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SCORING OF MILKFISH (*Chanos chanos*) GILL DAMAGE ADDED WITH FRACTIONATED NONI LEAVES (*Morinda citrifolia*)

Penilaian Kerusakan Insang Ikan Bandeng (*Chanos chanos*) yang Ditambahkan Daun Mengkudu (*Morinda citrifolia*) yang Difraksinasi

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ABSTRACT

Red spot disease known as Motile Aeromonas Septicemia is a serious challenge often faced by freshwater fish farmers. This disease is caused by infection with the bacteria *Aeromonas hydrophila* and can be fatal if not treated properly. Therefore, it is important to find alternative solutions that are more environmentally friendly, one of which is through the use of natural ingredients or herbal medicines. One of the natural ingredients that has the potential to be used to treat this infection is noni leaves (*Morinda citrifolia*). This study aims to explore the content of active compounds in noni leaf extract and test its effectiveness in overcoming tissue damage in milkfish infected with *A. hydrophila*. In this study, the Thin Layer Chromatography method was used to identify active compounds, and scoring was carried out on the level of damage to the fish gills. The results of the phytochemical test showed that noni leaf extract contains compounds from the Terpenoid and Flavonoid groups, which are suspected of having antibacterial activity. Data analysis was carried out quantitatively using the Analysis of Variance (ANOVA) method to ensure the accuracy of the results. From histopathological analysis, several tissue damages such as vacuole degeneration, congestion, and necrosis were found. Observations showed that the optimal dose of mengkudu leaf extract for the treatment of infected milkfish was 360 ppm. From these results, it can be concluded that mengkudu leaf extract has the potential as a natural therapeutic agent to overcome *A. hydrophila* bacterial infections in milkfish (*Chanos chanos*).

Keywords: *Morinda citrifolia, Aeromonas hydrophila, Chanos chanos, TLC test, Gill histopathology*

ABSTRAK

Penyakit bercak merah yang dikenal sebagai Motile Aeromonas Septicemia merupakan tantangan serius yang sering dihadapi oleh pembudidaya ikan air tawar. Penyakit ini

disebabkan oleh infeksi bakteri *Aeromonas hydrophila* dan dapat berakibat fatal jika tidak ditangani dengan tepat. Oleh karena itu, penting untuk mencari solusi alternatif yang lebih ramah lingkungan, salah satunya melalui penggunaan bahan alami atau obat herbal. Salah satu bahan alami yang berpotensi digunakan untuk mengobati infeksi ini adalah daun mengkudu (*Morinda citrifolia*). Penelitian ini bertujuan untuk mengeksplorasi kandungan senyawa aktif dalam ekstrak daun mengkudu dan menguji efektivitasnya dalam mengatasi kerusakan jaringan pada ikan bandeng yang terinfeksi *A. hydrophila*. Dalam penelitian ini, metode Kromatografi Lapis Tipis digunakan untuk mengidentifikasi senyawa aktif, dan dilakukan skoring terhadap tingkat kerusakan pada insang ikan. Hasil uji fitokimia menunjukkan bahwa ekstrak daun mengkudu mengandung senyawa dari golongan Terpenoid dan Flavonoid, yang diduga memiliki aktivitas antibakteri. Analisis data dilakukan secara kuantitatif menggunakan metode *Analysis of Variance* (ANOVA) untuk memastikan keakuratan hasil. Dari analisis histopatologi, ditemukan beberapa kerusakan jaringan seperti degenerasi vakuola, kongesti, dan nekrosis. Hasil pengamatan menunjukkan bahwa dosis optimal ekstrak daun mengkudu untuk pengobatan ikan bandeng yang terinfeksi adalah 360 ppm. Dari hasil ini, dapat disimpulkan bahwa ekstrak daun mengkudu berpotensi sebagai agen terapeutik alami untuk mengatasi infeksi bakteri *A. hydrophila* pada ikan bandeng (*Chanos chanos*).

