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ANALYSIS OF SECONDARY METABOLIC COMPOUNDS OF PURPLE LEAF EXTRACT (*Graptophyllum pictum* (L.) Griff.) ON *Aeromonas hydrophila*

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ABSTRACT

Aquaculture production can be hampered due to outbreaks of pathogens such as Motile Aeromonas Septicemia (MAS) disease caused by Aeromonas hydrophila. One alternative to prevent it is to use natural ingredients such as purple leaf extract (Graptophyllum pictum (L.) Griff). The purpose of this study was to test the content of secondary metabolites by phytochemical screening test and determine its effect as an antibacterial agent through the MIC (Minimum Inhibitory Concentration) method. Purple leaf extract is known to be bacteriostatic because it is only able to inhibit the growth of bacteria. From the results of the phytochemical screening test, the crude extract of purple leaves was positive for active compounds in the form of alkaloids, flavonoids, saponins, triterpenoids and tannins in which these active substances functioned as antibacterials in this study.

Keywords: Handeuleum, Phytochemistry, MIC (minimum inhibitory concentration)

INTRODUCTION

The fisheries sector is one of the strategic sectors in supporting the development of Indonesia's development through fishery commodity trading activities. Aquaculture has developed rapidly. Currently, fish farming is carried out intensively. Along with the development of the aquaculture business, problems arise such as the increased risk of fish disease outbreaks. The disease outbreak resulted in a decrease in production and product quality as well as the destruction of fish species that have important economic value. This is due to resource management that is not well controlled (KKP, 2020).

Aeromonas hydrophila is known as the cause of Motile Aeromonas Septicemia (MAS) disease in freshwater fish. The symptoms of this disease include decreased appetite, wounds on the body surface, bleeding from the gills, enlarged stomach due to fluid content, loose scales, damaged caudal fin, and if surgery is performed, swelling and damage will be seen in liver, kidney and spleen tissue. This disease can cause fish mortality rates of up to 80% (Bariyyah *et al*., 2020).

To overcome this problem, in addition to improving the conditions of the aquaculture environment, treatment by giving antibiotics has often been carried out, but this treatment can cause bacteria to become resistant and increase residues in the fish so that it has a negative impact on consumers and the environment (Muslim, 2009). Therefore, it is necessary to find alternative materials as a substitute for antibiotics from natural ingredients to control these bacterial attacks. An alternative material that can be used is to use purple leaf extract (*Graptophyllum pictum* (L.) Griff). Based on this, it is necessary to test the secondary metabolite analysis of purple leaves through phytochemical screening and dilution testing of Minimum Inhibitory Concentration (MIC) as an antibacterial agent.

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MATERIALS AND METHODS

Research Tools and Materials

Purple Leaf Extract (*Graptophyllum pictum* (L.) Griff), *Aeromonas hydrophila*, TSA (*Trypticase Soy Agar*), TSB (*Trypticase Soy Broth*), Petri dishes, Test Tubes, Incubator branded “redLINE by Binder”, and Spectrophotometer branded “Thermo Scientific GENESYS 20".

Preparation of Purple Leaf Extract (*Graptophyllum pictum* (L.) Griff)

Purple leaves are picked directly from the third leaf to the tenth leaf and then washed clean, then chopped into small pieces. A total of 500 grams of chopped purple leaves were then macerated with 96% ethanol for 3 x 24 hours with daily stirring and then the filtrate was taken by filtering. The filtering results are then evaporated in a rotary evaporator at a temperature of 50°C.

Bacterial Suspension Manufacturing

The manufacture of *A. hydrophila* culture was carried out by inoculating the cultured loops that had been regenerated and suspended in 10 ml of sterile NaCl water with the Farland MC standard (10^8 CFU/ml) (Bariyyah, 2019).

