

UTILIZATION OF ROSELLE (*Hibiscus sabdariffa* L.) METHANOL EXTRACT ON HEMATOLOGY OF CARP (*Cyprinus carpio*) INFECTED BY *AEROMONAS HYDROPHILA*

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ABSTRACT

The aim of this study was to examine the ability of roselle (*H. sabdariffa* L.) flower methanol extract in treating carp infected with *A. hydrophila* through erythrocyte and leukocyte blood profile. The research method uses a completely randomized design, with one independent variable (different doses of methanol extract of roselle flowers). Treat the dose of methanol extract of roselle flowers given by immersion method in this study were C - (bacterial infection of *A. hydrophila* and without extract dose), 75, 150, 225, 300, 375 mg/L and C + (without bacterial infection and without extract doses). Each treatment was repeated 3 times. The density of *A. hydrophila* as a pathogen is 107 CFU/ml. Hematological observations of carp are observed in healthy fish, after infection and after treatment. The results showed that the methanol extracts of the roselle flower petals were able to treat carp infected with *A. hydrophila* by normalizing the hematology of carp. The best dose of methanol extract of Roselle flower is effective in treating carp infected with *A. hydrophila* until the end of the study significantly ($P < 0.05$) i.e. 300 mg/L.

Keywords: Treatment, Erythrocytes, Leukocytes, Soaking, *Hibiscus* sp.

INTRODUCTION

An infectious disease caused by *A. hydrophila* or called Motile Aeromonas Septicemia (MAS) in carp (*C. carpio*) is one of the diseases that can cause no small amount of loss. Besides being able to kill fish, the disease can reduce the meat quality of infected fish. This disease apparently can cause fish death around 80-100% within 1-2 weeks (Maisyaroh *et al.*, 2018).

General efforts to overcome diseases caused by *A. hydrophila* are usually treated with chemicals such as antibiotics, but

improper use of antibiotics can cause resistance to bacteria and also pollute the environment. So, another alternative is to use natural ingredients that are antibacterial such as roselle flower (*H. sabdariffa* L.). Roselle flowers are known to contain phenolic active substances in the form of flavonoids and tannins which function to kill and inhibit bacterial growth (Bariyyah *et al.*, 2019). Several studies have shown this plant has antibacterial activity against gram-

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positive and negative bacteria (Abdallah, 2016).

Based on the antibacterial content of the roselle (*H. sabdariffa* L.) flower, its use needs to be applied in the treatment of fish. Hematology is closely related to pathology, especially in knowing the condition or health status of fish that is in a healthy or sick condition. So that the purpose of this study was to examine the hematological profile and effectiveness dose of methanol extract of roselle flowers in carp (*C. carpio*) infected with *A. hydrophila*.

MATERIAL AND METHODS

Blood Sampling

Fish blood was taken at the front of the caudal fin and dorsal fin using syringes which were first given anti-coagulant with 0.1 ml Na Citrate or Na₂ EDTA, the syringe was made with a slope of 45°. The blood drawn is put in a tube and then stored in a refrigerator.

Erythrocyte Count

The blood that has been taken is then diluted in an erythrocyte pipette, after which it is put into the counting room. The number of erythrocytes is calculated in a certain volume by using a conversion factor, the number of erythrocytes per μl of blood. The diluent solution used is HAYEM solution, with 5 g sodium sulfate (aqueous crystalline) composition; sodium chloride 1 g; mercury chloride 0.5 g, 200 ml aquadest. GOWERS solution can also be used: sodium sulfate 12.5 g; glacial acetic acid 33.3 ml; 200 ml aquadest. Filtered before use.

Leukocyte Count

Blood is diluted in a leukocyte pipette, then put into a counting chamber. The number of leukocytes is calculated with a certain volume by wearing a conversion factor the number of leukocytes per μl of blood can be. TURK solution is used as a diluent solution with a composition of 1% gentian violet solution in 1 ml water, 1 ml glacial acetic acid, 100 ml aqua dest. Filtered before use.

Data Analysis

Hematology analysis uses ANOVA (Analysis of variance) analysis. The software used for data analysis is SPSS (Statistical Program for Social Science) Version 25.

RESULT AND DISCUSSION

Erythrocytes

Based on observational data in Table 1, it can be seen that the average number of healthy carp erythrocytes ranged from 1.46 to 1.66×10^6 cells/mm³ which showed no significant differences between treatments. Then for fish infected with *A. hydrophila* known the average number of erythrocytes in the range of 0.85 - 0.90×10^6 cells/mm³, the value experienced drastic decline even though there were no significant differences between treatments. The decrease in the number of erythrocytes that occur is thought to be caused by the pathogenicity of *A. hydrophila*. This bacterium produces the enzyme hemolysin which can lyse red blood cells, so the number of red blood cells in blood vessels can be reduced (Anisha Minaka, Sarjito, 2012).