Kata kunci: *Morinda citrifolia, Aeromonas hydrophila, Chanos chanos*, uji KLT, Histopatologi insang

INTRODUCTION

Milkfish (*Chanos chanos*) is a type of freshwater fish that has high economic value and is widely cultivated in Lamongan. Fisheries and Marine Statistics data states that in East Java, milkfish is included in the leading commodities with a production volume reaching 160,989.57 tons in 2023 (East Java Provincial Marine and Fisheries Service, 2024). However, in the cultivation process, this fish is susceptible to disease attacks that can cause a significant decrease in production to fish death, resulting in economic losses. *Aeromonas hydrophila* is a pathogenic bacteria that is often found attacking milkfish (Fikri *et al*., 2022). This bacteria is known to be the main cause of Motile Aeromonas Septicemia (MAS) disease (Rozi et al., 2018). Infection by this bacteria often causes serious disorders in fish health which can result in fish death, causing losses in fish farming. According to Stratev and Odeyemi (2016), MAS disease will cause symptoms such as bleeding, ulceration and abscesses on the surface of the fish's body, fluid in the stomach, and anemia. However, in controlling this disease, farmers still use antibiotics or other chemicals which if used in the long term can cause bacterial resistance to the type of antibiotic. In addition, antibiotics in their use cause other side effects, namely leaving residues on exposed fish, this can endanger human health as consumers. Antibiotic residues can also pollute the aquatic environment (Satyantini *et al*., 2022). Therefore, alternative control efforts are needed, which are more environmentally friendly and safe, one of which is by utilizing natural ingredients. The noni plant (*Morinda citrifolia*) is known to have various health benefits, including antibacterial, antiviral, antifungal, anti-inflammatory, antitumor, analgesic (pain reliever), anthelmintic, and immune system enhancer properties. Parts of this plant ranging from fruit, seeds, leaves, flowers, and bark have been proven to have potential as a source of nutrition as well as a natural ingredient for the treatment and prevention of various diseases (Assi *et al*., 2017). The purpose of this study was to explore the content of active compounds in crude extracts of mengkudu leaves (*M. citrifolia*) and to determine the effect of its use on histopathological changes in gill tissue in milkfish (*C. chanos*) infected with *A. Hydrophila* bacteria.

RESEARCH METHODS

Place and Time of Research

The research was conducted at the Organic Chemistry Laboratory of the Faculty of Science and Technology of the Universitas Islam Negeri Maulana Malik Ibrahim Malang from October to December 2024. The Fish Disease and Health Laboratory and the Aquatic Environment and Biotechnology Laboratory at the Faculty of Fisheries and Marine Sciences Universitas Brawijaya from December 2024 to February 2025. The Aquatic Resources Management Laboratory of the Universitas Islam Lamongan from February to April 2025.

Tools and Materials

The tools used include aquarium, thermometer, DO meter, aerator, aquarium heater, aquarium filter, blender, refrigerator, sectio set, falcon tube, rotary evaporator, vacuum, hot plate, magnetic stirrer, micropipette, sterile micropipette tip, micro tube, oven, ruler, pencil, analytical balance, digital scale, borosilicate glass chamber, 250 mL drinking glass, measuring cup (10 mL, 50 mL and 250 mL), vacuum desiccator, 1 cm diameter column chromatography tube, 50 cm high, funnel, spatula, test tube, test tube rack, petri dish, ose needle, bunsen, dropper pipette, spectrophotometer, erlenmeyer flask (250 mL, 500 mL and 1000 mL), beaker glass (500 mL and 1000 mL), autoclave, laminary airflow, eppendorf tube, object glass, cover glass, light microscope, trinocular microscope with camera, hand tally counter.

The materials used include Milkfish (*C. chanos*), Fish Feed, Noni Leaves (*M. citrifolia*), maceration solvents (methanol and ethyl acetate), pure culture bacteria A. hydrophila obtained from the Center for Brackish Water Aquaculture (BBPBAP) Jepara, culture materials using Tryptic Soy Agar (TSA) media, Nutrient Broth (NB) media, Aquades, Alcohol and Na-fis 0.9%.

Research Design

This study applies a Completely Randomized Design (CRD) as the experimental design used. RAL was chosen because it is suitable for experimental conditions that take place in a uniform or homogeneous environment, such as in a laboratory.