Phytochemical Screening Test

a. Alkaloid Test

The purple leaf extract was put in a test tube, added 0.5 ml of 2% HCL and the solution was divided into two tubes. Tube I added 2-3 drops of Dragendroff's reagent, tube II was added 2-3 drops of Mayer's reagent. If in tube I an orange precipitate is formed and in tube II a yellowish precipitate is formed, it indicates the presence of alkaloids.

b. Flavonoid Test

The purple leaf extract was put in a test tube and then dissolved in 1-2 ml of 50% hot methanol. After that, Mg metal and 4-5 drops of concentrated HCL were added. An orange or red colored solution is formed, indicating the presence of flavonoids.

c. Saponin Test

The extract was put into a test tube plus water (1:1) while shaking for 1 minute, if it causes foam, 1 N HCL is added. If the foam formed can last for 10 minutes with a height of 1-3 cm, it indicates the presence of a group of saponin compounds.

d. Triterpenoid/Steroid Test

The purple leaf extract was put in a test tube, dissolved in 0.5 ml of chloroform, then added with 0.5 ml of anhydrous acetic acid. This mixture is then added with 1-2 ml of concentrated H2SO4 on the tube wall. If the results obtained in the form of a brownish or violet ring on the boundary of the two solvents indicate the presence of triterpenoids, whereas if a bluish green color is formed, it indicates the presence of steroids.

e. Tannin Test

Purple leaf extract was added with 3 drops of 1% $FeCl_3$ solution. If the solution produces a blackish green or ink blue color, it contains tannins.

MIC (*Minimum Inhibitory Concentration*) Test

Prepare a test tube and fill it with sterile TSB (*Tryptic Soy Broth*) media, then make 2 test tubes for positive control (*Chlorampenicol* 5 ppm) and negative control in the form of *A. hydrophila*. The crude extract of purple leaf was made with various concentrations of 1, 10, 100, 500, 1000 ppm. For each concentration test tube, 1 ose of bacterial isolate was given. After that, all test tubes were incubated using an incubator at 37°C for 24 hours. Then the media was checked for turbidity and measured the absorbance value using a spectrophotometer.

RESULTS AND DISCUSSION

Phytochemical Screening Test

Based on the results of the phytochemical tests that have been carried out, the class of chemical compounds contained in the purple leaf extract (*G. pictum* (L.) Griff.) which is indicated by the occurrence of color changes can be seen in Table 1 below:

Table 1. Results of Phytochemical Screening Test of Purple Leaf Extract (*Graptophyllum pictum* (L.) Griff.)

No	Metabolites	Observation Results	Note (+/-)
1	Alkaloids	Orange colored precipitate (Dragendroff)	+
		Yellowish precipitate (Mayer)	+
2	Flavonoids	Reaction Brick Red	+
3	Saponins	There is a stable foam	+
4	Triterpenoids	Brown reaction	+
5	Steroids	Reaction is Green	-
6	Tannins	The reaction is blackish green	+

Note : (+) = color change occurs
(-) = no color change

The results of the phytochemical screening test showed that the purple leaf extract had secondary metabolites in the form of alkaloids, flavonoids, saponins, triterpenoids and tannins (Table 1). This is in accordance with the statement of Ruzana, (2017) the results of the phytochemical test of compounds of the Alkaloid, Triterpenoid, Tannin, Flavonoid and Saponin groups contained in purple leaf extract (*G. pictum* (L.) Griff.)

MIC (*Minimum Inhibitory Concentration*) Test

The MIC test is a way to determine the smallest concentration of medicinal ingredients (purple leaf extract) so that it can inhibit the growth of microorganisms (*A. hydrophila*) macroscopically. The indicator for the observation of the MIC test is the condition of a mixture of TSB (*Tryptic Soy Broth*) which has been inoculated with *A. hydrophila* with purple leaf extract. Treatments: 1, 10, 100, 500, 1000 ppm and measured using a spectrophotometer with a wavelength of 600 nm. The results of the MIC test observations showed that the concentration of purple leaf extract 100 ppm was able to inhibit the growth of *A. hydrophila*.

The results of the MIC test of purple leaf extract against *A. hydrophila* for 24 hours are presented in Table 2. The results of the MIC test of purple leaf extract on the growth of A. hydrophila are presented in Figure 1. Treatment of 100 ppm purple leaf extract showed the tube containing the extract was clear and getting better. The higher the concentration of the treatment in the tube media, the clearer the condition of the resulting media. This is supported by the opinion of Pelezar and Chan (1986), the higher the concentration of antimicrobial used, the faster the bacteria are killed.

