendroff's reagents. Conversely, samples containing alkaloids were altered and turned white or orange due to interaction with these conventional reagents, as documented in reference [31].

When *P. pinnata* extracts were exposed to Lieber-Burchard reagent, the colour changed to red. This colour change to red was caused by acid oxidation and was promoted by the presence of H₂SO₄. During this process, in these compounds electrons are separated from hydrogen, which expanded the conjugation of the compound and was visually reflected in the red colour [32]. The presence of terpenoids can be seen from the red color that forms in samples from the oxygenation and dehydrogenation processes.

The compounds contained in *P. pinnata* extract are categorized by changing the color of the extract, to peach color. Furthermore, the red color formed is caused by benzopyrylium salt. The reduction of polyhydroxy groups from flavanols produces a red color due to the existence of the Mg element in HCl [33].

Foam formation occurred when *P. pinnata* extract was mixed with HCl. The positive extract contained saponin, when the extract formed foam around 5 minutes after adding the reagent [34]. Height of the foam produced in the extract after adding 1 drop of 1N HCl varied between 1 to 5 cm.

P. pinnata extract was positive for containing phenolics, this was known by observing the color change in the extract to dark blue when reacted with ferric chloride. The colour formed occurs due to a reduction process involving phosphomolybdic acid and phosphotungstate with the reagent, resulting in the formation of a blue colour. The blue color formed indicates the presence of phenolic content. This happens because phenolic ions reduce heteropoly acid, which forms molybdenum tungsten, so the color becomes dark [35]. The dark blue color is caused by the formation of sugar bonds in the extract.

FT-IR spectrum results are assumed that at a length of 3420 cm⁻¹ there is an O-H group, where flavonoid, phenolic, terpenoid and saponin compounds have C bonds with O-H. The 2891 cm⁻¹ spectrum shows C-H. The spectrum of 1753 cm⁻¹ shows the C=O group possessed by flavonoids. The spectrum of 1075 cm⁻¹ shows the C-O group found in structure of flavonoids, phenolics and saponins [36].

3.2 Inhibitory Activity

Inhibition test results showed that *P. pinnata* was able to inhibit the growth of *A. V. alginolyticus* bacteria by 13.0 mm to 14.7 mm. On *P. aeruginosa* by 14.5 mm to 17.1 mm. On *A. salmonicida* by 12.4 mm to 14.9 mm. On *E. ictaluri* by 13.5 mm to 15.5 mm. On *E. tarda* by 13.6 mm to 15.5 mm. On *A. hydrophila* 13.9 mm to 17.1 mm (Table 2). The inhibitory ability of *P. pinnata* extract on pathogenic bacteria is classified as strong [37].

Table 2. Inhibition activity of *P. pinnata* extract against pathogen bacteria

<i>P. pinnata</i> Extract concentration (mg.mL ⁻¹)	Inhibition zone diameter (mm)					
	<i>Vibrio alginolyticus</i>	<i>Pseudomonas aeruginosa</i>	<i>Aeromonas salmonicida</i>	<i>Edwardsiella ictaluri</i>	<i>Edwardsiella tarda</i>	<i>Aeromonas hydrophila</i>
100	13.0 ± 0.14	14.5 ± 0.07	12.4 ± 0.28	13.5 ± 0.57	13.6 ± 0.07	13.9 ± 0.49
200	14.0 ± 0.14	15.3 ± 0.35	13.1 ± 0.28	14.3 ± 0.42	14.5 ± 0.07	15.2 ± 0.07

300	14.5 ± 0.28	15.8 ± 0.07	13.7 ± 0.42	14.4 ± 0.28	15.0 ± 0.14	15.7 ± 0.21
400	14.6 ± 0.28	16.8 ± 0.42	14.6 ± 0.07	14.8 ± 0.07	15.2 ± 0.07	16.3 ± 0.78
500	14.7 ± 0.42	17.1 ± 0.85	14.9 ± 0.64	15.5 ± 0.42	15.5 ± 0.14	17.1 ± 0.92

The most notable result from testing the inhibitory effect of *P. pinnata* extract inhibitory activity was significant activity against *P. aeruginosa*. The amount of inhibitory power for each bacteria varies, this is because the reaction of pathogenic bacteria to *P. pinnata* extract is not the same. The concentration and type of antimicrobial substance are very influential in inhibiting microbial growth. The size of the inhibition zone formed is directly proportional to the increase in the concentration of the extract given [38]. The presence of a clear zone in the culture media for pathogenic bacteria indicates that the *P. pinnata* extract contains antibacterial compounds like terpenoids, saponins, phenolics, and flavonoids.

