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Effect of Treatmen of Akway Bark Extract (*Drymis piperita*) on the Amount of Erythrocytes and Hemoglobin of *Orochromis niloticus* Infected with *Aeromonas hydrophila*

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ABSTRACT

This research was conducted to prove the phenolic compounds contained in the bark akway (*Drymis piperita*) on blood hematology of tilapia. In this study we used the size of the fish with a length of 5 cm, 7 cm and 9 cm, with a dose treatment of akway bark extract namely 0.09 ppm, 0.9 ppm 9 ppm, 0.112 ppm, 1.12 ppm and 11.2 ppm and carried out for 14 days observation. The analysis was carried out on the hematological components which included erythrocytes and hemoglobin. The results showed that at the end of the observation the number of erythrocytes at the size of fish 5 cm = 1134.30 ± 6,739, fish size 7 cm = 1208.30 ± 4,138 and at fish size 10 cm = 1272.79 ± 3,928. The average hemoglobin value at the end of the observation for fish size 5 cm = 6.17 ± 0.153, fish size 7 cm = 7.10 ± 0.100 and fish size 10 cm = 7.80 ± 0.000. Thus, there is difference in the number of erythrocytes and hemoglobin between treatment doses.

Key words : *Drymis piperita, Erytrocyte, Hemoglobin, Orocromis niloticus, Aeromonas hydrophila*

Introduction

Tilapia nile (tilapia) is the second most widely cultivated fish worldwide world because it has high economic value (Wang and Lu, 2016). But In recent times, the production has started to decline causing economic losses (Silva *et al.,* 2012). Presence of microorganisms and disease caused by bacteria is thought to be the main cause (Van Hai, 2015). Aeromonas hydrophila is a pathogenic bacterium that can cause high fish mortality by the main feature of motile or hemorrhagic septicemia (Yardimci and Aydin, 2011). Material use Synthetic antibiotics have not been able to suppress fish mortality caused by this bacterium. But the continuous use of antibiotics is a serious problem that arises because it can cause bacteria to become resistant (Dias *et al.,* 2013). Fish infected with *A. hydrophila* can be detected

through hematological components in addition to environmental factors (Vaishali and Punita, 2013). Fish blood can be used as an indicator of health status caused by endogenous stressors and changes due to environmental factors. Approaches to fish health monitoring is done by observing the etymology and biochemistry of the blood profile. haematological changes and biochemistry in the blood can be used as an important indicator to monitor physiological and pathological changes in fish (Fransesco *et al.*, 2012). Analysis of the index biochemistry and hematology in the blood of cultured fish is important to identify and know the health status of fish. This is because with knowledge of blood hematology, can provide information about disorders and changes in metabolic function (Bahmani *et al.*, 2001). Under normal circumstances, the composition of fish blood fairly constant and only slightly varies due to environmental fluctuations (Vaseem and Banerje, 2013). However, the composition of the blood can change with treatment or infection by bacteria and viruses (Acharya and Mohanty, 2018). Use plants that have phenol content have begun to be developed as an alternative in bacterial control so that it is expected to be applied in the field of cultivation (Baydar*et al.*, 2004), Akway wood (Zakaria *et al.*, 2018).

Materials and Methods

Phenol Compound Extract

Extraction was carried out using MERCK ethyl acetate solvent, 109623, mixed with extraction was carried out by a maceration process with a ratio of akway wood powder and solvent 1:4 for 72 hours while in a shaker. The extraction process is carried out by the maceration method. The ratio of akway bark powder and solvent was 1:4 for 72 hours. evaporated at 40 ^{o}C. the final product was stored in a dark bottle (Cepeda *et al.*, 2015). A. Hydrophila A. hydrophila bacteria were imported from the Surabaya Quarantine Center, East Java, Indonesia. after the LD was tested, the bacterial density used was 8.3 x 106 cfu/ml.

Erythrocytes

The calculation begins with filling the erythrocyte pipette, i.e. the blood is sucked up to the 0.5 and mark lines Hayem's solution to 101 lines. homogenized 5-10 minutes to ensure proper mixing appropriate. Discard the first 3-4 drops, and count the filled chambers using a microscope at magnification (40x) (Maftuch and Nurin, 2020). Total erythrocytes obtained from the formula Erythrocytes = (A/N) x (1/V) x Fp

A = cell count
V = hemocytometer field volume
N = the observed hemocytometer field
Fp = dilution factor

Hemoglobin

The measurement of the amount of hemoglobin (Hb) refers to the Sahli method, the blood is sucked with a pipette sahli up to a scale of 20 mm^3 or on a scale of 0.02 ml, the blood is transferred into an Hb-meter which is containing 0.1 N HCl, stirred and allowed to stand for 3-5 minutes. added aquabides into blood and HCl color as the color of the standard solution on the Hb meter. The scale is read with Look at the surface of the liquid and match it with the Sahli tube scale. number on scale shows the amount of hemoglobin in grams per 100 ml of blood (Maswan, 2009).