Table 2. MIC (*Minimum Inhibitory Concentration*) of Purple Leaf Extract against *Aeromanas hydrophila* During 24 Hours Observation

Concentration (ppm)	**MIC Value (10^8 cells/ml) Bacteria**	**MIC Results**
1000 ppm	0,142	Clear
500 ppm	0,131	Clear
100 ppm	0,289	Clear
10 ppm	1,777	urbid
1 ppm	1,892	Turbid
Positive Control (C+)	0,085	Clear
Negative Control (C-)	2,816	Turbid

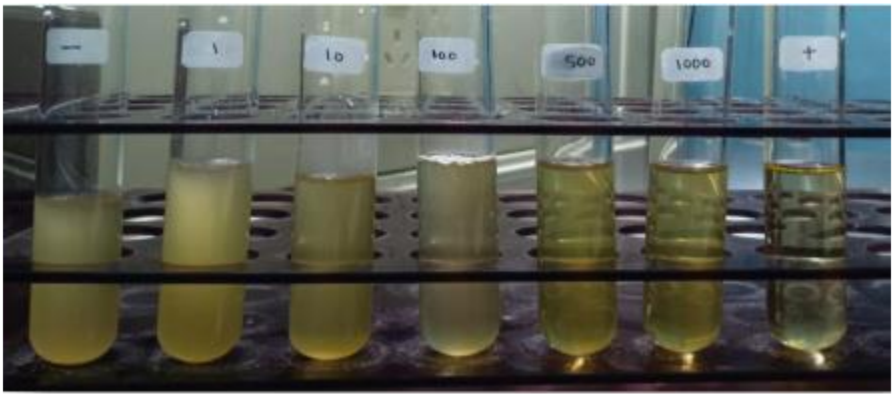

Figure 1. MIC Test Results of Purple Leaf Extract (*Graptophyllum pictum* (L.) Griff.)

Then a test was carried out to find out that purple leaf extract (*Graptophyllum pictum* (L.) Griff.) could inhibit bacteria that were bacteriostatic or bactericidal, which were tested using TSA (*Trypticase Soy Agar*) media and were given bacteria in a petri dish which was observed for 24, 48 hours. and 72 hours. The results of these observations can be seen in Table 3.

Table 3. Results of MIC (*Minimum Inhibitory Concentration*) Test on TSA (*Trypticase Soy Agar*) Media Tested for 24, 48 and 72 Hours.

Concentration (ppm)	**Observation Time (Hours)**		
	24	**48**	**72**
1000 ppm	-	+	+
500 ppm	-	+	+
100 ppm	-	+	+
10 ppm	+	+	+
1 ppm	+	+	+
Positive Control (C+)	-	-	-
Negative Control (C-)	+	+	+

Description: (+) = Bacteria Grow on Media
(-) = Bacteria Not Growing on Media

The results of the MIC test using TSA (*Trypticase Soy Agar*) media can be seen that at concentrations of 100 - 1000 ppm observed for 24 hours, bacteria do not grow (Table 2). Observations for 24 hours showed purple leaf extract (*G.raptophyllum pictum* (L.) Griff.) was bacteriostatic because it was only able to inhibit *A. hydrophila* at that time. While the observation for 48 hours bacteria began to grow on the media. According to Goering *et al.*, (2013) bacteriostatic is the nature of antibiotics that can inhibit the growth of bacteria temporarily (*reversible*). The inhibitory concentration was lower than the bactericide concentration. Kee and Hayes, (1996) added that the bacteriostatic properties of antibacterials were only able to inhibit the growth or development of bacteria but did not kill the bacteria. While bactericidal, able to play a role in killing bacteria so that they cannot grow or proliferate again.

CONCLUSION

Based on the results and discussion of the research above, it can be concluded that purple leaf extract (*Graptophyllum pictum* (L.) Griff.) contains active compounds in the form of alkaloids, flavonoids, saponins, triterpenoids and tannins that function as antibacterial. The results of the MIC test, the purple leaf extract concentration of 100 ppm and above showed a clear color that was able to suppress the growth of *Aeromas hydrophila*, but the purple leaf extract was bacteriostatic because it was only able to inhibit the growth of bacteria.

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