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Identification of the Use of Noni Leaf Extract (*Morinda Citrifolia*) in Blood Observations in Gouramy Fish (*Osphronemus Gouramy*) Infected with *Aeromonas Hydrophila*

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ABSTRACT

Aeromonas hydrophila is a facultative anaerobic bacteria, namely bacteria that can grow in conditions with or without oxygen. One of the diseases that fish farmers fear is red spot disease or *Motile Aeromonas Septicemia* (MAS) which is caused by *A. hydrophila*. Therefore, it is necessary to use alternative materials that can be used to eliminate *A. hydrophila*, one of which is by using herbal medicines. A natural ingredient that can be used to treat fish attacked by bacteria is noni leaves (*Morinda citrifolia*). The aim of this research was to determine the active compound content in noni leaf extract (*M. citrifolia*) and the use of noni leaf extract (*M. citrifolia*) on the hematology of gouramy fish. The results of the Column Chromatography (KK) test showed that the crude extract of noni leaves was positive for alkaloids, saponins, triterpemoids and flavonoids. And in the GC-MS test, it was discovered that the most dominant phenolic compounds contained in noni leaf extract. The results of hemotological data analysis based on the ANOVA test showed that administration of noni leaf extract (*M. citrifolia*) had a significant effect ($p<0.05$) on hematocrit, erythrocytes, leukocytes and hemoglobin. Based on the results of observations of the parameters seen, treatment of gouramy fish (*Osphronemus gouramy*) infected with *A. hydrophila* bacteria at an optimal dose of 360 ppm.

INTRODUCTION

Gouramy (*Osphronemus gouramy*) is one of the freshwater fish commodities that is widely cultivated by the community and is superior because it has important economic value and is widely favored by the community (Nurhudah *et al*., 2023). The problem of fish disease is one of the main obstacles faced by farmers because it has the potential to cause very large losses such as decreased production and mass mortality in fish (Azhar *et al*., 2020).

Aeromonas hydrophila is one type of pathogenic bacteria that is often found infecting gouramy fish. This bacteria can cause *Motile Aeromonas Septicemia* (MAS) disease (Rozi *et al*., 2018). 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, the presence of fluid in the stomach, and anemia.

Unfortunately, in controlling this disease, fish farmers still use antibiotics or other chemicals. Continuous use of antibiotics and chemicals can cause bacterial resistance to these types of antibiotics. The use of antibiotics can also have other side effects, namely leaving residues that can later endanger human health as consumers and the environment (Satyantini *et al*., 2022). Therefore, safer control alternatives are needed, one of which is by using natural ingredients.

Noni (*Morinda citrifolia*) is known to have antibacterial, antiviral, antifungal, antitumor, anthelmintic, analgesic, hypotensive, anti-inflammatory and immune-boosting effects. Parts of the noni plant including fruit, seeds, bark, leaves, and flowers are used to obtain nutritional and therapeutic value (Assi *et al.,* 2017).

This study aims to determine the active compounds in noni leaf extract (*M. citrifolia*) which are antibacterial and identify their use in hematology and histopathology of gouramy (*O. gouramy*) infected with *A. hydrophila.* This study is expected to provide safer and more effective alternative solutions in controlling *Motile Aeromonas Septicemia* disease due to *A. hydrophila* bacterial infection in gouramy, as well as provide new insights into the use of natural or herbal ingredients in aquaculture.

METHOD

Research was conducted at the Organic Chemistry Laboratory, Faculty of Science and Technology (Saintek), Maulana Malik Ibrahim State Islamic University, Malang from January to February 2024. Fish Disease and Health Laboratory and Aquatic Environment and Biotech Laboratory at the Faculty of Fisheries and Marine Sciences (FPIK) Brawijaya University, Malang. March to May 2024.

The research method used is an experimental method. Data analysis used in the core research is a simple Completely Randomized Design (CRD) with a treatment of 5 doses of crude extract of noni leaves (*M. citrifolia*) namely A (90 ppm); B (180 ppm); C (270 ppm); D (360 ppm); and E (450 ppm), as a control treatment, namely K- (gourami fish infected with *A. hydrophila* without treatment) and K+ (gourami fish without infection and without treatment). the study was conducted with three replications.

This research was divided into two stages, namely stage 1 and stage 2. In stage 1 of the research, extraction was carried out to see the content of the best solution extract, column chromatography (KK) and Gas Chromatography–Mass Spectrometry (GC-MS). Meanwhile, in stage 2, observations are made on the main

hematological parameters consisting of observations of erythrocytes, leuococytes, hematocrit and hemoglobin.

RESULT AND DISCUSSION

Phase I Research

Methanol Extraction of Noni Leaves (*M. citrifolia*) Column Chromatography (CC) Analysis

Column Chromatography was carried out to produce pure fractions in abundant quantities, the method used was based on Bigoniya and Singh (2014); and Vasconcelos *et al*. (2010).