Results

The fish size is 5 cm there is a significant difference in the dose treatment and days with values (p=0.000 and p=0.000). Doses of 11.2 ppm and the last day had the highest mean erythrocyte higher than the other doses, namely 1134.30 ± 6.739 (Figure 1a). In Figure 1b, The number of erythrocytes in fish size 7 cm, there is a significant difference in the treatment dose and day with a value (p = 0.000 and p=0.000). Doses of 11.2 ppm and the last day had the highest mean values of erythrocytes compared to doses of the other is 1208.30 ± 4,138 while in Figure 1c, it is known that at size 10, there is a difference which was significant in the treatment dose and day with a value (p = 0.000 and p = 0.000). Dose 11.2 ppm and last had the highest mean value of erythrocytes compared to other doses, namely 1272.79 ± 3.928. Differences between treatments with dose during the time of observation.

In Figure 2a for fish with a length of 5 cm, there is a significant difference in treatment dose and day with a value (p = 0.000 and p = 0.000). Doses of 11.2 ppm and last day had a mean value of the highest hemoglobin dose compared to other doses was 6.17 ± 0.153. For fish size 7 cm (Figure 2b), there is a significant difference in the treatment dose and day

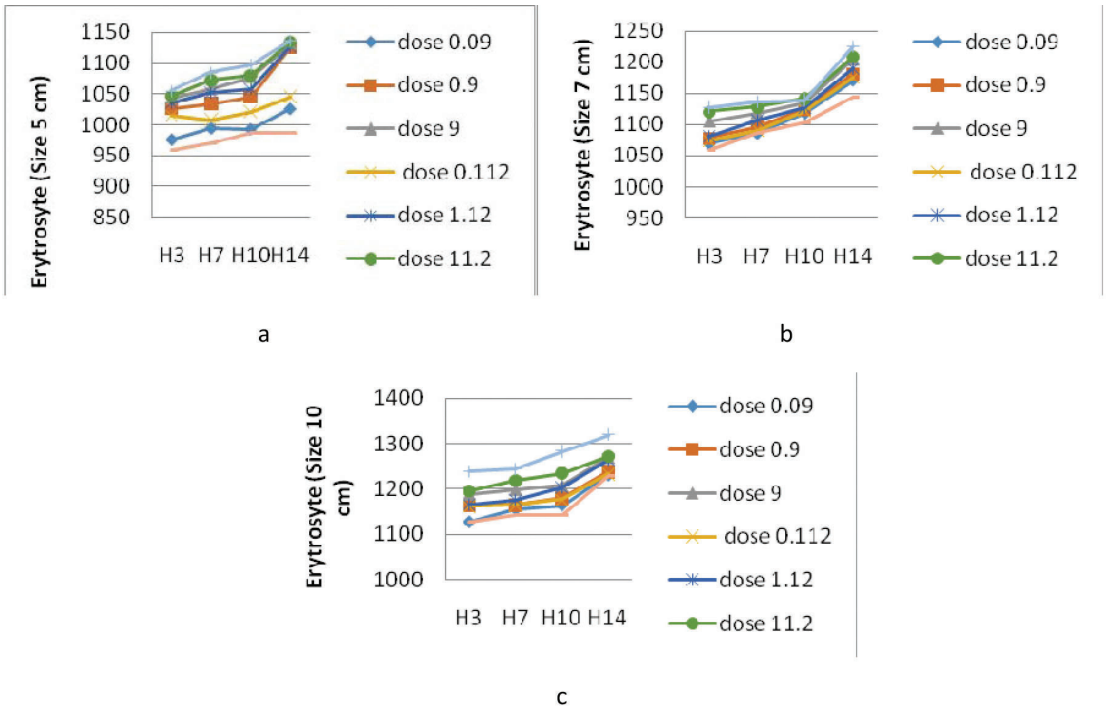

Fig. 1. a) The total number of fish erythrocytes is 5 cm; b) Fish erythrocytes are 7 cm; c) Fish erythrocytes measuring 10 cm.