Research Procedure

Research Part 1
(Characteristics and Optimization of Noni Leaf Extract Dosage (*M. citrifolia*)

1. Acclimatization of Test Fish
2. Preparation of Noni Leaf Extract *(M. citrifolia)* (Harborne, 1998)
3. Phytochemical Test of Noni Leaves *(M. citrifolia)* (Harborne, 1998)
4. Thin Layer Chromatography (TLC) Fractionation Method (Stahl, 1969)
5. Pathogenicity of A. hydrophila with 50% Lethal Dosage Test (LD_{50}) (Austin and Austin, 2012)
6. Optimization of Noni Leaf Extract Dosage (M. citrifolia) with 50% Inhibition Concentration (IC_{50}) (Finney, 1971)

Research Part 2
(The Best Mengkudu Leaf Extract (*M. citrifolia*) for the Treatment of Milkfish (*C. chanos*) Infected with *A. hydrophila*)

1. Soaking with Optimum Dose of Noni Leaf Extract (*M. citrifolia*)
2. Histopathology (Bancroft and Gamble, 2008; Ferguson, 2006)

a) Tissue Preparation
b) Tissue Fixation
c) Tissue Processing

d) Tissue Embedding
e) Sectioning
f) Staining
g) Observation

Data Analysis

Quantitative analysis was employed in this study, utilizing Analysis of Variance (ANOVA) to assess the impact of *Moringa citrifolia* leaf extract treatment on the histopathology of *Chanos chanos* (milkfish) challenged with *Aeromonas hydrophila*. All data processing and analysis were conducted using SPSS (Statistical Package for the Social Sciences)

RESULTS

Table 1. Percentage acquisition of Noni Leaf Extract Fractionation (*M. citrifolia*)

Solvent	Percentage (%)
Methanol	8,32
Ethyl Acetate	3,72

Thin Layer Chromatography (TLC) analysis in this study is shown in Figure 1.

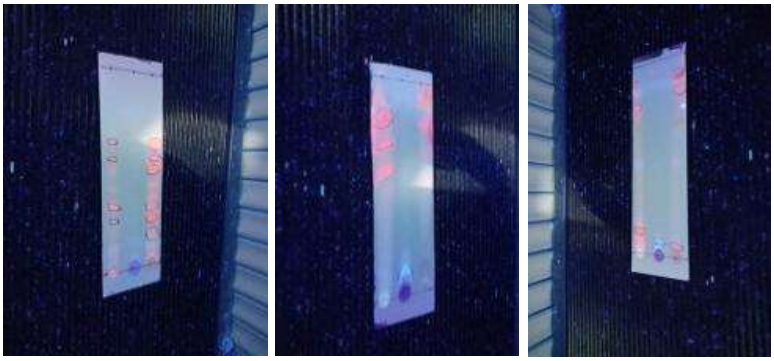

Figure 1. Thin Layer Chromatography (TLC) Analysis Determination of Solvents or Solvent Mixtures

Description :
Left = N-Hexane : Ethyl Acetate (8:12)
Middle = Chloroform : Methanol (16:4)
Right = N-Hexane : Ethyl Acetate (14:6)

The results of the Thin Layer Chromatography (TLC) Analysis test obtained the results of the Retardation factor (Rf) as shown in Table 2.

Table 2. Retardation Factor (Rf) Results

Solvent or Solvent Mixture	*Retardation factor* (Rf)
N-Hexane : Ethyl Acetate (8:12 v/v)	0,68
Chloroform : Methanol (16:4 v/v)	0,86
N-Hexane : Ethyl Acetate (14:6 v/v)	0,95

a

Table 3. Inhibition Zone Diameter Data of Bacteria Using Fractionation

Treatment	Inhibition Zone (mm)
Positive Control	24,8
Fractionation 4	21,7
Fractionation 3	45,1
Fractionation 2	42,9
Fractionation 1	37,2

Table 4. Value of Vacuole Degeneration Damage Scoring

Treatment (ppm)	Vacuole Degeneration
Positive Control	1,22±0,02 [a]
90 ppm	2,33±1,17 [cd]
180 ppm	2,10±1,02 [bc]
270 ppm	2,15±1,02 [bc]
360 ppm	1,83±1,54 [b]
450 ppm	2,03±1,17 [b]
Negative Control	2,63±3,00 [d]

Description: The numbers in the same column followed by the same letter are not significantly different (P>0.05) with a 95% confidence interval.