Figure 1. Column Chromatography Analysis of Methanol Extract of Noni Leaves (*M. citrifolia*)
Source: Research Documentation (2024)

The solvent or eluent used for Column Chromatography analysis is the eluent used in the Thin Layer Chromatography test, namely the eluent n-hexane: ethyl acetate (7:3; v/v) as the mobile phase and the stationary phase uses 60F254 silica gel powder. The results of Column Chromatography based on the color obtained are 4 (four) types of fractions (Figure 1; Figure2).

Gas Chromatography Mass Spectrophotometry (GC-MS) Analysis

The compounds obtained from the GC-MS analysis results are presented in the Table 1.

Table 1. Compounds in noni leaves GC-MS analysis

Retention time	Area %	Chemical Component
9,374	1,08	*Phenol, butyphenol*
12,575	30,05	*Triacontane, tetracontane*
14,718	11,05	*N-isobutyl*

Source: Processed Data (2024)

The results of GC-MS show that the most dominant compounds are from the phenol, triterpenoid and flavonoid groups. In a review conducted by Assi *et al*., (2017) it was found that the compounds contained in the mengkudu leaves (*M.*

citrifolia) include the triterpenoid group which has an anticancer function, while flavonoids function as antibacterials.

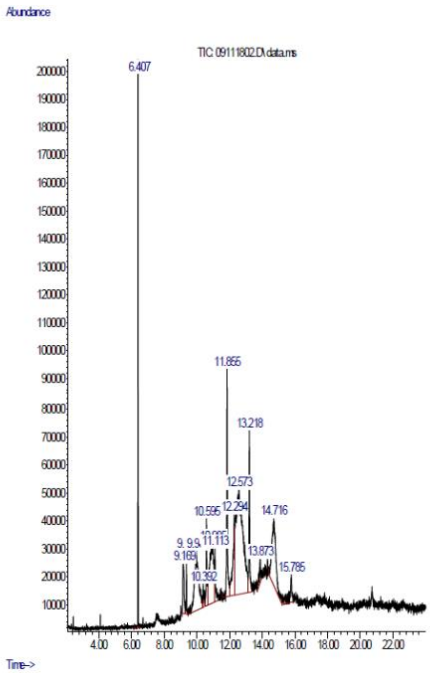

Figure 2. Results of GC-MS analysis of noni leaves (*M. citrifolia*)
Source: Processed Data (2024)

Phase II Research
Main Parameters
Erythrocytes

Fish blood consists mostly of red blood cells, the number of which is estimated at 4 million cells/ml. Fish red blood cells have a cell nucleus whose size varies between species.

Table 2. Erythrocyte Values of Gourami Fish (*O. gouramy*) during the Research

Treatment (ppm)	Healthy Fish	After Infection	After Treatment
K-	1,78±0,05 ^{a}	1,32±0,02 ^{a}	1,44±0,08 ^{a}
A (90 ppm)	1,77±0,03 ^{b}	1,35±0,05 ^{a}	1,75±0,05 ^{a}
B (180 ppm)	1,93±0,07 ^{cd}	1,79±0,09 ^{b}	1,95±0,06 ^{b}
C (270 ppm)	1,99±0,07 ^{d}	1,40±0,08 ^{a}	2,06±0,04 ^{a}
D (360 ppm)	2,51±0,03 ^{e}	2,25±0,05 ^{c}	2,45±0,05 ^{c}
K+	2,44±0,04 ^{e}	2,40±0,02 ^{d}	2,36±0,05 ^{d}

Description: Erythrocyte value (x10^{6} cells/ml)
Numbers in the same column followed by the same letter are not significantly different (P>0.05)
Source: Processed Data (2024)

In treatment D (360 ppm), the number of erythrocytes in gourami fish had the highest number of erythrocytes (Table 2). This is thought to be due to the influence of giving noni leaf extract. Noni leaves can be used as an antibacterial because noni leaves contain active substances, one of which is flavonoids.

Leukocytes

Leukocytes have various functions that are closely related to eliminating foreign objects (including pathogenic microorganisms). Leukocytes have several functions in fighting foreign objects that have entered the body (Johnny *et al*., 2005).

In treatment E (450 ppm) the total leukocytes were the highest compared to treatments A (90 ppm), B (180 ppm), C (270 ppm) and D (360 ppm). So the leukocytes in treatment E (450 ppm) provided defense. which is stronger and faster for the body's immune response system (Table 3). This is in accordance with the statement of Moyle and Cech (2004), leukocytes are blood cells that play a role in the immune system. Leukocytes help cleanse the body of foreign objects, including pathogen invasion through the immune system and other responses. Sick fish will produce many leukocytes to synthesize antibodies and phagocytose bacteria.