Table 1. Erythrocyte value during the research ($\times 10^6$ cells/mm³)

Treatment (mg/L)	Healthy	After being infected	After being treated
0 (C -)	1.58 ± 0.03 ^a	0.88 ± 0.01 ^a	0.80 ± 0.02 ^a
75	1.65 ± 0.14 ^a	0.85 ± 0.02 ^a	1.06 ± 0.03 ^b
150	1.59 ± 0.12 ^a	0.87 ± 0.05 ^a	1.22 ± 0.05 ^c
225	1.66 ± 0.14 ^a	0.89 ± 0.03 ^a	1.34 ± 0.04 ^d
300	1.57 ± 0.12 ^a	0.90 ± 0.06 ^a	1, 60 ± 0.05 ^f
375	1.46 ± 0.11 ^a	0.90 ± 0.05 ^a	1, 59 ± 0.01 ^e
0 (C +)	1.60 ± 0.01 ^a	1.55 ± 0.03 ^b	1.61 ± 0.06 ^f

From this table, it was known that the methanol extract of roselle flower (*H. sabdariffa* L.) had a significant effect ($P < 0.05$) on erythrocytes in the range of 1.06 - 1.60 $\times 10^6$ cells/mm³. This increase is still within the normal range and indicates a homeostatic effort on the body of the fish due to pathogenic infections so that the body produces more blood cells to replace erythrocytes undergoing lysis due to infection (Hardi *et al.*, 2011). The normal amount of erythrocytes in Teleostei fish between 1.05 - 3.0 $\times 10^6$ cells/mm³ (Matofani *et al.*, 2013). Besides the increase in erythrocyte levels in the blood of carp is the effect of the activity of the methanol extract of roselle flower (*H. sabdariffa* L.). Sundaryono, (2011) explains that flavonoids are compounds that can increase erythropoiesis (the formation of erythrocytes) in the bone marrow. So that the treatment that can increase erythrocyte goldfish infected with *A. hydrophila* is a dose of 300 mg/L which is 1.60 $\times 10^6$ cells/mm³.

Total Leukocytes

Leukocyte values in healthy fish ranged from 11.21 - 11.56 $\times 10^4$ cells/mm³ (Table 2). But after being infected with *A. hydrophila*, the number of leukocytes increases to the range of 16.91 - 17.24 $\times 10^4$ cells/mm³. Increasing the number of leukocytes can provide clues to the first phase of infection, stress, and leukemia (Priyatna *et al.*, 2018). According to Zou *et al.* (2000) an increase in the number of leukocytes is caused by increased cell division activity because leukocytes play a role in eliminating pathogens that enter the body. Lengka *et al.* (2013) added that the number of leukocytes in certain types of fish can change according to the health level of the fish. If fish are infected by pathogenic bacteria, there will be an increase in the total number of leukocytes or the number of leukocytes away from their normal limits and will suffer from leucopenia.

Table 2. The value of leukocytes during the research ($\times 10^4$ cells/mm³)

Treatment (mg/L)	Healthy	After being infected	After being treated
0 (C -)	11.47 ± 0.50 ^a	16.91 ± 0.52 ^b	16.93 ± 0.54 ^f
75	11.21 ± 0.10 ^a	17.24 ± 0.22 ^b	15.21 ± 0.18 ^e
150	11.48 ± 0.24 ^a	16.92 ± 0.08 ^b	14.54 ± 0.01 ^d
225	11.56 ± 0.19 ^a	16.79 ± 0.31 ^b	13.32 ± 0.01 ^c
300	11.39 ± 0.05 ^a	17.04 ± 0.42 ^b	12.29 ± 0.02 ^b
s375	11.29 ± 0.08 ^a	17.01 ± 0.29 ^b	13.01 ± 0.01 ^c
0 (C +)	11.49 ± 0.23 ^a	11.29 ± 0.03 ^a	11.33 ± 0.33 ^a

ANOVA test results (Analysis of variance) treatment of rosella flower (*H. sabdariffa* L.) methanol extract showed a significant effect ($P < 0.05$) in the range of 15.21 - 12.29 x10⁴ cells/mm³. In general, the total number of leukocytes in fish is 2 - 15 x10⁴ cells/mm³. The number of leukocytes in healthy carp ranges from 3.2 - 14.6 x10⁴ cells/mm³ (Harikrishnan *et al.*, 2003; Hartika *et al.*, 2014). The decrease in the number of leukocytes that occur is suspected to be due to the activity of the methanol extract of rosella flowers (*H. sabdariffa* L.) making the bacteria die and the infection stops, besides the fish are not under stress. When the infection stops, leukocytes as a phagocytosis are no longer needed so that the number of leukocytes returns to normal. Therefore, the treatment that can reduce fish leukocytes infected with *A. hydrophila* is a dose of 300 mg/L of 12.29 cells/mm³.

CONCLUSION

From the results of the study, it can be concluded that the administration of roselle (*H. sabdariffa* L.) methanol extract is able to control *A. hydrophila* infection in carp, seen from a decrease in the number of leukocytes and an increase in the number of erythrocytes at the best dose of 300 mg/L.

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