Table 5. Value of Congestion Damage Scoring

Treatment (ppm)	Congestion
Positive Control	1,22±0,02 [a]
90 ppm	2,01±1,02 [b]
180 ppm	1,94±1,54 [b]
270 ppm	1,87±0,59 [b]
360 ppm	1,81±0,02 [b]
450 ppm	1,94±0,59 [b]
Negative Control	2,34±0,59 [c]

Description: The numbers in the same column followed by the same letter are not significantly different (P>0.05) with a 95% confidence interval.

Table 6. Value of Necrotic Damage Scoring

Treatment (ppm)	Necrosis
Positive Control	1,22±0,00 [a]
90 ppm	2,14±1,17 [bc]
180 ppm	2,01±1,02 [b]
270 ppm	1,87±3,00 [b]
360 ppm	1,80±0,02 [b]
450 ppm	1,94±0,59 [b]
Negative Control	2,47±3,00 [c]

Description: The numbers in the same column followed by the same letter are not significantly different (P>0.05) with a 95% confidence interval.

DISCUSSION

Phytochemical screening was conducted to determine the content of plant metabolic by-products needed for growth, which are found in the fractionation of mengkudu leaves (*M. citrifolia*) which can be used in the treatment of Milkfish (*C. chanos*) infected with *A.*

hydrophila including: Alkaloid Screening, Flavonoid Screening, Saponin Screening, Steroid Screening, Tannin Screening, and Triterpenoid Screening.

From the results obtained, it is known that the highest value is in Fractionation 3 with a value of 45.1 mm, while the lowest value is in Fractionation 4 with a value of 21.7 mm. Bastian *et al.,* (2018) stated that an inhibition zone can form around the disc which is marked by a clear/transparent area (a term used in biology to describe a clean area around a well or place containing antimicrobial substances in a culture medium that inhibits the growth of bacteria or other microorganisms) which indicates that there is bacterial sensitivity to antibacterial materials.

Treatment with a dose of 360 ppm showed the lowest vacuole degeneration score in the gills, which was 1.83. Meanwhile, the 180 ppm dose treatment had a score of 2.10, the 270 ppm dose was 2.15, and the 450 ppm dose was 2.03. Fish in healthy conditions generally have low vacuole degeneration scores in the gills, indicating that the gill tissue is still functioning and well structured. Mora *et al.* (2022) stated that vacuole degeneration is the most severe type of histopathological change because it can indicate necrosis or cell death. In the congestion parameter, the 90 ppm dose treatment showed the highest score, which was 2.01. Conversely, the 360 ppm dose showed the lowest score of 1.81. For other treatments, the 180 ppm dose had a score of 1.94, the 270 ppm dose was 1.87, and the 450 ppm dose was 1.94. Healthy fish will show a low gill congestion score. According to Lukmini *et al.* (2024), long-term congestion can trigger necrosis, because obstruction of blood flow causes a lack of oxygen to cells, which ultimately leads to cell damage and death.

The highest score for gill necrosis was in the 90 ppm dose treatment of 2.14, while the lowest score was found in the 360 ppm dose treatment of 1.80. The 180 ppm dose treatment had a score of 2.01, the 270 ppm dose of 1.87, and the 450 ppm dose of 1.94. Healthy fish usually have a low gill necrosis score, indicating no serious damage to the tissue.

Based on the results of observations of gill histopathology including Vacuole Degeneration, Congestion, and Necrosis, treatment of milkfish (*C. chanos*) infected with A. hydrophila bacteria with the most optimal dose is at a dose of 360 ppm.

CONCLUSION

Based on the results and discussions in the research that has been done, the following conclusions can be drawn: Based on the results of identification using TLC (Thin Layer Chromatography) analysis on the fractionation of mengkudu leaves (*M. citrifolia*) using methanol solvent, the dominant content of Terpenoid compounds and other compounds from the Flavonoid group. Then, during histopathological observations on milkfish gills, there were several scoring of tissue damage, including: vacuole degeneration, congestion and necrosis. The addition of mengkudu leaf extract (*M. citrifolia*) can affect the increase in cellular health in milkfish (*C. chanos*) that have been infected with *A. hydrophila.*

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