Table 3. Leukocyte values of gourami fish (*O. gouramy*) during the study

Treatment (ppm)	Healthy Fish	After Infection	After Treatment
K-	72,66±1,68 ^{b}	83,06±2,53 ^{d}	80,70±1,14 ^{c}
A (90 ppm)	58,50±1,65 ^{a}	61,27±3,30 ^{a}	59,93±2,11 ^{a}
B (180 ppm)	59,16±3,36 ^{a}	63,24±2,24 ^{a}	61,23±2,66 ^{a}
C (270 ppm)	70,36±1,55 ^{b}	71,77±1,57 ^{b}	71,44±1,25 ^{b}
D (360 ppm)	76,38±1,62 ^{c}	77,60±1,45 ^{c}	69,15±0,78 ^{b}
E (450 ppm)	69,65±0,65 ^{b}	71,38±1,37 ^{b}	71,64±0,38 ^{b}
K+	72,68±2,14 ^{b}	70,96±2,06 ^{b}	70,66±2,06 ^{b}

Information: Leukocyte value (x10^{3} cells/ml)
Numbers in the same column followed by the same letter are not significantly different (P>0.05)
Source: Processed Data (2024)

Hemoglobin

Hemoglobin is contained in red blood cells, where hemoglobin is a protein in erythrocytes. Hemoglobin plays a role in the process of transporting oxygen in the blood and hemoglobin levels in fish blood are related to the number of erythrocytes (Lagler *et al*., 1977).

Treatment D (360 ppm) had the highest amount of hemoglobin so that treatment D (360 ppm) was close to normal or healthy fish conditions (Table 4). This is in accordance with the statement by Bastiawan, *et al* (2001) that the amount

of hemoglobin in gourami fish in healthy conditions is 12.0-14.0 g/dl. However, there is a difference in values between healthy gourami fish and gourami fish that are attacked by bacteria, namely the low Hb value in gourami fish that are affected by bacteria.

Table 4. Hemoglobin Value (hb/100 ml) of Gourami Fish (*O. gouramy*) during the Research

Treatment (ppm)	Healthy Fish	After Infection	After Treatment
K-	7,63± 0,15 ab	2,70± 0,10 c	2,46±0,23 a
A(90 ppm)	7,50±0,10 a	2,66±0,05 c	4,20±0,17 b
B (180 ppm)	7,70±0,10 ab	4,46±0,05 b	4,63±0,85 bc
C (270 ppm)	7,63±0,15 ab	2,33±0,05 b	5,16±0,75 cd
D (360 ppm)	7,80±0,10 b	2,10±0,10 a	5,66±0,11 d
E (450 ppm)	7,50±0,26 a	2,13±0,05 a	4,67±0,56 bcd
K+	7,66±0,11 ab	7,50±0,10 d	7,50±0,10 e

Note: numbers in the same column followed by the same letter are not significantly different (P>0.05)

Source: Processed Data (2024)

Hematocrit

Hematocrit levels can be used to indicate fish health (Bond, 1979), Wells *et al*. (2005) added hematocrit which is the ratio of blood cells and blood plasma, so that hematocrit levels can be used to indicate fish health (Hastuti *et al*. 2003).

Table 5. Hematocrit Value (%) of Gourami Fish (*O. gouramy*) during Research

Treatment (ppm)	Healthy Fish	After Infection	After Treatment
K-	34,00±2,00 a	20,33±0,57 bc	21,00±1,00 b
A (90 ppm)	35,66±1,52 a	22,33±0,57 c	24,33±0,57 c
B (180 ppm)	35,00±3,00 a	16,33±3,21 a	17,00±1,00 b
C (270 ppm)	35,00±3,00 a	19,33±1,15 b	21,00±1,00 b
D (360 ppm)	35,33±0,57 a	16,00±1,00 a	22,00±2,00 b
E (450 ppm)	33,66±1,52 a	18,33±1,52 ab	20,33±0,57 b
K+	32,33±2,51 a	33,33±1,15 d	33,00±1,00 d

Note: numbers in the same column followed by the same letter are not significantly different (P>0.05)

Source: Processed Data (2024)

Normal fish have a high hematocrit number, in the graph above treatment D (360 ppm) has the highest hematocrit number so that treatment D (360 ppm) is close to normal or healthy fish conditions (Table 5). This is in accordance with

Randal's (1970) statement in Dopongtanung (2008) which explains that if the fish's hematocrit value is below 22%, it indicates that the fish is anemic and possibly infected with a bacterial disease.

CONCLUSION

Based on the results and discussion in this research, the following conclusions can be drawn: Based on identification using column chromatographic analysis and GC-MS of noni leaves (*Morinda citrifolia*) with methanol solvent, they predominantly contain terpenoid compounds and other compounds from the Quercetin/Flavonoid group. Giving noni leaf extract (*M. citrifolia*) had an effect on improving cellular health in gourami fish (*Osphronemus gouramy*) infected with *Aeromonas hydrophila*, where the research results showed that the erythrocyte value in treatment D (360 ppm) was close to the erythrocyte value in healthy fish (control +) namely 2.45 ppm) is close to the erythrocyte value in healthy fish (control +), namely 22.0%, the hemoglobin value in treatment D (360 ppm) is close to the erythrocyte value in healthy fish (control +), namely 5.66 hb/100 ml.

Based on the results and discussion in this research, it is recommended that further tests be carried out on noni leaf extract (*M. citrifolia*) in order to obtain more specific results of molecular structure characteristics and it is also necessary to carry out histopathological observations on the gills and liver of gourami fish infected with *A. hydrophila*.

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