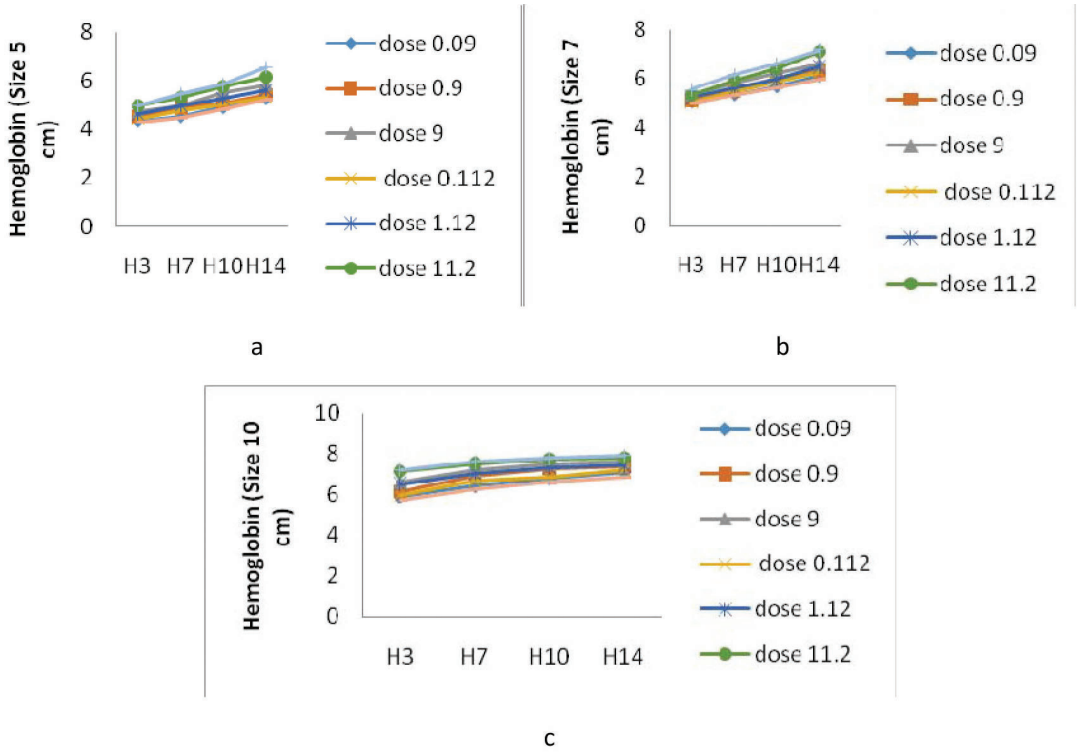

Fig. 2. a) Hemoglobin (fish size 5 cm); b) Hemoglobin (Size 7 cm); c)Hemoglobin (Size 10cm)

with values (p = 0.000 and p=0.000). Doses of 11.2 ppm and last day had the highest average hemoglobin values compared to doses of another, namely 7.10 ± 0.100 and for fish size of 10 cm (Figure 2c) also found significant differences significant on the treatment dose and day with a value (p = 0.000 and p = 0.000). Dose 11.2 ppm and last day has the highest average hemoglobin value compared to other doses, which is 7.80 ± 0.000 but not significantly different with a dose of 9 ppm.

Discussion

As a result of *A. hidrophila* infection causing anemia which has an impact on ihibition of growth and metabolism of fish due to the low of number of erythrocytes resulting in the supply of food to cells, tissues and organs (Harikrishnan *et al.,* 2010). Besides that phagocytic processes that require oxygen so that it affects the decrease normal erythrocyte counts in fish and the production of exotoxins in the form of hemolysin enzymes which are capable of lyse red blood cells on the surface of the gills, kidneys and liver by liberate hemoglobin (Akram *et al.,* 2021). Erythrocytes will then begin to increase due to the administration of akway wood extract containing phenolic compounds. This is presumably because phenol compounds are able to increase erythrocyte by stimulating the work function of the gill organs, kidneys and liver to can produce erythrocyte that can be use to replace erythrocyte that lysis due to infections (Francesco, 2019). Gad and Saad (2008), that phenol compounds can increase the number of erytrhrocyte in rainbow fish trout (*Onchorynchus mykiss*). Phenol content provides inhibition of erythropoiesis in hemopoetic organs.

The low hemoglobin value of toilapia at the beginning of the observation was thought to be due to *A. hydrophila* infection which cause anemia. Anemia in fish is caused by induction of cysteine proteinase enzymes that can prey on red blood cells that causes damage to red blood cell in the form of hemorrage (Ruszczyk *et al.,* 2008). The decrease in hemoglobin concentration is also directly proportional to the decrease in the red blood cells leukocytosis which results in erythroblastosis (Harikrishnan *et al.,* 2003). In addition, there was a decrease in hemoglobin on the first day. The observation are thought to be due to the adaption of the fish to the environmental changes that occur caused by bacterial infections. The second observation until the end of the observations showed an increase in the number of hemoglobin of tilapia infected with *A. hidrophila*. An increase in the amount of hemoglobin is suspected, this was caused by the fish being treated with akway bark wich contained phenolic compounds causes an increase the immunity of Tilapia so that it can tolerate infections cause by Tilapiareactivate the function of erythropoiesis in the lymph tissue and liver of fish in producing hemoglobin to blind oxygen and subsequently involved in the process of transporting oxygen to the peripheral network. The increase hemoglobin is linear with the increase in erythrocytes (Frandson, 1992).

Conclusion

Administration of akway bark extract on erythrocyte and hemoglobin hematological parameters showed the ability to reduce reduce the level of damage that occurs so that it can be applied to fish that infected. this is proven by the reduced level of damage so that it can be used in treating sick fish.

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