Terpenoids are antibacterial, because terpenoids have lipophilic properties which can damage cell membranes [39]. Terpenoids have the ability to interact with porins, namely transmembrane proteins found in the membrane layer of bacterial cells. Damage to porins is caused by strong polymer bonds from these interactions. Consequently, the bacterial cell wall permeability system is disrupted, and the supply of nutrients to the cell is reduced, so that bacterial growth stops.

The terpenoids present in this fraction possess antibacterial properties due to their ability to inhibit lipid production and alter the structure of cell membranes by impeding the synthesis of ergosterol [40, 41]. The antimicrobial efficacy of terpenoid compounds stems from their lipophilic characteristics. Terpenoids can disintegrate and disrupt the lipid component within the bacterial plasma membrane, leading to an imbalance in cell membrane permeability. This disruption results in the disturbance of ion homeostasis within bacterial cells [42].

Flavonoids have demonstrated the ability to form complex compounds with characteristics that can damage microbial cells. The interaction of flavonoids with proteins in bacterial cells can damage the components inside the cells. Additionally, this compound can direct energy transfer into the bacterial cytoplasm, thus hindering bacterial mobility [43]. Flavonoid compounds inhibit bacterial growth by binding to the cell surface, causing damage to cell walls and enzyme activity. The hydroxyl group and beta ring elements in flavonoids are involved in antibacterial mechanisms. Flavonoids can influence porin activity, biofilm formation, permeability, thereby disrupting the cytoplasmic membrane and inhibiting the formation of nucleic acids. [44].

Flavonoid compounds, when acting as antibacterial agents, employ several mechanisms to disrupt bacterial functions, including damaging cell walls and interfering with metabolic processes. These mechanisms encompass three primary categories: inhibition of nucleic acid synthesis, disruption of cytoplasmic membrane function, and interference with energy metabolism [45]. Furthermore, flavonoid compounds can also destroy the permeability of bacterial cell.

Saponins exhibit antimicrobial properties. As an antibacterial, saponin damages the cell membrane permeability system, so that cells lose proteins and enzymes [31, 46]. If saponin interacts with the sterol membrane, it can destroy the cell wall structure, resulting in the detached of important materials of the cell. The inhibitory mechanism for saponins in bacteria is to damage the permeability of bacterial cell membranes. Apart from that, saponins also have hydrophilic and lipophilic properties and can bind to the lipid components of bacterial cells, causing lysis and rupture of bacterial cells [46]. Consequently, when this interaction takes place, the cell wall becomes susceptible to breaking or undergoing lysis, allowing antibacterial substances to enter the cell easily and disrupt its metabolism, ultimately leading to bacterial death [47].

The hydroxyl groups contained in phenolics show strong antibacterial characteristics. Hydroxyl groups damage protein structures and prevent nutrients from entering bacterial cells. Additionally, hydroxyl groups dissolve fats in cell walls, thereby disrupting bacterial cell membranes [48].

The hydroxyl groups in phenolic compounds can impair the permeability from the cytoplasmic membrane system, which disrupts the transport of essential organic ions into cells, ultimately leading to the death of bacterial cells. Phenolic compounds also possess biological functions such as anti-carcinogenic, anti-inflammatory, and antioxidant properties. Numerous phenolic compounds have been demonstrated to be effective in inhibiting a variety of pathogenic bacteria [49]. Phenolics cause the flagella to break in bacteria, causing damage to the membrane and cell walls, resulting in macromolecules leaving the cell [50].

4 Conclusion

P. pinnata leaf extract contains terpenoid, flavonoid, saponin and phenolic compounds. *P. pinnata* leaf extract is effective in inhibiting the accretion of pathogenic bacterial in fish like *V. alginolyticus*, *E. ictaluri*, *P. aeruginosa*, *A. salmonicida*, *A. hydrophila*, *E. tarda*.

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